ISAG 34th XI'AN 2014

ABSTRACT

2014.7.27 - 8.1

The 34th International Society for Animal Genetics Conference

Author Index

Numbers following names refer to abstract numbers; an S prior to the number indicates an invited speaker presentation, and a P prior to the number indicates a poster presentation. The author index is created directly and automatically from the submitted abstracts. If an author's name is typed differently on multiple abstracts, the entries in this index will reflect those discrepancies. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.

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Invited Speaker

S001 - S017

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S001 How animal genomics can shed light on important biological mechanisms. Leif Andersson (Uppsala University)

Genome research in domestic animals is well justified because of their agricultural importance but also because of their unique role as model organisms for biological research. Animal breeding during the last 10,000 years is by far the most extensive screen for genetic variants with phenotypic effects that has been performed in animals. This selection scheme has led to genetic adaptations to a life in captivity, to new environments well outside the species range and different production forms. The majority of genetic variants that have been under selection have small phenotypic effects and are therefore difficult to reveal and study at the molecular level. However, some of the genetic variants have large phenotypic effects and have been crucial during the selection process. Humans have also cherry-picked genetic variants with prominent effects on the trait under selection such as the *Gait Keeper* (*DMRT3*) mutation in horses or because of appealing effects on the appearance such as *Greying with age* (*STX17*) in horses. In this presentation I will describe some examples of gene discoveries we have made in domestic animals that have revealed previously unknown biological mechanisms. I will also argue that domestic animals are still an underutilized resource for basic biological research.

S002 Genetic mapping of complex traits using mosaic populations. Jonathan Flint (Wellcome Trust Human Genetics)

Mosaic populations are those descended from a small set of founders, typically 8 inbred individuals. In mosaic populations, chromosomes are a fine-grained mosaic of the founders, providing unique opportunities for genetic mapping and exploring the relationship between phenotype and genotype. Genetic analysis can take place from two perspectives: from the point of view of the ancestral haplotypes (the progenitor chromosomes) and from the point of view of the individual sequence variant (human geneticists only work with the latter). Under the assumption that a single casual variant is responsible, then the association between phenotype and causal variant will be more significant than the association between phenotype and the ancestral haplotype. This permits a statistical test for the presence of causal variants. I will describe the compilation of complete catalogues of sequence variants, and the analysis of their joint and individual effects in mosaic populations. Using the causal variant test, we have established the distribution of effect sizes of causal variants, and related this to their molecular nature and their position in the genome. The most striking finding about the relationship between sequence variant and phenotype is that the majority of genetic effects cannot be attributed to a single causal variant.

S003 The Impact of NGS Technology on Animal and Plant Breeding. Gengyun Zhang (Beijing Genomics Institute, Shenzhen(BGI-SZ))

The quick advances of sequencing technology, based on NGS, make us easier to elucidate whole genome sequence of a species, even for neglected species. The genotyping (molecular diversity) of different individuals could be obtained economically through a sequencing based procedure. The breakthrough of genome sequencing technology dramatically improved our capacity to recognize and exploit biodiversity in germplasms. The largest high throughput sequencing and bioinformatics platforms in the world have been established in BGI. The success of our series breeding projects indicated that there's no further technical obstacles for applying whole genome molecular marker assisted selection, no matter for animal or plant species . Whole genome marker assisted selection system can dramatically increase our genetic gains in breeding procedure. Our successes indicate a new era for animal and plant breeding is coming. The technical platforms established in BGI could provide practical and economic support for global breeding efforts.

S004 Mapping of Functional Elements in the Human and Mouse Genomes. Bing Ren (University of California, San Diego)

A major challenge confronting the biomedical research field is to elucidate how genome sequence directs temporal and tissue specific gene expression programs during development. A large number of potential cis regulatory sequences have been annotated in the human genome, thanks to advances in high throughout technologies, but the function of these elements and their roles in health and diseases remain largely uncharacterized. In this presentation I would discuss results from experiments designed to understand the gene regulatory programs in the human and mouse cells. We have used high throughput approaches to characterize the transcriptome, DNA methylation, chromatin modification and higher order chromatin structure in a broad panel of cell types and tissues. Integrative analysis of these datasets revealed widespread remodeling of the epigenome and reorganization of higher-order chromatin structure among distinct cell types. More over, pervasive allelic gene activities are detected in the genome. The allelic gene expression patterns can be correlated to epigenetic state at distal enhancers, supporting the role of these elements in regulating gene expression over a distance.

S005 Introgression mapping in pigs: Selection of Asian haplotypes in European pigs following 18th – 19th century human mediated hybridization. Martinus Groenen (Wageningen University, Animal Breeding and Genomics Centre)

The genomes of domestic pigs have been shaped by a complex demographic history, independent domestication in Asia and Europe and, more recently, human mediated admixture. About 10,000 years ago pigs were domesticated independently in Europe and Asia from local wild boars that had diverged around 1My ago. This resulted in distinct European and Asian pig breeds, each with discrete phenotypic characteristics. In the late 18th - early 19th century, the domesticated descendants of these divergent populations were hybridized as Asian pigs were used to improve Western pigs. This hybridization can be identified in the genome of European pigs as relatively long haplotypes shared with Asian pigs. Since the initial introgression, subsequent bottlenecks and selection have resulted in specific Asian haplotypes to reach high frequencies in European breeds. Using whole genome sequence data, we demonstrate both the presence of introgressed Asian haplotypes in these European domestic pigs and selection signatures on loci in those regions. These signatures are often subtle and rarely result in a complete sweep, possibly because of the quantitative nature of the associated traits. The identified Asian introgressed haplotypes are associated with regions harboring genes involved in meat quality, development and fertility. To be able to distinguish between drift and selection, we analysed some of these regions in an association study in a large pig population. Individuals were genotyped with the Illumina 60K SNP beadchip and measured for growth, fatness and fertility traits. Our findings suggest that increased fertility was an important breeding goal for early nineteenth century pig farmers, and that specific Asian variants were selected during the development of modern European pig breeds.

S006 Long history of natural and artificial selection shapes Chinese pigs with immense diversified phenotypes and vast genomic sequence variation. Lusheng Huang (Jiangxi Agricultural University)

China is one of the main domestication centers for pigs. During a long (~10,000 years) history of natural selection and human-driven artificial selection, Chinese indigenous pigs have developed immerse phenotypic diversity in morphology, fertility, growth, palatability, and local adaptability. Now China is the leading country with the richest genetic resource of domestic pigs, having more than one-third (~100 breeds) of total global breeds. To understand how the long history of natural and artificial history shapes phenotypic and genomic diversity in Chinese pigs, we first constructed a genomic DNA bank of more than 12,000 samples covering all Chinese native pig breeds. We then conducted systematic investigations on phenotypic characteristics for the Chinese representative local breeds, with the design covering all the sire lines and

the majority of the dam lines in the studied breeds, and all the animals were transported to NanChang, raised in the same farm for the characterization of 200 phenotypic traits . We also measured over 400 phenotypic parameters in 6 experimental crosses, including a unique 8-breed heterogeneous mosaic population and a large scale F2/F3 intercross. Using the above-mentioned experimental populations and a battery of genetic assays, we demonstrated that the Chinese brown and white belt coat color were caused by a 6-bp deletion in the coding region of TYRP1 and a regulatory mutation in EDNRB respectively. We showed that a truncating mutation in HOXA1 causes congenital microtia in Chinese Shaziling pigs. Our RNA-Seq analysis further provided a list of candidate genes for human microtia and its associated syndromes. We also revealed that the VRTN QTN increasing porcine vertebral number was originated from Chinese pigs. As a multigenic effect example, we illustrated that a missense mutation in PPARD and a copy number variation covering MSRB3 are two causative variants for external ear size. To uncover the genetic diversity, we re-sequenced the genomes of 69 pigs from 15 geographical divergent locations in China. The 25 × genome coverage enabled us to detect 41 million variants, substantially expanding the catalogue of porcine genetic variants. We identified a genome-wide set of loci that likely have a role in regional adaptations to high- and low-latitude environments within China. These loci correspond to a list of genes related to thermostatic regulation like hair cell differentiation, energy metabolism and blood circulation. Interestingly, we found an exceptionally large (14 Mb) and low-recombination region on the X chromosome that appears to have two distinct sweeps in the high- and low-latitude populations, possibly underlying their adaptation to cold and hot environments respectively. Surprisingly, the adaptive sweep in the high-latitude regions has acted on DNA probably introgressed from an extinct Sus species, providing the first example of adaptive evolution triggered by intergeneric introgression in mammals. These findings significantly improve our understanding of the genetic basis of diverse phenotypic variations in Chinese indigenous pigs.

S007 Targeted Genome and Epigenome Editing Using CRISPR and TALE Technologies. Jae Joung (Department of Pathology, Massachusetts General Hospital and Harvard Medical School)

Targeted genome and epigenome editing technologies have recently emerged as important tools for biomedical research and as potential reagents for therapies of gene-based diseases. In this talk, I will present our recent work using the clustered regularly interspaced short palindromic repeat (CRISPR) RNA-guided nuclease platform for introducing targeted genome sequence alterations, including discussion about the latest specificity improvements developed by our group. I will also describe the creation and validation of new technologies for modifying specific epigenomic marks on histones and DNA that can be used to induce targeted alterations in endogenous human gene expression. Taken together, these methodologies provide transformative tools for understanding human biology and offer promising pathways forward for developing therapies based on targeted alterations of gene sequence and expression.

S008 Unraveling the swine genome: implications for human health. Lawrence Schook (University of Illinois)

The pig (*Sus scrofa*) was first used in biomedical research in ancient Greece and has quickly grown into an important biomedical research tool over the past few decades. Their importance as a biomedical model is due to their anatomical, behavioral, genetic and physiological similarities with humans, as well as their availability, short generation interval and large litter size. Studies using pigs have been shown to be more predictive of therapeutic treatments in humans than rodent studies, and are currently being used to study a variety of human diseases including Huntington's, Alzheimer's and cardiovascular diseases. Due to genomic and physiological similarities, the pig is emerging as a valuable translational biomedical research animal model.

Completion of the pig genome sequence, the ability to genetically modify and somatically clone provides the foundation for further development of validated porcine models. In addition, the similarity in size and physiology allows pigs to be used for many experimental approaches not feasible in mice. Research areas utilizing pigs range from neonatal development to translational models for cancer therapy. Increasing numbers of porcine models are being developed with the release of the swine genome sequence and the development of additional porcine genomic and epigenetic resources will further their use in biomedical research. The porcine genome provides the foundation for development of novel animal models to validate human conditions and to support clinical trials to expedite new drugs, devices and diagnostics. Clearly, further refinement of the pig genome will be critical to fully exploiting the physiological characteristics of the pig to develop Quantitative Trait Nucleotide (QTN) causative for human diseases. Also, essential will be the development of innovative bioinformatic tools that are linked to emerging new genetic modeling tools. The opportunities are endless with validating existing models and rapidly testing QTN based hypotheses.

S009 Evolution of the immunoglobulin heavy chain genes in jawed vertebrates. Yaofeng Zhao (State Key Laboratory of Agrobiotechnology, China Agricultural University)

Immunoglobulins (Igs) are pivotal defending molecules of adaptive immune system and are only expressed in jawed vertebrates including mammals, birds, reptiles, amphibians, teleost (bony fish) and cartilaginous fish. Although it is well known that mammals express five isotypes of immunoglobulin heavy chains (IgM, IgD, IgG, IgA and IgE), only IgM and IgD are now recognized to be the most ancient Ig classes emerged at approximately 500 million years ago. While IgM is found in nearly all jawed vertebrates examined so far (with coelacanth as the only exception) and exhibits a conserved structure (always containing four constant domains), IgD is missing in birds and a selected number of mammalian species, and surprisingly shows a great structural variation across species. IgG and IgE are two mammal-specific Ig classes, which are both believed to be originated from a common precursor, IgY, expressed in non-mammal tetrapods such as birds, reptiles and amphibians. IgA, the mucosal antibody, is widely found in amphibians, birds and mammals, but is surprisingly lost in most reptiles. Although the IgA orthologue was not identified in fish, a number of teleost fish are shown to develop a distinct Ig class, IgZ/T, which can perform similar functions to IgA in mucosal immunity, showing a typical case of convergent evolution. With extensive and comparative studies of the immunoglobulin genes in many species, we are now able to draw a clear picture describing the evolution process of immunoglobulin genes in jawed vertebrates.

S010 Engineering Fat Cell Fate to Fight Obesity and Metabolic Diseases. Shingo Kajimura (University of California, San Francisco)

All mammals harbor two types of adipose tissues that serve distinct physiological functions: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT functions mainly in the storage of excess energy, while BAT specializes in dissipating energy in the form of heat and functions as a defense against hypothermia. Recent studies report that adult humans also have significant amounts of active BAT and it's mass inversely correlates with adiposity, indicating the potential importance of BAT in human obesity.Our lab aims to understand the developmental and molecular circuits that regulate fate specification of brown adipocytes and to investigate their roles in energy homeostasis under pathological conditions such as obesity and diabetes. Recent studies in our lab will be reviewed and discussed in the lecture.

S011 Antiviral RNAi – A new antiviral immunity mechanism in mammals. Shou-Wei Ding (University of California - Riverside)

Diverse eukaryotic hosts produce virus-derived small interfering RNAs (siRNAs) to direct antiviral

immunity by RNA interference (RNAi). Until recently, however, it was unclear whether the mammalian RNAi pathway has a natural antiviral function. In a paper published in October 2013, my lab has presented two main lines of evidence to support a natural antiviral function of RNAi in mammals (Li et al., *Science* 342:231). First, we detected abundant production of canonical viral siRNAs during mouse infection by Nodamura virus (NoV), a mosquito-transmissible positive-strand RNA virus. Second, we found that without viral suppression of RNAi by the B2 protein of NoV, suckling mice are able to launch an antiviral RNAi response sufficiently potent to terminate lethal viral infection. Using the suckling mouse model for NoV infection, we have recently characterized the function and mechanism of antiviral RNAi in mammals and the in vivo mechanism of RNAi suppression by the viral B2 protein. Results from these recent studies and other related studies will be presented.

S012 Exploring genetic control of swine responses to viral diseases. Joan Lunney (ARS USDA), Bob Rowland and Benjamin Trible (Kansas State University), Igseo Choi and Samuel Abrams (ARS, USDA), Carlos Souza (Embrapa Pesca e Aquicultura), James Reecy, Eric Fritz-Waters, James Koltes, Chris Eisley, Christopher Tuggle, Andrew Hess, Jenelle Dunkelberger and Jack Dekkers (Iowa State University), Nicholas Boddicker (Genesus, Inc.), Juan Steibel and Catherine Ernst (Michigan State University), Le Luo Guan, Hua Bao, Arun Kommadath, Paul Stothard and Graham Plastow (University of Alberta) and Andrea Ladinig and John CS Harding (University of Saskatchewan)

Our goal is to understand genomic control of viral disease responses focusing on the economically most important disease of pigs, porcine reproductive and respiratory syndrome (PRRS) (annual losses of $664M). The PRRS Host Genetics Consortium (PHGC) was established to combine efforts of scientists from university, government and commercial pig genetics and animal health companies to assess the role of genetics in determining pig resistance/ susceptibility to PRRS virus (PRRSV) infection, pathology and growth effects. We utilized a nursery pig PRRSV infection model with deep sampling for phenotypic analyses, extensive genotyping (60K SNPchip) and a shared database http://www.animalgenome.org/lunney/. We have completed 15 trials using ~200 PRRSV-infected pigs each and identified a genomic region on SSC4 which has a significant impact on variation in viral load and growth response following challenge with each of 2 different PRRSV isolates. More recent trials involve complex challenges (PRRSV and porcine circovirus) combined with PRRS vaccines, as well as field trials; each comparing pigs with different SSC4 haplotypes. To address disease resistance mechanisms we probed serum protein expression (antibody and cytokine) and the blood transcriptome (using microarrays and RNAseq) of PHGC pigs. We have verified proteins and genes that are differentially expressed in PRRS resistant versus susceptible pigs and are probing this data for alternate control and regulatory networks. This data will help us identify new resistance pathways that may be used for new vaccines and biotherapeutics. An alternate gilt PRRSV infection model has been established to determine the effects of third trimester infection on fetal development and viability. Using deep phenotypic analyses and genotyping of gilts and fetuses, new predictors of PRRS severity in gilts and fetuses are being identified. Support: US National Pork Board, USDA ARS and NIFA, Genome Canada, Genome Alberta, Genome Prairie, pig breeding companies.

S013 Editing miRNAs target sequence with SNPs for animal breeding. Zhiying Zhang (Northwest A&F University)

miRNAs are small non-coding RNAs and emerge as key regulators for many developmental processes. The mechanism that miRNAs regulate target gene expression is either affecting target gene mRNA stability or inhibiting mRNA translation upon binding mRNA 3'UTR target site. Previous studies demonstrated that SNPs identified within either miRNAs or their target sequences jeopardized

miRNA regulatory function. Based on the miRNA function and SNP effect, we propose a hypothesis that editing miRNA target sequence with CRISPR/Cas9 technology will eliminate miRNA regulatory effect on target gene expression, which high level of expression enhances animal performance. This will provide a novel approach for animal breeding. We will first establish a system to examine the interaction between miRNAs and their target sequences. Then, we will study the effects of the SNPs on IGF-2 gene expression, and generate the IGF-2 gene edited mice with SNPs to prove the concept. Finally, we will employ this technology to generate miRNA target gene edited pigs. This study will provide an insight into improving animal performance by editing miRNA target sequences with SNPs.

S014 Animal domestication in the context of evolutionary biology, population genetics and gene flow. Greger Larson (Durham University)

The domestication of plants and animals over the past 11,500 year has had a significant effect not just on the domesticated taxa,but also on human evolution and on the biosphere as a whole.Decades of research into the geographical and chronological origins of domestic animals have led to a general understanding of the pattern and process of domestication,though a number of regarding the different pathways animals followed to become domesticated,I will present a large-scale synthesis that addresses the global pattern of animal domestication alongside a dicussion of the differential evolutionary processes that have shaped domestic animal population.More specifically,I will present a framework for understanding how unconscious selection characterized the earliest steps of animal domestication.In addition,Iwill discuss the underappreciated role of introgression and the importance of relaxed and positive selection in shaping modern domestic phenotypes and genomes.

S015 Integrated prediction of genetic value and mapping of causal mutations. Michael Goddard (University of Melbourne)

ayesian methods that fit all SNPs simultaneously can be used both for prediction of breeding values in genomic selection and for mapping and identification of mutations causing variation in quantitative traits (QTL). The power of these methods can be increased by using genome sequence data instead of SNP chip genotypes and by incorporating prior biological information about genes and genomic sites into the analysis. In this paper we illustrate these methods and use them to find QTL for milk production traits.

S016 The inheritance and programming of DNA methylome in vertebrates. Jiang Liu (Beijing Institute of Genomics, Chinese Academy of Sciences)

Epigenetics plays crucial roles during animal development, cell differentiation and human diseases. But limited is known whether epigenetic information can transferred across generations. Our laboratory is using genomic technology to study the inheritance and programming of DNA methylome in different vertebrates. In zebrafish, our results show that the paternal DNA methylation pattern is maintained throughout early embryogenesis. The maternal DNA methylation pattern is maintained until the 16-cell stage. Then, the oocyte methylome is gradually discarded through cell division, and progressively reprogrammed to a pattern similar to that of the sperm methylome. By the midblastula stage, the embryo's methylome is virtually identical to the sperm methylome. Therefore, besides DNA sequences, sperm DNA methylome is also inherited in zebrafish early embryos. Our data also show that the inheritance of the sperm methylome facilitates the epigenetic regulation of embryogenesis. Additionally, our group is also trying to understand how parental DNA methylomes are reprogramming in mammals.

S017 NGS-based screening for de novo and embryonic lethal mutations in livestock. Michel Georges (Unit of Animal Genomics, GIGA-R and Faculty of Veterinary Medicine, University of Liège,

Belgium)

We are in the process of resequencing the genome of > 1,000 cattle of the Belgian, Blue, Holstein-Friesian and Jersey breeds.
The resulting sequence data are being used for a number of purposes, including the identification and characterization of (i) de novo mutations in the bovine germline, and (ii) segregating embryonic lethal mutations compromising fertility.
In an initial study, we have searched for de novo mutations in four-generation pedigrees including four grand-parents, two parents, a proband and five of his offspring. All animals were sequenced at minimum 20-fold depth. The pedigree structure allows for unprecedented evaluation of the rate of de novo mutations in the male and female germ-lines, the importance of recurrent mutations, and the level of mosa ̈ism accompanying de novo mutations.
All available sequence data were used to mine for segregating variants predicted to disrupt the function of essential genes that might be enriched in embryonic lethals. Hundreds such candidates were genotyped in 3,000 to >10,000 animals to identify variants characterized by a significant underrepresentation of homozygous animals. The segregation of the corresponding variants is being tested in matings between carrier sires and dams to demonstrate their embryonic lethality.

Bioinformatics, Statistical Genetics, and Genomic Technologies

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P1001 Gene actions in crosses of Nigerian indigenous and exotic pigs on growth traits. Victor Mela Obinna Okoro (Federal University of Technology, Owerri, Nigeria.)

Two breeds of pig - Large white (LW) and Landrace (LR) breeds and the Nigerian indigenous pigs were crossed in full diallel arrangement to evaluate the effects of cross, sex and parity on growth traits, and also establish the nature of gene action due to the growth traits at birth, weaning and 20 weeks of age. The full 3x3 diallel cross resulted in a total of 132, 107 and 105 pigs at birth, weaning and 20 weeks of age respectively. General Combining Ability (GCA), Specific Combining Ability (SCA) and Reciprocal Effects (RE) were estimated for eight traits which includes Body weight (BWT), Ear length (EL), Tail length (TL), Heart girth (HG), Snout circumference (SC), Snout length (SL), Height at wither (HW) and Body length (BL). There were significant differences ($P < 0.05$) among the various crosses, sex and parity but no significant interaction. The LRxLW cross consistently expressed higher body weight and morphometric traits than other crosses at birth, weaning and 20 weeks of age, while the INxIN expressed least body weight at birth and 20 weeks of age, while LWxIN was the least at weaning. There was no significant GCA effect ($P>0.05$) on all the traits measured, but SCA was significant ($P<0.01$) for all morphometric traits and body weight. RE was significant for body weight at birth and weaning, while at 20 weeks, was significant for SC and HW. The non-significant GCA estimates and significant SCA estimates suggest that the genes governing the eight traits measured act non-additively, hence improvement may be attained by exploiting heterosis through planned crossbreeding. However, the significant reciprocal effect in body weight and some morphometric traits indicates maternal and sex-linked effect at the affected ages, implying that significant reciprocal cross ensures better performance for the growth traits in the progeny.

P1002 Identification of molecular circuits involved in epigenetic re-modelling in the ovine pars tuberalis using next generation sequencing data. Le Yu and Dave Burt (Roslin Institute, University of Edinburgh)

Seasonally breeding mammals use photoperiod as a critical cue to drive hormone rhythms and synchronize reproduction to the optimal time of year. Photoperiod is encoded by the nocturnal secretion of melatonin (MEL). MEL acts on the pars tuberalis (PT) of the pituitary, regulating PT-specific expression of thyrotropin, controlling hypothalamic thyroid hormone metabolism in adjacent ependymal cells, which drives reproductive changes.
We employed Next Generation Sequencing(NGS) data to define the full repertoire of transcriptional changes within the PT following exposure to long photoperiod(LP). Sheep were housed for 12 weeks on a short photoperiod(SP) and cohorts were exposed to LP for 1, 7 or 28 days. RNASeq reads using the Illumina platform were mapped to the sheep genome. The number of reads mapping to each gene was calculated and normalised to the total number of reads generated. Using edgeR a statistical analysis was performed to identify genes differentially expressed at LP compared to SP. Further to this, an analysis using Biolayout was performed, this takes into account small changes in expression and identifies genes exhibiting similar patterns of expression, for subsequent cluster analysis. The goal is to examine the data as a whole and identify groups of genes that are co-expressed, and may have similar functions.
Our results suggest there may be dramatic tissue/vasculature remodelling of the PT in response to photoperiod. Furthermore, we hypothesise that the observed epigenetic changes may play a role in these seasonal tissue re-modelling responses. To sum up, using bioinformatics analysis based on NGS data we

have identified dynamic changes of circuits involved in epigenetic changes, and possible tissue/vasculature remodelling of the PT in response to photoperiod.

P1003 Genetic parameters and genetic trends for growth and fur quality trait in sliver blue mink in China. Zongyue Liu (Institute of speical economic animal and plant.CAAS)

Samples were obtained from Da Lian Ming Wei Marten Industry Company, based on 1686 sliver blue mink of six generation between 2005 and 2011. The phenotypic trends of body weight, body length, guard hair length, under fur length, and the rate of two hairs were analyzed by year and sex. Genetic parameter of growth and fur traits in the sliver blue mink was estimated with multi-traits animal model. The fixed effects, which were included the year, sex effects and the animal additive genetic effects were calculated. The results showed as following: Heritability estimates for body weight body length, guard hair length, under fur length and the rate two hair lengths was 0.41, 0.53, 0.53, 0.52, and 0.52. Except for the phenotypic correlation between the rate of two hair lengths and body weight, body length, guard hair length and under fur length were negatively (− 0.218, − 0.178, − 0.074, − 0.425), among the other four traits were positively (0.298, 0.882, 0.869, 0.806, 0.788, 0.93). The genetic correlation between guard hair length and under fur length and the rate of two hair length was negatively (− 0.941,− 0.983), others were positively (0.983, 0.731, 0.972, 0.981, 0.622, 0.992, 0.641, 0.987). The genetic and phenotypic tendency of all traits was estimated and analyses by regressing mean of annual breeding value. The genetic trend of body weight and body length was close to zero, and positive. The genetic changes for GL, UL, and RATE were negative and parallel. Our results herein form a practical basis for designing optimal breeding schemes in Chinese sliver blue mink.

P1004 Genome-wide association study identified the *BCL11B* gene as a positional candidate gene affecting GPT levels in pigs. Jae-Bong Lee, Beom-Mo Kim and Chae-Kyoung Yoo (Gyeongsang National University), Hee-Bok Park (Chungnam National University), In-Cheol Cho (Rural Development Administration) and Hyun-Tae Lim (Gyeongsang National University)

Serum glutamic pyruvic transaminase (GPT) levels can be used as an indicator of muscle and liver cell damage affecting health status. In this study, a genome-wide association study (GWAS) was performed to detect significant single nucleotide polymorphisms (SNPs) affecting GPT in a large F2 intercross between Landrace and Korean native pigs (N=1,105) using the porcine 60K chip, and a mixed-effects model approach accounting for familial relationships between individuals in this intercross. The significant SNPs were identified on SSC7. Two candidate genes, *BCL11B* and *AHNAK2*, were subsequently selected and further investigated. Within these candidate genes, three SNPs were identified and genotyped using the pyrosequencing. The results revealed that the SNP in the *BCL11B* (nominal P=7.23×10-8) and two SNPs in the *AHNAK2* gene (nominal P=3.51×10-6, 5.64×10-6) were associated with GPT, respectively. The SNP marker in *BCL11B* can be a positional promising candidate gene for further functional investigations.

P1005 Association test for the single nucleotide polymorphisms in *RUNX1*, *DYRK1A*, and *KCNJ15* with blood related traits in pigs. Jun-Ho Shin, Chae-Kyoung Yoo and Jae-Bong Lee (Gyeongsang National University), Hee-Bok Park (Chungnam National University), Su-Yeon Kim (Gyeongsang National University), In-Cheol Cho (Rural Development Administration) and Hyun-Tae Lim (Gyeongsang National University)

The aim of this study was to detect positional candidate genes located within the support interval regions based on the results of RBC (red blood cell count), MCV (mean corpuscular volume), and MCH (mean corpuscular hemoglobin) QTL in SSC13. The flanking markers of the three QTL SI regions are *SW38* and *S0215*; 44 genes were located, and *RUNX1*, *DYRK1A*, and *KCNJ15* were selected as positional candidate genes. The ten SNPs located in the exonic region of the three genes were detected by next generation sequencing (NGS). A total of 1,232 pigs of a resource population between Landrace and Korean native pigs were genotyped. To investigate the effects of the three genes on each phenotype, a mixed-effect model which is the considering family structure model was used to evaluate the associations between the SNPs and three traits in a large F2 intercross population. Among them, the MCV level was highly significant (nominal P = 9.8×10-9) in association with the *DYRK1A-SNP1* (c.2989 GP < 0.05 for MCV and MCH were also obtained by marker-assisted association test and *F*-drop test of SNP in *KCNJ15*. However, we cannot conclude that this polymorphism is causative for the effects observed, as either *F*-drop test or marker-assisted association has limited power to distinguish one positional candidate gene from QTL at a distance of several centi-morgans.

P1006 Genome-wide association study of thoracic vertebrae in a large F2 intercross between Landrace and Korean native pigs. Hyun-Tae Lim, Jae-Bong Lee and Chae-Kyoung Yoo (Gyeongsang National University), Hee-Bok Park (Chungnam National University) and In-Cheol Cho (Rural Development Administration)

The number of lumbar and thoracic (THO) vertebrae is known to have a substantial phenotypic variations in pigs. The aim of this study was to identify quantitative trait loci (QTL) affecting the number of THO in pigs. A genome-wide association study (GWAS) was conducted in an F2 intercross population between Landrace and Korean native pigs (N=1,105) using the porcine SNP 60K chip and a mixed-effects model approach accounting for familial relationships between individuals in this intercross. After implementation the quality control criteria, 39,474 SNP markers were left for GWAS. On SSC7, we detected association between SNP markers and the number of THO at genome-wide significance level (nominal *P*-value=4.09×10-28). The identified SNPs can potentially be applicable to improve the numbers of THO in this intercrossed population after being verified using the independent study.

P1007 Effect of diet on ovarian transcriptome profiles in domestic sheep (*Ovis aries*) breeds. Kisun Pokharel (Biotechnology and Food Research, MTT Agrifood Research Finland, Jokioinen, Finland; Department of Biology, University of Eastern Finland, Kuopio, Finland), Jaana Peippo (Biotechnology and Food Research, MTT Agrifood Research Finland, Jokioinen, Finland), Johanna Rautiainen (Pro Agria Rural Advisory Centre, Tampere, Finland), Mervi Honkatukia (Biotechnology and Food Research, MTT Agrifood Research Finland, Jokioinen, Finland), Magnus Andersson (Department of Production Animal Medicine, University of Helsinki, Saarentaus, Finland), Göran Andersson (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden), Meng-Hua Li (Institute of Zoology, Chinese Academy of Sciences (CAS), Chaoyang District, Beijing, China) and Juha Kantanen (Biotechnology and Food Research, MTT Agrifood Research Finland, Jokioinen, Finland; Department of Biology, University of Eastern Finland, Kuopio, Finland)

Ovulation rate and litter size in sheep (*Ovis aries*) are complex traits affected by endocrinological, genetic and environmental conditions. We

analyzed factors affecting fecundity of the prolific Finnsheep breed and searched for related structural (SNP genotyping) and functional (mRNAs and miRNAs) variations in its genome. A total of 31 ewes representing three breed groups (Finnsheep, Texel and their F1-crossbreds) and two diets (control and flushing) were included. Experiments were focused on two different time points during establishment of pregnancy: follicular growth phase (the first phase) and early pregnancy prior to implantation (the second phase). Blood parameters were used to monitor ovary function and energy status of the ewes. In the first phase, one ovary of each ewe was surgically removed determined by individual progesterone profiles. After estimation of follicular counts, a sample of each ovary was used for RNA extraction followed by mRNA and miRNA sequencing using HiSeq200 Illumina technology. This revealed a total of 13 537 ovine genes expressed in all 31 samples. The gene expression profiles were then compared within and between breed-diet pairs. In the second phase, the sheep were inseminated and slaughtered followed by collecting another set of tissue samples (pituitary gland, CL, oviductal and uterine epithelial cells, preimplantation embryos) for RNA extractions and sequencing. In the final phase, SNP genotyping data will be correlated to transcriptome and phenotypic data. Preliminary results from the first phase show no significant effects of the diet (control vs flushing) on follicular counts within pure breeds ($p>0.05$). However, 503 genes showed significantly different expression levels between Finnsheep and Texel having flushing diets compared to one gene significantly differentially expressed between Finnsheep and Texel having control diets. This study provides new information on effects of flushing diet on fertility in the high-prolific Finnsheep breed, a valuable genetic resource for global sheep farming.

P1008 Genome-wide analysis of imprinted genes based on transcriptome sequencing in pigs. Jing Wang (China Agricultural University), L. Chen (ChongQing Academy of Animal Science), Chao Wang (Tsinghua University) and Xiaoxiang Hu, Ning Li, Yaofeng Zhao and Yiqiang Zhao (China Agricultural University)

Genetic imprinting is a specific epigenetic phenomenon in which a subset of genes is expressed depending on their parent of origin. More and more studies have revealed that the imprinted genes are important for prenatal growth control, normal brain function and postnatal energy homeostasis. However, the current studies of imprinted genes are limited in mammalian species other than human and mouse. In an effort to identify novel imprinted genes in pig (*Sus scrofa*), we analyzed the allelic gene expression for individuals from reciprocal crosses between Duroc and Rongchang pigs. Gene expression was measured by high throughput sequencing on poly-A selected RNA (RNA-Seq) and we studied 3 tissues including hypophysis, hypothalamus and longissimus dorsi. In total, we identified more than 60 genes with potential parent-of-origin specific expression. By analyzing the Ka/Ks ratio of the maternally expressed genes (MEGs) and paternally expressed genes (PEGs), we show that certain candidate MEGs and PEGs are under possible positive selection. Finally, by functional analysis for candidate or confirmed imprinted genes, such as *DIO3*, we also assessed the effects of genomic imprinting on growth/reproduction traits.

P1009 Metagenomic animal species determination using Next Generation Sequencing approaches in food samples. Rainer Schubbert, Katrin Juling and Christine Käppel (Eurofins Medigenomix)

The use of DNA-based methods for the analysis of plant, animal and also microbial species in food, as well as in environmental samples has become more and more popular during the last years. Especially since the horse meat scandal in

2013, species identification by modern DNA-based methods is of growing interest for authenticity testing and to detect contamination from animal or plant sources. Sanger sequencing of several barcoding regions is the state of the art method for animal species identification in samples with unknown ingredients. Nevertheless, DNA fragmentation in processed samples and the overlay of several sequences in samples with more than one species limits the applicability of the Sanger sequencing method. Realtime PCR targeting species - specific regions allows determining only the ingredients that have been searched for. Hence, the identification and determination of species mixtures in complex food samples is challenging due to the widespread diversity of the species that are used in food production and therefore might be present as ingredient. We have compared different deep amplicon sequencing methods based on independent technical platforms like Roche 454 or Illumina MiSeq for species identification in mixed samples. Constantly declining costs in the NGS field makes the methods attractive also for routine analysis of food or environmental samples in the near future. For validation of the methods we have analyzed artificial mixtures with known contents of DNA from different mammals, birds, fishes and molluscs and real samples like fish meal and complex fish products. In order to evaluate the influence of diverse barcoding targets, imbalanced amplification due to variable PCR efficiencies and different DNA contribution from the various species we validated our results additionally with a realtime PCR approach. We focused also on the determination of detection limits of diminutive admixtures with the different analytical methods.

P1010 Detection of inbreeding, admixture and selection signature in four dairy cattle breeds. Qianqian Zhang (1Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, 2Animal Breeding and Genomics Centre, Wageningen UR Livestock Research) and Bernt Guldbrandtsen, Mogens Sandø Lund and Goutam Sahana (1Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University)

The level of inbreeding has been increasing for generations in cattle population due to intense use of a limited number of bulls for breeding. The increasing level of inbreeding causes inbreeding depression. Inbreeding results in runs of homozygosity (ROH) within individual genomes with near-zero nucleotide diversity. Also, haplotypes from breeds with better characteristics have been introduced into different breeds. Admixture between breeds results in the selected shared haplotypes which are identical by descent (IBD). For the first time, we utilized whole genome sequence of 104 bulls from four cattle breeds to detect ROH and IBD shared segments. On average 19.5% of the genome was found to be in ROH. We studied IBD haplotypes reflecting ancestral original haplotypes or due to admixture between breeds. Modern Danish Red Cattle is a composite breed with contributions from Old Danish Red, Brown Swiss and other red breeds as well as Holstein. The average nucleotide diversity in Modern Danish Red Cattle is significantly higher than in Holstein, Jersey and Old Danish Red Cattle ($p<0.01$). Certain haplotypes from other breeds have risen to prominence in Modern Danish Red Cattle. A number of these haplotypes from other breeds must have been favored by selection in Modern Danish Red Cattle. Presumably they hold important favorable genetic variants. Subsequently, the genomic regions under selection are fixed in genome. We used NGS data to detect selection signature by computing Fst in cattle genome. We demonstrate that introgressed Holstein and Old Danish Red haplotypes are preserved in Modern Danish Red Cattle. Selection signatures are prominent in

these regions. Some of the identified regions in our study validate the regions found in previous selection signature studies and overlap with certain milk QTLs in dairy breeds. The results can be used to maintain genetic diversity and further discover selected and shared genomic regions across breeds.

P1011 Genome-wide Association Studies with Somatic Cell Score in Chinese Holstein Cows. Xiao Wang, Peipei Ma, Qin Zhang, Jianfeng Liu, Dongxiao Sun, Xiangdong Ding, Yachun Wang, Yi Zhang, Shengli Zhang and Ying Yu (College of Animal Science and Technology, China Agricultural University)

Bovine mastitis is a costly disease in modern dairy farms worldwide. In general, Genome-wide association study (GWAS) for somatic cell scores (SCSs) or mastitis has been conducted with different association methods in different groups, while the identified single nucleotide polymorphisms (SNPs) are various. This study is attempted to identify SNPs of significant effects on mastitis resistance and susceptibility in Chinese Holstein cows. To avoid uncertain effects' influence, estimated breeding values (EBVs) of SCS based on a multiple trait random regression test-day model were provided as the phenotypes. A total of 2093 SCS EBVs of cows were performed with mixed model based on single locus regression model analysis (MMRA) while 1267 *vs* 667 were analyzed by ROADTRIPS software based on case-control association testing. After quality control, 43885 SNPs was totally available for MMRA in contrast to 43881 and 43887 SNPs for half of/one standard deviation (SD) of SCS EBVs for ROADTRIPS. In total, 54 significant SNPs on chromosome level were detected including 44 SNPs by MMRA, 5 SNPs by ROASTRIPS and the rest 5 SNPs by both methods, which are mainly located on the BTA 14 and X. *TRAPPC9* gene detected by both methods reveals the new candidate gene associated to mastitis resistance. In addition, GO analysis confirms one pathway participating in regulation of inflammatory response. To our knowledge, it is the first study aiming at unraveling the genetic mechanism of the mastitis resistance and susceptibility using a case-control association testing combined with MMRA based on a high density SNPs. Such findings herein provide novel methods for discovering candidate genes in dairy cattle. It was financially funded by the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-37), the National Natural Science Foundation of China (31272420), the Fund for Basic Research from the Ministry of Education of the People's Republic of China (2011JS006) and the National Key Technologies R & D Program (2011BAD28B02).

P1012 Widespread differential maternal and paternal genome effects on fetal bone phenotype at mid-gestation. Ruidong Xiang (Robinson Institute, Research Centre for Reproductive Health, The University of Adelaide, South Australia, Australia. JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, South Australia, Australia.), Alice Lee (Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia.), Tanja Eindorf (tanja.eindorf@student.adelaide.edu.au), Ali Javadmanesh and Mani Ghanipoor-Samami (Robinson Institute, Research Centre for Reproductive Health, The University of Adelaide, South Australia, Australia. JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, South Australia, Australia.), Madeleine Gugger (JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, South Australia, Australia.), Carolyn Fitzsimmons and Zbigniew Kruk (Robinson Institute, Research

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Parent-of-origin dependent (epi)genetic factors are important determinants of prenatal development that program adult phenotype. However, data on magnitude and specificity of maternal and paternal genome effects on fetal bone are lacking. We used an outbred bovine model to dissect and quantify effects of parental genomes, fetal sex and non-genetic maternal effects on the fetal skeleton and analyzed phenotypic and molecular relationships between fetal muscle and bone. Analysis of 51 bone morphometric and weight parameters from 72 fetuses recovered at Day153 gestation (54% term) identified six principal components (PC1-6) that explained 80% of the variation in skeletal parameters. Parental genomes accounted for most of the variation in bone wet weight (PC1, 72.1%), limb ossification (PC2, 99.8%), flat bone size (PC4, 99.7%) and axial skeletal growth (PC5, 96.9%). Limb length showed lesser effects of parental genomes (PC3, 40.8%) and a significant non-genetic maternal effect (gestational weight gain, 29%). Fetal sex affected bone wet weight (PC1, $P<0.0001$) and limb length (PC3, $P<0.05$). Partitioning of variation explained by parental genomes revealed strong maternal genome effects on bone wet weight (74.1%, $P<0.0001$) and axial skeletal growth (93.5%,$P<0.001$), while paternal genome controlled limb ossification (95.1%, $P<0.0001$). Histomorphometric data revealed strong maternal genome effects on growth plate height (98.6%, $P<0.0001$) and trabecular thickness (85.5%, $P<0.0001$) in distal femur. Parental genome effects on fetal bone were mirrored by maternal genome effects on fetal serum 25-hydroxyvitamin D (96.9%, $P<0.001$) and paternal genome effects on alkaline phosphatase (90.0%, $P<0.001$) and their correlations with maternally controlled bone wet weight and paternally controlled limb ossification, respectively. Bone wet weight and flat bone size correlated positively with muscle weight (r=0.84 and 0.77, $P<0.0001$) and negatively with muscle *H19* expression (r= −0.34 and −0.31, $P<0.01$). Since imprinted maternally expressed *H19*

regulates growth factors by miRNA interference, this suggests muscle-bone interaction via epigenetic factors.

P1013 Generation of mammary gland bioreactor of lactoperoxidase in transgenic mice. Shengzhe Shang, Dan Lu, Yunping Dai, Min Zheng and Ning Li (China Agriculture University)

As a member of peroxidase superfamily, lactoperoxidase plays an important role in innate immune system, mainly exists in mammary, salivary, lachrymal, respiratory tract, cervical mucus and other mucosal glands. Lactoperoxidase together with thiocyanate ions (SCN-) and hydrogen peroxide is known as the lactoperoxidase system (LPS) which can kill or inhibit growth of some bacteria and protect lactating mammary gland and the intestinal tract of the newborn infants against pathogenic bacterium. Lactoperoxidase system is applied in preservation of milk or other food, health care products and clinical medicine. The expression of native lactoperoxidase is under 30mg/L in cow and even lower in human. In our study, we try to construct some effective expression vectors to increase the amount of lactoperoxidase in mouse milk as mammary gland bioreactor model. The structure of tissue specific expression vectors consist of milk protein gene regulation elements and lactoperoxidase whole genome sequence. We compared the capacity of different milk gene promoters which include the original bovine lactoperoxidase gene promoter and alpha-s1 casein gene promoter to drive the expression of bovine lactoperoxidase gene. The existence of exogenous gene was estimated by PCR. Expression level of rLPO in mouse mammary glands was tested by RT-PCR and Western blot. Activity of rLPO in transgenic mouse milk was verified by comparing with purified LPO and wild type non-transgenic mouse milk. The results showed that transgenic mice which can express bovine lactoperoxidase in a tissue and time specific way. The accurate number of protein level and biological function need to be tested in future experiments. The ultimate goal of our research is to generate transgenic cow to achieve industrialization of lactoperoxidase.

P1014 High-level human α-lactalbumin expressed in milk of transgenic pigs can improve the lactational performance of sucking pigs. jin ma and Ning Li (china agriculture university)

α-Lactalbumin（α-LA）is a whey protein of milk in all mammalian subdivisios. α-LA and β1, 4-galactosyltransferase compose lactose synthase in the Golgi complex of mammary epithelial cells. Affecting osmotic force in milk volume establishment, the content of lactose is important for milk production. The concentration of α-lactalbumin is 2 to 3 g/L in human. As a most abundant protein in human milk, α-lactalbumin comprises 25 to 35% of the total protein content. 63% of α-LA amino acids composition are essential amino acids for human nutrition. After partial digestion in gastrointestinal-tract, the peptides from α-lactalbumin are assumed to have various biological activities, such as antimicrobial activity, inhibition of angiotensin-converting enzyme activity, and opioid activity. Using RP11-346L11 bacterial artificial chromosoe clone which has the full-lenth human α-lactalbumin, we generated transgenic cloned pigs (F0) by somatic cell nuclear transfer and the first-generation hybrids (F1). From d0 through d 21 of lactation, the average concentration of human α-LA was 1 to 2g/L in the milk of two tested F0 sows. Lactose concentrations in colostrum and mature milk from transgenic sows are greater than control sows. Protein and fat concentrations in colostrum and mature milk from transgenic sows are smaller than control sows. The reduction in protein and fat content may due to a dilution effect that high-level human α-LA leads to more lactose and more water into milk. Piglets reared

by transgenic sows grew faster than piglets reared by control sows. The next steps are analyzing more details on the growth performance between the piglets reared by transgenic sows and control sows, and finding the how human α-lactalbumin affects these details.

P1015 Dissection of genetic architecture of the major QTL for saturated fatty acids composition (C20:0) in longissimus dorsi on porcine chromosome 16. Zhiyan Zhang, Feng Zhang, Yuna He, Wanchang Zhang, Bin Yang, Junjie Zhang, Junwu Ma, Jun Ren and Lusheng Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University)

Saturated fatty acids increase blood cholesterol and triglyceride concentrations, resulting in arterial atherosclerotic and occurrence of cardiovascular disease.To map and fine map quantitative trait loci (QTL) for fatty acid composition in longissimus dorsi and uncover their genetic mechanism, we performed a whole-genome scan on 667 F2 animals in a White Duroc X Erhualian intercross population using 194 microsatellite and detected one major QTL controlling C20:0 fat acid on chromosome 16 at position of 45 cM. That QTL explained 15.75% of the phenotypic variance with the confidence interval (CI) of 10.5 cM by linkage analysis. Recently, We've refined this QTL to 41.3 ~ 43.4 Mb on SSC16 using the IBD strategy and linkage & linkage disequilibrium (LALD) method, on basis of genotyping F2 resourse population and Sutai population of 357 individuals with hign density marker of 60K procine beadchips. Seven genes were annotated in the detected CI regions and one of them - elongation of very long chain fatty acids protein 7 (*ELOVL7*) gene stood out as a candidate based on its physiological and biochemical functions. Expression level of *ELOVL7* in longissimus dorsi muscle was tested in 112 F2 individuals and eGWAS analysis was subsequently performed. The top SNP of eGWAS result on SSC16 was exactly the same as phenotype association top SNP.This implied that *ELOVL7* was involved in synthesis of C20:0 fat acid. To further uncover potencial caustive mutation, We re-sequenced the whole 80Kb gene in six F0 with known QTL genotype and identified 11 clustered co-segregated candidates out of 433 discovered variants. We eliminated 9 candidate QTNs by genotyping known QTL genotype individuals in Sutai populations. The effects of remain two candidate QTNs were further comfirmed in Laiwu population of 342 individuals, Erhualian population of 343 individuals and Duroc X Large white X Landrance commercial population of 678 individuals.This study will help to reveal the genetic mechanism for pig fatty acids metabolism and benefit marker-assisted selection (MAS).

P1016 A genome wide association study for beef marbling and fatty acid composition in Japanese Black cattle using pooled DNA. Ayaka Nakajima (Kobe University), Yoshinobu Uemoto (National Livestock Breeding Center), Moriyuki Fukushima, Emi Yoshida, Eiji Iwamoto, Takayuki Akiyama and Namiko Kohama (Hyogo Prefectural Technology Center for Agriculture), Eiji Kobayashi (NARO Institute of Livestock and Grassland Science) and Kenji Oyama, Shinji Sasazaki and Hideyuki Mannen (Kobe University)

Beef marbling and fatty acid composition of bovine adipose tissue are important traits for improving meat quality. However, A few responsible genes with major effect for beef marbling have been found until now. Recent studies have reported that a percentage of fat in rib-eye area, which is measured by image analysis, has strong phenotypic correlation with Beef Marbling Standard and it would be an attractive alternate for evaluation of beef quality.

In addition, fat tissue containing abundant monounsaturated fatty acid, especially oleic acid, reflects lower melting points of fat, leading to favorable beef flavor and decreasing blood concentration of LDL cholesterol. The objective of this study was to identify genomic regions associated with percentages of fat content and oleic acid in rib-eye area using closed Hyogo population of Japanese Black cattle. Each of 100 animals with higher and lower values was selected from 1836 animals based on corrected phenotype, and then pooled as the high and low groups, respectively. We performed a DNA pool-based genome-wide association study using Illumina BovineSNP50 BeadChip v2 with three replicate assays for each pooled sample. The values of inflation factor were 1.12 on the fat percentage and 1.09 on the oleic acid percentage, suggesting that our analysis method successfully accounted for population stratification. Association analysis revealed that three and four SNPs were found to be associated with fat percentage and oleic acid, respectively, at 5% genome-wide significance level. In the fat percentage, significant SNPs were located on BTA7 (2 SNPs) and BTA12 (1 SNP). In the oleic acid percentage, significant SNPs were located on BTA9 (3 SNPs) and BTA14 (1 SNP). There were no previously reported genes related to fat metabolisms around these regions. Therefore, our results suggested novel candidate regions for beef marbling and fatty acid composition in Japanese Black cattle.

P1017 Genomic Allele-Specific Expression Analysis in Chickens Based on RNA-seq Data. Qiong Wang, Kaiyang Li, Dezhi Fu and Lujiang Qu (Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University)

Allele-specific expression(ASE) is a phenomenon that parental copies of a gene are expressed at unequal levels. It can be monoallelic gene expression, or express in a preferential allelic expression manner. To detect ASE in chickens, we analysed the RNA-seq data of 12 chickens which come from two reciprocal cross groups by using White Leghorn and Cornish highly inbred lines. We found there are a fraction of genes behaving as allele-specific expression. In our research, brains of 6 progenies (3 males and 3 females) at the age of 1 day from each group were collected to establish RNA-seq libraries. At the same time, DNA samples of 4 parents from the two groups were used for whole genome re-sequencing. With the data of re-sequencing (a total of 82.5Gb), 726884 SNPs between the two strains of parents have been checked out at first. The transcriptome sequencing reads (a total of 47.4Gb) were aligned to the parental alleles. Totally, 310 SNPs in females and 365 SNPs in males have been selected as ASE SNPs, which revealed 133 ASE genes on autosomes in females, and 159 ASE genes in males. There are 90 ASE genes identical in males and females. To analyze the molecular and biological functions of ASE genes, we performed Gene Ontology (GO)-enrichment analysis. The results showed that ASE genes in both sexes are mostly connected with protein transport and lipid metabolism, while the genes show allele-specific expression only in males are mainly involved in carbohydrate metabolism and lipid binding. The ASE genes which shared by males and females are greatly related to cell death and protein binding. The results revealed that genes expressing in an asymmetrical way play important roles in substance metabolism process and homeostasis.

P1018 Genome sequencing of Yesso scallop *Patinopecten yessoensis*: generating a genomic resource for understanding the biology and evolution of Pectinidae (Mollusca: Bivalvia). Zhenmin Bao and Shi Wang (Ocean University of China)

The Pectinidae family, also known as scallops,

consists of more than 300 extant species worldwide. Scallops represent one of the oldest and evolutionarily most successful groups of invertebrates and many of them are also important fishery and aquaculture species. However, this group of animals remains unexplored in terms of genome sequencing. Here we report the whole genome sequencing and assembly for Yesso scallop (*Patinopecten yessoensis*, Jay 1857), one of the most important maricultural shellfish in the north of China. Totally, 467 Gb sequencing data (equivalent to ~338x genome coverage) were produced based on the Illumina's HiSeq2000 platform. Due to the high genome heterozygosity (~1.3%), an efficient assembly approach was adopted based on the idea of assembling the two haploid genomes separately. The length of final assembly is 999 Mb, consisting of 463,681 contigs (N50 = 20 Kb) and 362,434 scaffolds (N50 = 748 Kb) and covering genomic and genic regions with 98% and 97%, respectively. A high-resolution genetic linkage map containing 937 SNPs was simultaneously constructed to assist chromosome assembly, and all genetic markers matched to the genomic scaffolds. The Yesso scallop genome presumably contained 23,359 genes with an average CDS (coding DNA sequence) length of 1,737 bp. Repetitive sequences are dominant in the genome with transposable elements and tandem repeats accounting for 44% of the whole genome. Comparative genome analysis based on the resequencing data from four other scallop species revealed that Pectinidae genomes are highly divergent. Weathervane scallop (*Patinopecten caurinus*) is the closest relative to Yesso scallop, followed by Zhikong scallop (*Chlamys farreri*), Bay scallop (*Argopecten irridians*) and Purple scallop (*Argopecten purpuratu*). Our work represents the first effort toward fully decoding of a scallop genome and will pave the way for profound understanding the biology and evolution of Pectinidae.

P1019 The preliminary results of GGRS for some indigenous pig breeds in Zhejiang Province. Jiucheng Chen (Zhejiang university)

INTRODUCTION The indigenous pig breeds in Zhejiang Province are famous of high reproductive capacity and some other advantages. The next generation sequencing (NGS) technology, which enables the discovery of thousands and thousands of SNPs with plummeting cost, was wildly used in genetic studies in order to explore the genetic foundation. Genotyping by genome reducing and sequencing (GGRS) as a new method of NGS may provide a more effective way used in SNPs discovery, genotyping and genome-wide association study (GWAS) for indigenous pig breeds.
MATERIALS AND METHODS The genomic DNA were extracted from ear tissue of 168 indigenous pigs in Zhejiang Province (60 Jiaxing pigs, 48 Chalu pigs, 60 Jinhua pigs) and equally divided into two lanes (lane 6 and lane 8). Each DNA sample was cut by restriction enzyme AvaII and jointed unique adapter-barcode, respectively. The fragments ranged of 300-400 bp were selected for next sequencing.
RESULTS A number of 394,785,787 raw reads were generated in lane 6 and 357,085,701 raw reads were generated in lane 8 of an Illumina High-seq 2000 sequencer for the pig population. 296,089,340 (74.9%) reads in lane 6 and 280,868,561 (80%) in lane 8 complying with the filtering rules were high-quality reads. The base average quality score was at least 20 (error rate of base calling of 1 in 100), in which the average quality score of the first 65 bp was at least 30 (error rate of base call of 1 in 1000). On average, the sequence depth and coverage of lane 6 were 5.9x and 1.55%, which of lane 8 were 6.2x and 1.40%, respectively. In the light of the consequence of sequencing, genetic data had reached the requirement of GWAS and could do the further analysis.

P1020 Molecular characterization, expression and functional analysis of *NOD1 in* Qingyuan

partridge chicken. Tao zhiyun (Yangzhou University), Zhu chunhong, Shi zuhao, Song chi, Xu wenjuan and Song weitao (Jiangsu Institute of Poultry Sciences) and Qin aijian (Yangzhou University)

Nucleotide-binding oligomerization domain-containing proteins-1 (NOD1) is a cytoplasmic pattern recognication recepteors(PRRs) and a key member of the NOD-like receptors (NLRs) family. It has been reported sensing a large variety of microbial infections or danger molecules to induce host innate immune response by modulating NF-κB signalling. But no study on the chicken *NOD1*(*chNOD1*) has been reported to date in chicken. In the current study, the full-length cDNA sequence of *chNOD1* was cloned, and the putative amino acid sequences were identified in Qingyuan partridge chicken. The complete open reading frame (ORF) of *chNOD1* contained 2856 bp encoding a 951 amino acid protein. Structurally, it comprised of one caspase recruitment domain (CARD) at N-terminal, seven leucine rich repeat (LRR) regions at C-terminal and one NACHT domain between N and C-terminals. Phylogenetic tree analysis showed that chicken NOD1 clustered with that of duck and turkey. Further more,tissue-specific expression analysis of *chNOD1* gene by quantitative real-time PCR(qRT-PCR) was detect in Qingyuan partridge chicken. The result showed that it wide distribution in various tissues and highest expression was detected in testical. At last, inductive expression of *chNOD1* and its associated adaptor molecule *RIP2* (receptor-interacting protein 2), and the effector molecules *NF-κB* (nuclear factor of kappa B)wereobserved followingS. enterica serovar Enteritidis (SE) infection inQingyuan partridgchicken. Together, these findings highlighted the important role of *chNOD1 in chicken in response to pathogenic invasion.*

P1021 Cloning and assembling the *Streptococcus thermophilus* CRISPR3/Cas locus for prokaryotic genome engineering. Lijun Guo, Kun Xu, Chonghua Ren and Zhiying Zhang (Northwest A&F University)

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) system has recently been used to engineer genomes of various organisms. Here we present a simple method to assemble the *Streptococcus thermophilus* CRISPR3 arrays for prokaryotic gene targeting. The *Streptococcus thermophilus* CRISPR3 consists of arrays of short conserved repeat sequences (36 bp) interspaced by unique spacer sequences of same size (30 bp). Considering that a guide sequence of 20 nt within sgRNA, which is derived from the spacer sequence, is longer enough for guiding both *Streptococcus pyogenes* and *Streptococcus thermophilus* CRISPR/Cas9 endonuclease activities, we hypothesized that the spacer sequence can be reconstituted with a restriction enzyme site (6 bp) and the interested guide sequence (24 bp). By employing compatible restriction enzyme strategy, we can simply assemble different numbers of target sequences in an array format for multiple gene targeting. Generally, a ~6.7 kb fragment (consisting of the tracrRNA gene, four *cas* genes and CRISPR leader sequence), the 103 bp CRISPR terminator, the first direct repeat and the interested guide-direct repeat were cloned and assembled into pBlue vector consecutively. By assembling a guide sequence from kanamycin resistant gene, the programmed *Streptococcus thermophilus* CRISPR3/Cas system was proved to function against different kanamycin resistant plasmids (pTrack-CMV, peGFP-C and pDsRed-C) in *E.coli*. Further applications of the system for engineering *E.coli, S. aureus* and bacteriophage genomes are currently being undertaken. The method makes it easier for programming the *Streptococcus thermophilus* CRISPR3 arrays for multiple gene targeting and may be adjusted to other CRISPR/Cas systems.

P1022 Whole-genome association study for milk protein composition in the Chinese Holstein cattle population. Chenghao Zhou, Shengli Zhang, Dongxiao Sun, Cong Li, Lingzhao Fang and Qin Zhang (China Agricultural University)

Until recently, the genome-wide association study (GWAS) was a useful approach to reveal the genetic architecture of quantitative traits in dairy cattle. The present study was conducted to find the candidate genes or quantitative trait loci (QTL) associated with milk protein composition(αS1-CN, αS2-CN, β-CN, κ-CN, α-LA, and β-LG), casein index, protein percentage, as well protein yield in Chinese Holstein cattle population using GWAS method. The present study was performed for average 54,001 SNPs to estimate maker effects. Genotype and phenotype information for 867 Chinese Holstein cows were included in the analysis. The phenotypic values of all 6 major milk proteins (αS1-CN, αS2-CN, β-CN, κ-CN, α-LA, and β-LG) for each individual were evaluated as weight-proportion of the total protein fraction (wt/wt%) using a commercial ELISA kit. The mean percentages of αS1-CN, αS2-CN, β-CN, κ-CN, α-LA, and β-LG were 36.21%,10.08%,27.45%,5.62%,2.76% and 8.82%, respectively. A two-step strategy was applied to estimate SNP effect, the first step involved a general linear model and the second step used a mixed model accounting for all family relationships. Moreover, the top 500 SNPs were used for the multiple QTLs evaluation. SNPs associated with these target traits (P-values < 0.01) were selected. Association analysis revealed that the main genomic regions associated with milk protein composition were found on BTA 5, 6, and 11. All 6 major milk proteins(αS1-CN, αS2-CN, β-CN, κ-CN, α-LA, and β-LG) were located in a genomic region on the BTA6. The present study demonstrated novel candidate regions in BTA14, 19 and 20 for milk protein composition.
The present study showed that association study with dense SNP markers in a mixed model analysis, which was observed to perform best for samples from complex pedigreed populations like cattle.

P1023 Differential gene expression analysis of RNA-seq on hypoxic adaptation in Tibet pig. Bo ZHANG (China Agricultural University), qiangba yangzong (College of Agriculture and Animal Husbandry of Tibet), Qing-gang LI (Anhui Academy of Agricultural Sciences) and Hao ZHANG (China Agricultural University)

Tibet pig that live in mountain and valley regions (2500~4300m altitude) of Tibet plateau located in the southwest of China show striking phenotypic and physiological differences from lowland pigs, and have well adapted to the extreme conditions such as hypoxia. In order to gain more insights into genetic characteristic, we performed transcriptome sequencing of heart tissue in Tibet pig. We collected heart tissues of four groups, Tibet pigs raising at highland (Linzhi, 3000 m) (TH, n=4), Tibet pigs raising at lowland (Beijing, 100 m) (TL, n=4), Yorkshire pigs raising at highland (Linzhi, 3000 m) (YH, n=4) and Yorkshire raising at lowland (Beijing, 100 m) (YL, n=4), at the age of 6 months. Two pools of total RNA from four individuals each group were sequenced in Ilumina HiSeq 2000. The reads data were analyzed or calculated some software, Bowtie2, Tophat, Cufflinks, etc. Through RNA-seq, we generated about 551 M high quality reads, and in which 80% were aligned on swine reference genome. According to a standard of fold-change (FC≥2) and significant (P<0.01, Fisher test), we gained 329, 214, 265 and 397 of significantly differentially expressed genes (DEGs) respectively in TH vs.YH, TH vs.TL, YH vs.YL, and TL vs.YL. There were 95, 78 and 69 genes respectively from the DEGs of TH vs.YH, TH vs.TL and YH vs.YL enriched to some GOs that contained hypoxic function

domains with oxygen reaction, cardiac hypertrophic response and oxygen transport activities, etc. Based on KEGG database, we found 10, 8 and 8 genes respectively above three groups enriched to KEGG pathways which involved in hypoxia-related regulation pathway of HIF-1 signaling pathway, hypertrophic cardiomyopathy and dilated cardiomyopathy. By considering their functions and differential expression in four groups, we selected 10 interested novel genes which were determined to be likely involved in hypoxia adaptation to do further function verification experiments. These genes will be useful to clarify the molecular mechanism of hypoxia in Tibet pig.

P1024 Genome-Wide Association Study for wool fiber diameter of Chinese Merino sheep. Shu-Dong Liu, San-Gang He, Lei Chen, Zheng Yuan, Wen-Rong Li and Ming-Jun Liu (Key Laboratory of Genetics, Breeding and Reproduction of Grass Feeding Livestock of Ministry of Agriculture, Xinjiang Academy of Animal Science, Urumqi, Xinjiang, P R China.)

Fiber diameter is the most important trait of fine wool sheep. It determines the economic value of fine wool. Here, a genome-wide association study (GWAS) was performed to identify the genetic variants of which putatively influence wool fiber diameter trait in Chinese Merino sheep. Genomic DNAs were extracted from 278 Chinese Merino sheep in a population based on pedigree and phenotypic information, and genotyped with the Illumina Ovine SNP50 BeadChip. Phenotypes of wool fiber diameter of the fine wool sheep were determined by OFDA 2000. SNP array test report displayed that the call rate was 99.86% and the main filtering parameters were the test mind (0.1), geno (0.05), minor allele frequency (0.01) and Hardy-Weinberg equilibrium (0.000001). A total number of 49441 SNPs were selected after removing the SNPs which were not met the quality control criteria. The association study was carried out by plink1.07 software package and the minimum threshold defined by nominal P-values of 1x10-6(P = 0.05/49441) was determined by Bonferroni correction. Expected genome-wide versus the distribution of obtained p-values were coincided by QQ-plots. Association analysis revealed that 11 significant SNPs that exceeded genome-wide significance (unadjusted p-value<8.5x10-5) were located on OAR1 (ovine chromosome 1), OAR3, OAR6, OAR7, OAR9, OAR15, OAR17, OAR26. Furthermore, functional annotation of these regions identified genes involving hair follicle signaling pathway, neuron development, cell division, cell growth, molecular transport and cytokines. Further statistical analyses with additional population of Chinese Merino sheep are worthwhile to validate these genetic variants and candidate genes.

P1025 Systematic study of TALEN N-terminal length with maximal cleavage efficiency in yeast and mammalian cells. Zhiqiang Zhang, Kun Xu, Yun Wu and Zhiying Zhang (Northwest A&F University)

TAL effector nucleases (TALENs) have been successfully applied for genome engineering in varieties of species with potential therapeutic applications. Studies have shown that the length of TALEN N-terminal can affect the level of transcription activity. We generated a series of truncated mutants by deleting 20 amino acids from the N-terminal each time and carried out a systematic study to optimize the length of N-terminal by using our yeast and mammalian reporter systems *in vivo*. Results on a TALEN targeting mouse *Gt(ROSA)26Sor* gene showed different optimistic lengths for maximal cleavage efficiency in yeast and mammalian cells. In the yeast reporter system, the mutant with 120 N-terminal residues showed the highest cleavage efficiency. Mutants with 200, 153 and 140 residues retained 70% ~ 60% of efficiency, whereas the mutant with 100 amino acids nearly

retained no cleavage activity. In the mammalian reporter system, same to the previous study, the mutant with around 153 amino acids showed the best length for cleavage. Mutants with 160 and 140 amino acids also retained the same efficiency while trimming the segment to 100 residues impaired the activity to almost zero.

P1026 Genome-wide discovery and characterization of porcine endometrial lncRNA in embryo implantation. Ruize Liu, Lijie Su, Jinjun Hong and Mei Yu (Huazhong Agricultural University)

Implantation and placentation are critical steps for successful pregnancy. Embryo implantation is directly affected by genes and miRNAs related to uterine receptivity. Studies have demonstrated the widespread roles of lncRNA(long noncoding RNA) in gene regulation and cellular processes. To explore the functional roles of lncRNAs in the course of initial conceptus attachment, we downloaded the dataset of RNA-seq of endometrium on Day 14 of pregnancy (GSE43667) and built a computational pipeline to identify lncRNAs. We identified 2209 lncRNAs candidates, including 1458 lincRNAs, 146 lncRNAs falling entirely within the intron of known genes and the other 605 lncRNAs having exon overlap with known genes. Of the 2209 lncRNAs candidates, 549 lncRNAs partially overlap the NONCODE lncRNAs, including Xist. All predicted lncRNAs share many of the characteristics of their mammalian counterparts: relatively low exon number, short length, and low expression, which indicated that the predicted lncRNAs were credible. Of the lncRNAs, 169 differentially expressed lncRNAs were obtained, 95 with higher and 74 with lower expression levels in the porcine endometrium on day 14 of pregnancy in comparison to day 14 of the estrous cycle. To predict the function of differentially expressed lncRNAs, we analyzed the correlation between the expression of each protein-coding gene with the expression of differentially expressed lncRNAs and performed GSEA to relate GO terms with lncRNAs. The GSEA revealed that the differentially expressed lncRNAs involved in several biological processes, such as cell proliferation, growth factor binding, immune response, chemokine activity, hemopoiesis, and apoptosis. Those results indicate that the differentially expressed lncRNAs are putative developmental regulators in endometrial development during implantation period. Over all, we found some implantation-related lncRNAs, which may play an important role during embryo implantation by regulating of the proliferation, apoptosis, and immune response related genes.

P1027 Influence of embryo transfer method on development of cloned porcine embryos. Xiaoyan He (South China Agricultural University), Junsong Shi and Rong Zhou (Guangdong Wens Foodstuff co. LTD) and Zicong Li, Dewu Liu and Zhenfang Wu (South China Agricultural University)

Somatic cell nuclear transfer(SCNT) can be used as a technology to reproduce genetically superior boars. Skin fibroblasts are usually used as nuclear donors for cloning of adult boar. To improve cloning efficiency of adult cells, the impacts of embryo transfer time, ovulation status and gestational age on the pregnancy rates(PRs) were evaluated in this study. The PRs of 350 surrogates in total were analysed from our experimental records in last year. The results showed that PRs were similar among three groups with different embryo transfer time. When transplant surgeries were performed on day 1, day 2 or day 3 after SCNT, the PRs were 66%, 65% and 71%. When day 2 after SCNT was chosen for transplant surgeries, the PRs represented significant differences between the group in post-ovulation stage(72%) and the group in pre-ovulation stage (50%). Gestational age of sows is also an influencing factor. When transplant surgeries were performed in sows

which had given birth once, twice, three times and fourth times or more, the PRs were 70%, 70%, 63% and 45% respectively. This study suggests that the ovulation status of sows is important for embryo implantation. Meanwhile, the development of cloned embryos in young sows is better. In conclusion, SCNT is a feasible way to multiply boars with desired phenotypic traits. However, the adult SCNT technology remains inefficient and cloning efficiency is about 1% of embryos transferred surviving to term. Based on the refined embryo transfer methods, we are trying to use medicine to regulate pregnancy-related hormone levels and nutrient levels of surrogates. These may be basal ways to improve porcine cloning efficiency.

P1028 Analysis of African chicken ecotypes using a 600k SNP chip. Damarius Fleming, J.E. Koltes and Alysta Markey (Iowa State University), C.J. Schmidt (University of Deleware), C. Ashwell (North Carolina State University) and M.F. Rothschild, J. Reecy and S. J. Lamont (Iowa State University)

Climate change is contributing to higher environmental temperatures, which negatively impact animal production. Greater understanding of the genomic control of response to heat stress will aid in breeding of animals that are better able to tolerate hot climates. Populations with a history of undergoing natural selection in hot climates are likely to have evolved mechanisms of tolerance that can be identified at the genomic level. We genotyped 72 Ugandan chickens of three distinct ecotypes by sampling location using the Axiom ® 600k Chicken Genotyping Array. Clustering analysis grouped the 72 birds into 3 clusters containing varying percentages of birds from each location, indicating genetic diversity within sampling areas. A case-control genome wide association analysis was conducted using PLINK to evaluate the genetic basis of observed phenotypes such as the naked neck phenotype. The naked neck phenotype is hypothesized to be an evolutionary adaptation amongst chickens in hotter climates that allows for better thermoregulation. We conducted a case-control, genome-wide association study to identify genomic regions that may show evidence of allelic co-segregation with the naked neck phenotype. Four cases (naked neck) and 66 controls (feathered neck) were analyzed using 508689 SNPs. Sixteen SNP associations (genome-wide significance -log10=5e-07) were detected on chromosome 1 and seven on chromosome 3. Four of the strongest signals on chromosome 3 are within 500kb of the candidate gene for the naked neck trait, *BMP12* (*GDF7)*. Identification of the novel region(s) associated with the naked neck phenotype on chromosome 1 suggests may indicate the existence there of genomic elements with possible epistatic interactions with the candidate gene on chromosome 3. Future studies on other phenotypes, and contrasting ecotypes from hot climates with populations developed in temperate climates, will shed additional light on the genomic basis of other traits.

P1029 Estimates of genetic parameters for liver fat content in broiler lines divergently selected for abdominal fat. Meijing Liang, Zhipeng Wang and Hui Li (Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture; Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang Province; College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, PRC)

The Northeast Agricultural University broiler lines were divergently selected for abdominal fat content (NEAUHLF), and the aim of the present study was to estimate the genetic parameters of liver fat content (LFC) trait collected from 462 birds for the 16th generation population. Two statistical methods were used in this process, Gibbs sampling and restricted maximum likelihood (REML). The REML method is a

common approach used in animal breeding practices, to estimate genetic parameters for the improvement of animal populations. Currently, the Gibbs sampling based on Bayesian method is gradually being employed by animal breeders. In this study, a Bayesian animal model was implemented via Gibbs sampling. We compared estimates obtained from both Gibbs sampling and REML, to verify the accuracy of the calculated values. The statistical model included line & hatch as fixed effects and additive genetic effects as random factors. The heritability estimate for LFC obtained from Gibbs sampling was 0.43±0.10, and the corresponding REML estimate was 0.36. There's slight difference between these two methods. Therefore, the LFC trait in this population has a moderate level of heritability. Based on the Gibbs sampling method, genetic correlations of LFC with body weight (BW) and liver weight (LW) at 7wk were 0.29±0.11 and 0.20±0.15, respectively, whereas a moderate positive genetic correlation (0.49±0.30) between LFC and abdominal fat weight (AFW) at 7wk was estimated. These results were in agreement with those calculated using the REML method.

P1030 Genetic diversity and classification of tibet yaks based on Mitochondrial ATP6 gene sequence. Qin Yang and Zhixin Chai (Southwest University for Nationalities), Qiumei Ji and Jinwei Xin (Tibet Academy of Agricultural and Animal Husbandry Sciences) and Qiaoqiao Song and Jincheng Zhong (Southwest University for Nationalities)

In order to investigate the genetic diversity and clustering relationships of Tibet yak, the mitochondrial ATP6 (Adenosine Triphosphate 6) gene from 367 yaks in 16 populations were sequenced and analyzed. There were 70 polymorphic sites within sequences. Among these polymorphic sites, 52 were singleton variable sites and 18 were parsimony-informative sites. A total of 67 haplotypes from 70 polymorphic sites were identified, and the nucleotide and the haplotypes diversities were 0.534 and 0.00181, indicating a relatively abundant genetic diversity in Tibetan yaks. The NJ phylogenetic tree revealed that the Tibet yak could be divided into Sibu and Cuona two types. Network relationship chart of 67 haplotypes presented two clusters, indicating that Tibet yaks may be derived from two separate maternal lineages.
tatement of funding:This research was funded by National Key Technology R&D Program (No.2012BAD13B06)

P1031 Phosphorylation, genome-wide occupancy and target gene activation of cAMP-response element binding protein (CREB) in porcine follicles. Kai Xue and Jiazhe Song (Dalian Medical University) and Ning Li (China Agricultural University)

As an important transcription factor, cAMP response element binding protein (CREB) is able to response to diverse stimulations from hormones and growth factors, and activates thousands of gene transcriptions. Here we found that the expression of CREB in porcine ovary was extensive, and increased gradually during ovarian follicle development. Furthermore, phospho-CREB-ser133 were only detected in the preantral and antral ovarian follicles, which were larger than 100μm (Fig.1). To further clarify the function of CREB, we applied Chromatin Immunoprecipitation (ChIP), Solexa sequencing and bioinformatics to detect porcine ovarian follicles. The results indicated that the CREB binding sites (peaks) of antral ovarian follicle cell sample (S6) and ovary cell sample (S7) were 72729 and 68602, respectively. And the peak coverages of sample S6 and S7 were 10.9Mb and 10.8Mb, occupying 4.03% and 4% porcine genome. Then, by the gene enrichment functional annotation analysis and pathway mining analysis, we targeted 36 main functional clusters of CREB target genes, referring to signal transducer,

internal secretion and cell proliferation et al in porcine ovarian follicle development. In addition, together with the data of ovarian follicle gene expression from Affymetrix Porcine Genome Genechips, we detected the variations of 1913 Affymetrix-CREB target genes in porcine ovarian follicle. The results showed that the up-regulations of 88% Affymetrix-CREB target genes were detected in the preantral and antral ovarian follicles, which also comprised the expressions of phospho-CREB-ser133. These results indicate that the activations of CREB target genes via phospho-CREB-ser133 occur during porcine ovarian follicle development. Our research provides new insights to understanding the gene expressions and regulations in the dynamic developing ovary.

P1032 Establishment of site-specific transgenic pig via homologous recombination mediated by CRISPR-Cas9. Linyuan Ma and Ning Li (China Agricultural University)

Transgenic technology is widely used in biological research. However, it has several limitations: the insertion site, integrity, and copy number of the transgene cannot be controlled. The transgenes are subjected to the local chromatin environment and can cause endogenous gene disruption, although the mutagenic properties of transposons can be desirable for particular applications. Moreover, transgenic DNA concatemerized into a large array is subject to repeat-induced gene silencing. Site-specific integration may overcome these hurdles by targeting the transgene to a specific chromosomal locus. Due to the specific integration site, transgene expression is consistent. To date, the research of site-specific integration is limited to model organisms, such as mice and human. There is little research on site-specific integration in livestock. In order to achieve site-specific integration in pig, we take advantages of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9(Cas9) system, a genome-editing technology. CRISPR-Cas9 system succeeded in genome-editing in multiple species, such as mice, human, bacteria, yeast and zebrafish. According to specific genomic loci, We successfully construct targeting vectors with short homologous arms and different transgene expression cassettes, and expect to achieve site-specific integration in pig with the help of CRISPR-Cas9 system and Cre/loxP system. Key words: site-specific, CRISPR-Cas9, homologous recombination

P1033 The abundance and species of gut microflora and nutrient digestibility during the growing phrase of Sutai pigs. Qing Niu, Pinghua Li, Bo Zhou and Ruihua Huang (Institute of Swine Science, Nanjing Agricultural University)

A large and diverse microbial population is contained in the intestinal tract of pig, which plays an important role in health and nutrient digestion. Here we characterized bacterial species in fecal samples of healthy Sutai pigs at the age of 60 days (n = 9), 90 days (n = 9) and 150 days (n = 9) using 16S rDNA gene sequencing, examined the differences of gut microflora and nutrition digestibility. A total of 3,398,670 effective sequences were obtained, and bacterial composition was analyzed from 3,169,578 modified sequences. It obtained 1,138,078, 1,1059,81 and 925,519 operational taxonomic units (OTU) at 3% distance cutoff in 60 d, 90 d and 150 d of age, respectively. Bacterial phylotype richness at 150 d of age was the greatest, and that at 60 d was the least. The most dominant bacteria flora was *Firmicutes* at three phrases of age. At 150 d of age, the abundance of *Clostridium* ($P < 0.05$) and *Ruminococcus* ($P < 0.01$), as well as the digestibility of *crude fiber* (CF) ($P < 0.01$) and *acid detergent fiber* (ADF) ($P < 0.01$), were higher than the other two phrases of age. Our study suggests that the abundance and quantity of

bacteria flora of intestinal tract and the digestibility of fiber increased during the growing phrase of *Sutai* pigs.

P1034 Screening of differentially expressed genes and enrichments analysis of microRNAs in *longissimus dorsi* of Yorkshire and Shaziling pig. DONG XU (College of Animal Science and Technology, Hunan Agricultural University)

MicroRNAs (miRNAs) are involved in the regulation of fat metabolism, In this paper we studied the genes andmiRNAs differentially expressed in *longissimus dorsi* by both microarray and deep sequencing, and analyzed the functional relevance of integrated miRNA and mRNA expression in relation to the physiological and biochemical parameters in two 25-day-old male Yorkshire (YS) and Shaziling (SZL) pig. According to the miRBase (release 19.0), 298 co-expression of miRNA from YS and SZL in small RNA libraries were filtered as candidate miRNA, miR-429 for YS and miR-105-1 for SZL were tissue-specific, and 9 miRNAs were differentially expressed with a more than 2.0-fold change (P-Value, 0.5, FDR, 10%), 5 up-regulated (mir-127, let-7e, mir-363, mir-493, mir-299) and 4 down-regulated (mir-204, mir-29a, mir-210, mir-183) miRNAs, 184 new conserved miRNAs and 21 pairs of novel miRNA/ miRNA were found. Genes showing over 1.5 fold change (P-Value, 0.5, FDR, 10%) were considered to be differentially expressed, and 553 genes were found, that is, 341 genes were up-regulated and 212 genes were down-regulated. These genes were mainly associated with the mitogen-activated protein kinase, cell cycle, P53, WNT, cell adhesion molecules pathway (P-Value, 0.5). Some of these genes were metabolic process-related, catalytic activity-related, hydrolase activity-related genes. Q-PCR validated the 4 differentially expressed miRNAs (DEMs) (Ssc-miR-127, Ssc-let-7e, Ssc-miR-204, Ssc-miR-29a), and 2 novel miRNA and 8 differentially expressed genes (DEGs) (GADD45, MYH1, MAPK14, SYMPK, SRPX, NRAP, EEA1 and IL17RD). We also found that there was significant correlation between mRNAs and miRNAs.This research would illustrate the molecular formation mechanisms of *longissimus dorsi* between the native and European pig.

P1035 Construction of the recombinant expression vector of Guanling cattle *MyoDI* gene promoters named pEGFP-N3-*MyoDI*. Congcong Huan

Guizhou Guanling cattle has the remarkable properties of strong constitution, limb hoof, flexibility in action, hardiness, drought-enduring, forage-resistance, well-adapted and so on. It is the local cattle which has been raised by most farmers. Nowadays, much attention has been paid to cultivate the cattle breed with advantageous traits. Myogenic regulatory factors (*MRFs*), consisted of *MRF4, MyF5, MyoG*, and *MyoD I*, are the key factor to control the generation of skeletal muscle. And the generation of muscle is controlled by these factors at the same time. A lot of research showed that muscle specific transcription factor had close relation with the muscle growth and meat quality. In this study, we designed four primers for the cloning of *MyoD I* gene promoters which had different length. The fragments, obtained by PCR, were ligated with the target gene. After transformation into *E. coli* DH5α, the positive colonies were picked up and confirmed by sequencing. Then the expression vector pEGFP-N3-*MyoDI*was constructed and transformed into eukaryocyte. It provided the molecular basis for the subsequent cell transfection and transgenic mouse model, and also promoted the further study of the activity and function of *MyoD I* gene promotors.

P1036 Analysis of differentially expressed genes controlling the regulatory mechanism of broiler pigments. Muhammad Tarique Tunio,

Shuming Yang and Mohsina Zubair (Chinese Academy of Agricultural Sciences , Beijing China)

The chicken is kind of important model organism that unites the evolutionary gap between mammals and other vertebrates and provide major source of protein from meat and eggs for all over the world's population. However, specific genes underlying the regulatory mechanism of broiler pigmentation have not yet been determined. In order to better understand the genes involved in mechanism of pigmentation in the muscle tissue of broilers, the Affymetrix microarray hybridization experiment platform was used to identify gene expression profiles at 7 weeks of age. Broilers fed with canthaxanthin, natural lutein and orange-II pigments (100mg/kg) helped to explore the gene expression profile ($P<0.05$). Our data showed that 6th week of age was very important phase for gene expression profiles. This leads to the identification of differentially expressed genes; 60 were known genes, 40 up-regulated and 20 down-regulated in canthaxanthin, while natural lutein had a differentially expresses gene; 30 were known genes, 15 up regulated and 15 down-regulated whereas orange-II had differentially expressed genes, 7 were known gens, 5 up-regulated and 2 down-regulated. Our data indicate that the numbers of differentially expressed genes were more up-regulated than the down-regulated and several genes showing conserved signaling to previously known functions. Thus, functional characterization of differentially expressed genes revealed several categories that are involved in important biological process of pigmentation, growth, molecular mechanism, fat metabolism, cell proliferation, immune response and lipid metabolism, protein synthesis and degradation. The present study results demonstrate that; canthaxanthin, natural lutein and orange-II genes are identified as key regulatory genes to control the regulatory mechanism of pigments. Hence, it will be important targets for further investigation to understanding the mechanism of pigmentation in broilers.

P1037 Connection and mechanisms of leptin and insulin resistance in *leptin* knockout pig obesity model. Tan Tan and Ning Li (China Agricultural University)

Insulin resistance has been an important research topic in type 2 diabetes. It is not only the significant characteristics of type 2 diabetes, but also the focus in insulin drug treatment. Leptin is closely related with obesity, diabetes and other diseases on the basis of the insulin resistance. Relationship between leptin and insulin are negative feedback, they constitute the Fat -Insulin Shaft. The specific mechanisms of cross-talk between leptin and insulin resistance and how to lead series complications of obesity has not been fully elaborated. In our lab's previous research(Song et, al),we get the pig obesity model via ZFN-mediated leptin gene knockout with the typical symptoms of obesity. My study content focus on related inquiry of leptin and insulin resistance on the basis of this mode. First we detected the leptin gene knockout pigs' blood physiological and biochemical indexes and pancreatic islets and glucose tolerance ability, etc. Important results show that the knockout pigs has high level blood sugar, high blood insulin, significantly insulin resistance and the disorder of glucose tolerance ability at present. Then we identified F1 genotype and hybridised, realized the species genetic and get the homozygote F2. It is expected to get a more obvious early onset type 2 diabetes model for subsequent experiments and research with high fat diet feeding. Because the pig has lot of similarities in individual growth, genetic characteristics, physiological and biochemical indexes with human, so our research breaks new ground for the prevention of metabolic disease and drug screening.

P1038 Detailed analysis of segmental duplications among domestic animals. Xiaotian Feng (China Agricultural University)

Segmental duplications (SDs) are duplicated sequences with high sequence similarity (≥90%) and ranged in length from 1 to 400 kb. Multiple studies reveal that segmental duplications play a momentous role in gene innovation and formation of genomic structural variation. Additionally, genes embedded within these duplicated regions show a significant enrichment of immunity, growth and responses to external stimuli. Here we perform the first detailed analyses of SDs among common domestic animals, providing resources for further studies. We identified SDs in the genomes of common domestic animals with two different methods--whole-genome assembly comparison (WGAC) and whole-genome shotgun sequence detection (WSSD)--based on UMD_3.1, Sscrofa10.2, EquCab2.0, OryCun2.0 Oar_v3.1, CHIR_1.0 and Gallus_gallus-4.0 genome assembly for cattle, pig, horse, rabbit, goat, sheep and chicken, respectively. GO and Pathway analyses in SD regions were performed by Bioconductor and David programs. We finally constructed SD maps using parasight v7.6. SD contents of domestic animal genome are 2.55%, 2.04%, 6.63%, 3.42%, 1.91% and 1.97% for cattle, pig, horse, rabbit, goat, sheep and chicken, respectively. SDs in subtelomeric and pericentromeric regions are 2-6 fold enriched compared to other regions of genome. GO and pathway analyses indicate that genes in SD regions show enrichment of metabolic process, responses to stimulus and olfactory transduction. Average copy number of genes within SD regions range from 3 to 7 among different domestic animals. SDs are common and vital duplicated sequences of domestic animal genome. They obstruct genome assembly due to their high sequence similarity. A large number of genes detected within SD regions imply that SDs are associated with several biology process and genomic rearrangement. Further validation studies need to be performed.

P1039 Whole-transcriptome, high-throughput RNA sequence analysis of the bovine mammary gland response to S. aureus infection in vivo. Lingzhao Fang, Xiao Wang, Yachun Wang, Shengli Zhang and Ying Yu (China Agricultural University)

Intramammary infections with *Staphylococcus aureus* (*S. aureus*) cause subclinical mastitis that can persist for long periods of time accounting for approach 80% of mastitis related costs (Shim et al., 2000). Therefore, there is a need to understand the immune response throughout the mammary gland during *S. aureus* infection. Although the transcriptional response of bovine mammary gland cells to *in vitro* infection has been studied, the interplay and consequences of these whole-transcriptomic responses in the *in vivo* environment of mammary gland are less clear. In the current study, we have analyzed and compared the transcriptomes of *S. aureus*-infected mammary tissue purified from udder quarters inoculated with two different concentration *S. aureus*, and from neighboring control udder quarters 24 h after infection. A mean of 23 million paired reads from each sample mapped uniquely and unambiguously to *Bos taurus* reference genome (UMD3.1.73) locations. After analysis of these mapped reads, differentially expressed genes (DEG) were detected in infected quarters *vs*control ones. 121 (76 upregulated and 45 downregulated), 418 (334 upregulated and 84 downregulated) were found in low and high concentration *S. aureus*-infected group *vs* control one. Meanwhile, 395 DEG (225 upregulated and 170 downregulated) were found in low vs high group (adjusted P-value < 0.05, fold difference >2). Gene ontology analysis of DEG using DAVID revealed an enrichment of immune, apoptotic and cell signaling genes (FDR <0.05).Pathway analysis of DEG using kobas2.0 showed the most significant pathway was

Cytokine-cytokine receptor interaction (FDR= 4.18E-04). The present study highlights the value of RNA-seq in identifying novel immunomodulatory mechanisms that underlie host- *S. aureus* pathogen interactions during infection. Funded by NSFC 31272420.

P1040 Repeatability of the major reproductive traits in silver foxes. huitao liu, Yiqing Li, Xiumei xing, Qiong Wu and Fuhe Yang (Institute of Economic Animal and Plant Science, Chinese Academy of Agriculture Sciences)

523 breeding records of 316 female silver foxes and 426 breeding records of 103 male silver foxes had been carried on the statistics and analysis in this study. Most of the silver foxes had three times or more than three times breeding records. Meanwhile, the repeatability of several main reproductive traits in silver foxes was estimated. The results showed that the average litter size at birth or weaning and the pregnancy duration in female silver foxes was 3.3, 2.7 and 51.8 days, respectively. The repeatability of the three traits in female silver foxes was 0.121, 0.009 and 0.213, respectively. The litter size at birth or weaning and pregnancy rate in the mating female foxes was 3.1, 2.6 and 75.3%, respectively. The repeatability of the three traits in the mating female silver foxes was 0.112, 0.307 and 0.202, respectively. It was concluded that the main reproductive traits of female foxes, the litter size at birth or weaning and pregnancy rate in the mating female foxes were much lower, which were mainly controlled by non-genetic factors like environment, nutrition and so on. Therefore, it will be more advantageous to improve the reproductive trait of silver fox by strengthening breeding management in production. This study would provide practical guidance for selective breeding of silver foxes.

P1041 QTL analysis of clinical-chemical traits in Korean native chicken. Hee-Bok Park, Dong-Won Seo, Shil Jin, Nu-Ri Choi and Muhammad Cahyadi (Chungnam National University), Chae-Kyoung Yoo, Jae-Bong Lee and Hyun-Tae Lim (Gyeongsang National University), Kang-Nyeong Heo (Poultry Science Division, National Institute of Animal Science, RDA), Cheorun Jo (Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University) and Junheon Lee (Department of Animal Science and Biotechnology, Chungnam National University)

Clinical-chemical traits are generally used biomarkers to examine the health status of individuals. There is a substantial range of variation in most clinical-chemical traits and the genetic factors of this variation have been relatively uninvestigated in chickens. The aim of this study was to identify quantitative trait loci (QTLs) affecting 8 clinical-chemical traits (glucose, total protein, creatinine, high-density lipoprotein cholesterol, total cholesterol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and amylase) in an F1 intercross established by purebred breeding among the 5 lines of Korean native chickens (KNC). Phenotypic data were collected from approximately 600 F1 animals. Genotype data on 161 DNA markers representing 28 linkage groups have been generated for this F1 intercross. The total map length was 2,813 cM. We used a multipoint variance components linkage approach to identify QTL for the traits. Currently, two significant QTLs affecting serum amylase levels were identified on chromosomes 7 (LOD =3.61, nominal *P*-value = 4.66x10-5) and 8 (LOD = 3.30, nominal *P*-value = 9.70x10-5), respectively. Although previous reports have shown the efficiency in mapping QTL that accounts for genetic differences between two divergent lines, studies using the F2 intercross design have provided less practical insight into whether these QTL segregate within commercial populations. For the successful implementation

of QTL information into selective breeding programs, segregation of QTL needs to be confirmed within the population of interest. Thus, identified QTLs in this study can provide practical information to identify molecular genetic factors affecting clinical-chemical traits within the KNC population.

P1042 The use of Wildlife Forensics to reveal a potentially deadly package! – Some case unusual case studies from the Australian Museum. Rebecca Johnson (Australian Museum, Australian Centre for Wildlife Genomics)

Australian enforcement agencies are increasingly embracing the use of DNA to assist in the investigation of wildlife cases. In certain instances, DNA can provide important information including species identification or pedigree information, which cannot otherwise be determined.
Wildlife crime is not only a risk to the wellbeing of individual animals targeted. It puts local and global biodiversity at risk as well as local industry through potential pest threats to agriculture. It can also pose significant disease risk to both human and animal health.
This presentation will outline two very different cases that demonstrate the utility of DNA identification in wildlife forensic cases. The first, is a NSW police case involving a threatening package – where the contents were identified using transfer DNA. The other is a Quarantine investigation – where we were asked to distinguish between a number of cryptic snail species and develop a diagnostic test to discriminate the invasive species from its non-invasive congeners.
Through sharing our insights and experience with the techniques used in these successful yet diverse cases, we hope to encourage further uptake of the use of DNA by authorities where the quite unique skills involved in wildlife forensic work are required.

P1043 Characterizing indigeneous livestock genetic resources by using whole genome data: the case of sheep and goats. Francois Pompanon, Badr Benjelloun and Florian Alberto (Univ. Grenoble Alpes), Ian Streeter (European Bioninformatic Institute), Wahid Zamani (Univ. Grenoble Alpes), Adriana Alberti and Stefan Engelen (CEA- Genoscope), Alessandra Stella (Parco Tecnologico Padano), Laura Clarke (European Bioninformatic Institute), James KIjas (CSIRO) and Frédéric Boyer and Pierre Taberlet (Univ. Grenoble Alpes)

Next Generation Sequencing technologies allow powerful characterization of genome diversity and detection of genes under selection that can be used for assessing livestock genetic resources. In this context, the NEXTGEN project (EU-FP7, http://nextgen.epfl.ch/) proposed a comparative analysis of whole genome data at the intraspecific level to optimise genetic management of farm animal diversity. Whole Genomes Sequences (WGS) at 10 X coverage were produced for about 200 sheep (*Ovis aries*) and 200 goats (*Capra hircus*) mainly from traditional breeds from Morocco and Iran, and with representatives of their wild ancestors (the Bezoar *Capra aegagrus* and the Asiatic mouflon *Ovis orientalis*). One goal of NEXTGEN was to address the key question of the genotyping strategy to choose for describing the diversity of indigenous breeds. Thus, we assessed how the choice of SNPs affects the bias and precision of population genetics diversity estimates (Heterozygosity, nucleotide diversity, Fst, LD,...) and the ability to detect signatures of selection. For that, we constituted panels of varying size (1K-5M) with random SNPs or with SNPs from the exome or from commercial SNP-BeadChips, and compared the results to the reference values obtained with WGS. As expected, high SNPs densities were necessary for detecting selection signature or estimating linkage disequilibrium (500K-1M) and lower size random sets (5k-10K) were sufficient to estimate whole genome

diversity. Using subsets of SNPs designed for describing the diversity of industrial breeds produced ascertainment biases. Then we showed that a panel of randomly chosen SNPs can be designed as a surrogate genome data source to accurately approximate genomic diversity and whole genome processes in both indigenous and industrial breeds. Finally, we showed the potential of wild populations and traditional breeds characterized within NEXGEN to act as Farm Animal Genetic Resources.

P1044 Genome wide association study revealed candidate regions for calving interval in Japanese black cattle. Yuya Kawasaki, Kenji FUKAZAWA and Takahiro FUJII (Graduate School of Agricultural Science, Kobe University), Yoshinobu UEMOTO (National Livestock Breeding Center), Moriyuki FUKUSHIMA, Takayuki AKIYAMA and Namiko KOHAMA (Hyogo Prefectural Technology Center for Agriculture), Eiji KOBAYASHI (NARO Institute of Livestock and Grassland Science), Kenji OYAMA (Food Resources Education & Research Center, Kobe University) and Shinji SASAZAKI and Hideyuki MANNEN (Laboratory of Animal Breeding and Genetics, Kobe University)

Fertility traits in beef cattle herds have been receiving increased attention due to recent rises in production costs. The objective of this study is to identify genomic regions associated with female fertility traits in Japanese Black cattle. We selected 236 animals from a Japanese Black cattle population (n = 526) based on pedigree and phenotypic information and these animals were genotyped using Illumina's BovineSNP50 v2 BeadChip. A total of 32,796 SNP were selected after removing SNP not meeting quality control criteria (call rate<0.95, MAF<0.05, HWE<0.001). We applied GRAMMAR and Genomic Control approaches to estimate the associations between genotypes and three fertility traits (age at first calving, calving interval, the number of calves produced at 4 years of age). Association analysis revealed that seven SNP on five chromosomes (BTA4, 5, 10, 15 and 26) were found to be associated with calving interval at 5% chromosome-wide significance level. In seven significant SNP, one SNP was located at 60.0 Mb on BTA4 (p = 2.9E-5) and two SNP were located at 60.4 Mb and 79.6 Mb on BTA5 (p = 3.1E-5 and p = 3.0E-5) and two SNP were located at 19.4 Mb and 32.2 Mb on BTA10 (p = 3.2E-5 and p = 1.3E-5) and one SNP was located at 26.5 Mb on BTA15 (p = 2.7E-5) and one SNP was located at 6.1 Mb on BTA26 (P = 2.8E-5). Out of these candidate regions, four were consistent with previously reported QTL associated with fertility traits. On the other hand, there were no QTL reports around the other three regions and it is suggested that novel candidate regions for calving interval would be in BTA5 and 26.

P1045 The cloning of cDNA of *GLP-2R* gene, expression profiles and its analysis of bioinformatics in porcine. Yulan Chai, Lingyu Wang, Hu Yang and Haiming Ma (Hunan Agricultural University)

This experiment obtained cDNA sequence from Yorkshire pig by technology of rapid amplification of cDNA ends (RACE)，and the length of cDNA was 1 868 bp，including 1665 bp length of open reading frame which encoded a protein of 554 amino acids, 203 bp length of 3' end. Using bioinformatics techniques to predict the homology with porcine *GLP-2R* gene of other species and phylogenetic tree was established by MEGA5.0 softwarewhich revealed that the pigs had the closest relationship with *Orcinus orca*and *Ovis aries*，while the farthest relationship with *Chrysemys picta*、*Taeniopygia guttata* and *Falco peregrinus*. Bioinformatics analysis of the amino acid sequence encoded by porcine *GLP-2R* gene indecated that it was water-soluble protein which contained 23 glycosylation sites and 7 transmembrane regions. In addition, according to

Smart prediction software, among the encoded amino acid sequence of porcine *GLP-2R* gene, the amino acid residues 92 to 168 was a piece of HormR domain, while amino acid residues 176 to 433 were a piece of PFAM: 7tm_2 domain.The secondary structure dominated by random coil and alpha helix, and the tertiary structure of *GLP-2R* protein showed a forniciform helix structure. Real time PCR analysis concluded that the porcine*GLP-2R* gene expressed to a certain degree in 10 different tissues of 25 day-age Yorkshire piglet and Shaziling piglet; and the expression in *longissimus dorsi* muscle was the highest in 10 different tissues, while the lowest in kidney. On the other hand,using statistical software SAS10.0 to statistical analysis, with P =0.05 as the critical point of a significant difference, significance differences of porcine *GLP-2R* gene among different tissues and different breeds of pigs was analyzed. The results exhibited that the porcine*GLP-2R* gene mRNA of *longissimus dorsi* muscle was significantly different with others($P < 0.05$) in the same breed and its pancreas, crureus, *longissimus dorsi* muscle are significantly different ($P<0.05$) in the above two breeds' *GLP-2R* gene.

P1046 The cloning of cDNA of *SLC13A5* gene, expression profile and its analysis of bioinformatics in porcine. Lingyu Wang, Yulan Chai and Haiming Ma (Hunan Agricultural University)

A recent study indicated that solute carrier family 13 (sodium-dependent citrate transporter member 5, *SLC13A5*) gene was related to the intramuscular fat content (IMF) in pigs. This study was carried out to clone the *SLC13A5* cDNA in pig, and analyzed its cDNA sequence and expression profile of ten tissues of 25-day-old Yorkshire and Shaziling pig. The full-length cDNA sequence of porcine *SLC13A5* gene was acquired from the *longissimus dorsi* muscle by Rapid Amplification of cDNA of End method in the Yorkshire pig, and its sequence was analyzed and predicted by bioinformatics method. Then we investigated the expression of *SLC13A5 in ten tissues* through qPCR analysis. The full-length of *SLC13A5* gene was 2118 bp, the open reading frame length was 1665 bp, 5'end length was 557 bp, the length of 3' end was 1320 bp, and the coding region encoded a protein of 333 amino acids. Pig *SLC13A5* gene and other species phylogenetic tree showed that pigs had the closest relationship with *Ovis aries*, and followed with*Macaca mulatta, Xenopus laevis, Columba livia,* and *Pseudopodoces humilis*. Amino acid sequence analysis revealed that *SLC13A5* protein was a kind soluble protein, and its relative molecular weight was 61183.7 Da, and theoretical pI was 7.45. The *SLC13A5*protein predicted included 18 phosphorylation sites and 8 transmembrane regions. The secondary structure was dominated by random coil, and the tertiary structure of *SLC13A5* protein showed a forniciform helix structure. *SLC13A5* gene were differently expressed in the Yorkshire and the Shaziling piglet. The expression of the *SLC13A5* gene in Cecum was extremely remarkable($p < 0.01$) in the above two breeds, and in its liver and crureus wassignificantly different ($p<0.05$). In the same breed, expression of this gene in *longissimus dorsi* muscle was significantly different with other 9 tissues ($p < 0.05$).This research had laid the foundation for further study on molecular genetic mechanisms of the *SLC13A5* gene pigs.

P1047 Genome association of scale pattern mutation in the Yellow River common carp. Chuanju Dong (Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences and College of Life Science and Technology, Shanghai Ocean University) and Jian Xu, Peng Xu and Xiaowen Sun (Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences)

The Yellow River common carp (YR) is one of the four most famous food fish in China with the feature of golden scale and red tail which is obviously different from other carp strains. However, there are a certain proportion of scale variation individuals emerging in domesticated YR population recently, which reduce the market price significantly. In order to solve this problem, genomics and molecular genetics approaches are employed to identified the potential causative mutations or alleles of scale variation, then eliminate them from domesticated YR strains through molecular assistant selection ultimately. In this study, we generated a number of the YR families and identified one family with scale pattern segregation. The parents have normal scale pattern, but the progenies is segregated with normal scale pattern (wild type, WT) and abnormal scale pattern (mutation type, MT) ratio of 3:1. High throughput SNP genotyping was conducted using the carp 250K Affymetrix Axiom SNP array on 40 WT, 40 MT progenies and two parents, and then obtained 199,577 polymorphic SNP markers for GWAS on scale pattern mutation. After a series of analysis, GWAS was performed on 75 samples and 96,206 SNPs remaining, then correction by BONF. Finally we obtained 33 significantly SNP loci ($P<0.05$) associated with scale pattern phenotype. We then scanned 50 kb on both downstream and upstream of the significant sites on common carp genome, and identified 20 genes on 8 chromosomes. Functional analysis of these genes revealed that four genes are likely associated with scale genesis and development. Further targeted exon re-sequencing will be conducted to identified the causative mutations, and gene function will be further confirmed with gene knockdown or knockout experiments on closely related model fish, zebrafish in the lab.

P1048 Development of genomic tools for systematic study of Pectinidae biology and evolution. Shi Wang, Xiaoteng Fu, Jinzhuang Dou, Wenqian Jiao and Yan Sun, Lingling Zhang, Xiaoli Hu and Zhenmin Bao

Recent advances in the next-generation sequencing (NGS) technologies now allow for rapid generation of extensive genomic resources for potentially any organism. With rapid decline in sequencing costs, there is an urgent need for developing cost-efficient genomic tools based on the NGS platforms to enable conducting omics-level analyses in less-studied organisms such as scallops. Here we introduce several genomic tools that have been recently developed by our group, including: (i) 2b-RAD technique, which adopts type IIB restriction enzymes in reduced genome sequencing, thus providing a flexible and reliable platform for genome-wide genotyping; (ii) RADtyping pipeline, which is an integrated package for accurate *de novo* codominant and dominant RAD genotyping in mapping populations; (iii) Sequencing-based gene network analytical approach, which reconstructs gene networks based on coexpression patterns to investigate complex physiological processes; (iv) MethylRAD technique, which enables cost-effective genome-wide profiling of DNA methylation by using methylation- dependent restriction enzymes to achieve reduced genome representation. We demonstrate the great utility of these tools in scallop genomic studies. Integration of these tools would greatly facilitate the systematic study of Pectinidae biology and evolution.

P1049 Use of genomic information to predict of breed composition in Australian beef cattle. Yuandan Zhang and Brue Tier (University of New England)

Australian beef cattle consists of B. Taurus and B. Indicus breeds and various crosses. B. Taurus breeds comprise British derived (Angus, Murray Gray, Hereford, Shorthorn) and Continental European derived breeds (Limousin, Charolais, Simmental). B. Indicus breeds include Brahman,

Africander and composite breeds (Tropical Composite, Belmont Red, Santa Gertrudis, Droughtmaster). Knowledge of breed composition, currently being identified by pedigree, is essential for genetic evaluation and other purposes. We studied a simple but effective method to predict breed composition using Illumina Bovine50K SNP genotypes. This method utilised breed specific allele frequencies to calculate the probability of an individual belonging to a breed. The odds ratio of probabilities "assigning to" to "not assigning to" a breed was used to assign an individual to a breed using Bovine50K (37072 SNPs after editing), BovineLD (6838 SNPs) and various subsets of SNPs chosen according to variation in breed specific allele frequencies. The accuracies of breed assignment were tested against the pedigree breed information. For crossbred or composite animals, components for the British Taurus, Continental European Taurus and Bos Indicus were estimated using the corresponding allele B frequencies and their SNP genotypes. Very high accuracies of breed assignment were achieved for British derived Taurus breeds, Simmental and Brahman (greater than 99%) and slightly lower accuracies for Charolais and Limousin cattle (93% to 97%). Use of 15% of 50K SNPs (BovineLD) could assign animals to breeds very accurately (>97%) for most Taurus breeds and Brahman but not for Limousin and Charolais cattle. Furthermore, use of small subsets of SNPs, selected on high variation in allele frequencies across breeds, can also very accurately assign individuals to their breeds. For example use of only 100 SNPs with highest standard deviation of allele frequencies across breeds identified 100% of Brahman cattle. However this method is not useful to assign individuals to their cross types or composite breed types yet.

P1050 Divergence, gene gain and lose in shrimps. Jianbo Yuan, Xiaojun Zhang, Chengzhang Liu, Fuhua Li and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

Except Insects, crustacean is one of the groups with most species in Athropoda. As the member of Crustacea, shrimp account for a great many of species, but few of the researches are focused on phylogeny and evolutionary biology of shrimps. As the development of next-generation sequencing technology, a growing number of shrimp genomes and transcriptomes are sequenced and published gradually, this may provide good resources for the exhaustive research on shrimp phylogenetic analysis. In this study, we performed phylogenetic analysis of arthropods and estimated the divergence time of shrimps based on the amount of transcriptome data. Using TreeFam program, we extracted 19,982 gene families from full genes of nine arthropods. Then, 85 single-copy genes were selected for the phylogenetic analysis of Athropoda. A consensus phylogenetic tree was constructed by the single-copy genes, which overcame the defects of lacking genetic information in single molecular phylogenetic analysis. After estimating the divergence time of each species, we found that Crustacea was divergent at about 395 million years ago. There were a great many of gene gain and lose when the ancestor evolved to *Daphnia* and shrimp, indicating that there is significant bias between the two genomes. Two shrimps, *Litopenaeus vannamei* and *Penaeus monodon*, were also divergent early and a lot of gained or losed gene families were found between them, which suggest these two Penaeidae shrimp are quite different each other. Furthermore, we found that there are many GO terms with one shrimp gene gained significantly while the other shrimp lost significantly. Among these GO terms, cellular component related gene families were significantly gained in *P. monodon*, while nervous system related gene families were significantly enriched in *L. vannamei*. The bias of gene gain and gene lose between two shrimps

may help them adapting to their new environments, respectively.

P1051 Selective breeding and transcriptome sequencing of Manila clam *Ruditapesphilippinarum*. Xiwu Yan, Zhongming Huo, Yanjie Qin, Hongtao Nie, Jianfeng Ding and Feng Yang (Dalian Ocean University)

The Manila clam is mainly distributed along the coasts of the Pacific and Indian oceans and is now cultured for commercial purposes in a number of European, America, and Asian countries, especially in China. By 2013, the annual production of Manila clams in China had increased to over 3,500,000 tons. Despite this economic importance, the industry has not yet benefited from genetic improvement. Here, we report on the selective breeding and transcriptome sequencing for the Manila clam.
A breeding program for the Manila clam was initiated in 2006 with the initial establishment of 145 full-sib families for selection for rapid growth and exclusive shell color. The results suggested that A large portion of the variance of growth trait in shell length was additive genetic. Three new strains of Manila clam with appealing shell colors were produced after selection, including orange, white, and zebra wave. Mass selection for shell length was conducted in the orange strain for five generations. The selected line grew significantly faster than the control line .
A normalized cDNA library representing a mixture of adult tissues was sequenced using aultra high-throughput sequencing technology (Roche 454). A database consisting of 5,060 Isotig and 19,509 Singlets were annotated. 1,626 molecular functions,3,458 cellular components and 7,400 biological processes were classified on Gene Ontology. A total of 59,122 SNPs sites were obtained, including 25,852 insertion, deletion and 33,270 transition, transversion sites. SNP markers associated with growth, shell color, and gender traits were developed for the Manila clam.

P1052 New duck genetic maps: a tool for QTL and proteinQTL (pQTL) detection. Alain Vignal, Yoannah Fran ̧ois, Caroline Molette and Christel Marie-Etancelin (INRA Chemin de Borde-Rouge)

Although the genome sequence of the common duck *Anas platyrhynchos* was recently published, the scaffolds resulting from the assembly are not yet organised along the chromosomes. Moreover, QTL detection in ducks is limited by the scarcity in genetic markers. As a typical example, a first genome scan in the INRA GENECAN backcross design could only be done with 91 microsatelllite markers, covering 778 cM in 16 linkage groups, whereas the duck karyotype is composed of 40 pairs of chromosomes, including the Z and W gonosomes. To increase the number of genetic markers and genome coverage, we developped a specific set of SNP markers. The GENECAN design is composed of 7 half-sib families, so we sequenced each of the 7 F1 sires, to detect SNP markers. Using reads with a mapping quality > 30, 11 million SNP were detected. In the Pence of genome maps in duck, the chicken was chosen to guide the choice of markers along chromosomes. Amongst the 10 million duck SNP mapped on the chicken genome, 500 000 (5%) are heterozygote in at least 6 of the 7 sires and 350 000 (3.5%) in all 7 sires. The choice of a final set of 384 markers for genotyping with the Illumina VeraCode technology was by: (1) maximization of the informativity in the sires; (2) no other SNP in the vincinity that could affect genotyping quality; (3) optimization of the genome coverage in cM, taking into account the differences in recombination rates between macrochromosomes and microchromosomes. The new genetic map now includes 311 SNP and 82 microsatellite markers, arranged in 28 linkage groups. It has been used to detect phenotypic QTL related to products quality and pQTL corresponding to the variation in the abundance

of proteins estimated by 2D gel electrophoresis.

P1053 Computational tools to exploit cattle exomes. Nádia Pinto, Shanyuan Chen and Lucía Pérez-Pardal (CIBIO), Félix Goyache (SERIDA) and Albano Beja-Pereira (CIBIO)

Next Generation Sequencing is rapidly becoming the tool of choice for research on animal genetics due to the huge amount of data provided. Indeed, in many cases, such method allows the identification of genome regions that have played a major role in the genetic make-up of individuals as part of the evolutionary process that lead into the adaptation of those organisms to extreme environments. Nevertheless, such extent of information makes the statistical analysis of the data a complex task since the tremendous number of variants lead, if the problem were crudely algorithmized, to the collapse of the informatics program after the study of few markers. In this work we are developing computational tools, resorting to Matlab language, to identify and compare phenotype relevant variants from the whole exomes of three main domestic cattle lineages (Indian zebu, African taurine and European taurine) and other extant bovine wild species. Our main goal is to be able to automatize the identification of the genome regions with greater evolutionary differences between these taxa, along with the proportion of divergences between them. Such statistics will give insights on the history of divergence between the different cattle lineages, as well as on the identification of adaptive and selective pressures suffered across their evolutionary history.

P1054 Linkage analysis to improve the chicken genome assembly. Xiaorong Gu (State Key Laboratory for Agro-Biotechnology, China Agricultural University), John Garbe (Minnesota Supercomputing Institute, University of Minnesota), Chenglong Luo (Institute of Animal Science, Guangdong Academy of Agricultural Sciences; State Key Laboratory of Livestock and Poultry Breeding), Zheya Sheng (State Key Laboratory for Agro-Biotechnology, China Agricultural University), Hao Qu (Institute of Animal Science, Guangdong Academy of Agricultural Sciences; State Key Laboratory of Livestock and Poultry Breeding), Xiquan Zhang (Guangdong Provincial Key Laboratory of Agro-Animal Genomics and Molecular Breeding), Dingming Shu (Institute of Animal Science, Guangdong Academy of Agricultural Sciences; State Key Laboratory of Livestock and Poultry Breeding), Ning Li (State Key Laboratory for Agro-Biotechnology, China Agricultural University), Yang Da (Department of Animal Science, University of Minnesota) and Xiaoxiang Hu (State Key Laboratory for Agro-Biotechnology, China Agricultural University)

Linkage analysis of the chicken 60K SNP data was conducted using 1279 F2 chickens from three F2 populations to map SNP markers with unknown positions, and to detect and correct SNP assembly errors. A total of 1254 SNP markers with unknown chromosome positions were mapped with LOD scores in the range of 10-388. All autosomes and two linkage groups had significant linkages with previously unmapped markers. The number of newly mapped markers ranged from 2 for Chr12 to 92 for Chr02. In many cases several previously unmapped markers had no recombination with a mapped marker. These tightly linked markers may need increased sample sizes to be separated. A potential assembly error on Chr01 was confirmed to be a true error.

P1055 Transcriptomic analysis of lung tissues in ducks highlights networks related to anti-avian H5N1 influenza A virus. Yinhua Huang (State Key Laboratory for Agribiotechnology, China Agricultural University, Beijing, China), Kang Yi (BGI-Shenzhen, Shenzhen, China), Hualan Chen

(National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Harbin, China), Xiaojuan Liu (State Key Laboratory for Agribiotechnology, China Agricultural University, Beijing, China), Huapeng Feng (National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Harbin, China), Jianwen Li (BGI-Shenzhen, Shenzhen, China) and Ning Li (State Key Laboratory for Agribiotechnology, China Agricultural University, Beijing, China)

We previously presented the duck genome sequence and performed deep transcriptome analyses to investigate immune-related genes. Herein, we compared global gene expression profiles of six lung transcriptomes from duck infected with a highly pathogenic (A duck/Hubei/49/05) or a weakly pathogenic (A/goose/Hubei/65/05) H5N1 virus to the corresponding from control animals (Huang et al., 2013). This analysis identified 5489 differentially expressed genes (DEGs) (FDR $\leq$ 0.001, fold change $\geq$ 2). We then constructed a gene co-expression network using these 5489 DEGs and identified 11 modules of varying sizes, from 65 to 1789 genes. The different modules are color-coded with 11 colors for presentation purposes. After that, we counted correlation and p values between these 11 modules and virus titers in brains, lungs, tracheas and duogenums of ducks used to perform the above transcriptomic analysis. Of these modules, the brown one including 753 genes showed strong negative correlation to virus titer in duogenums (r = -0.88, p value = 0.02) and lungs (r = -0.65, p value = 0.2). Detailed analysis found that the brown module included more than seven important immune genes (CXCL13, A2M, CD274, CCR2, MX1, RIG-I and TLR4) related to anti-influenza A virus, further supporting its critical role in host immune response to avian influenza A virus. Similarly, the red module including 274 genes was strong negative associated with virus titers in trachea (r = -0.74, p value = 0.09) and duogenum (r = -0.71, p value = 0.1). Moreover, the pink module including 176 genes was highly correlated to virus titers in all four tissues with r values ranging from 0.72 to 0.9. These analyses showed an example to characterize important networks and unravel biological modules of immune genes involving host immune response to viruses, such as avian influenza virus, using deep transcritpomic data.

P1056 Sex realted genes expression pattern in yellow catfish (Pelteobagrus fulvidraco) revealed by gonad transcriptome sequencing. Jianguo Lu, Peixian Luan, Xiaofeng Zhang, Shuqun Xue and Xiaowen Sun (Heilongjiang River Fisheries Research Institute)

Yellow catfish (P. fulvidraco) has been recognized as a vital freshwater aquaculture species in Southeast Asia. Genetically, yellow catfish harbors an XX/XY system. It is reported that males of yellow catfish can be up to 30-50% larger than female siblings under the same breeding condition in the first year, and one to two times larger than females in the second year. According to these specific sex-related growth phenomena, four cDNA libraries derived from XX and XY gonads in one year juveniles and two year adults were sequenced by using Illumina HiSeq2000 technology aiming to better understand the profiling of the transcriptome of yellow catfish. A total of 211,701,078 paired-end reads of 100 bp were generated. The raw transcriptome sequences in this study have been uploaded in the NCBI SRA site, the accession number is SRR1103702. Then those low-quality sequences (Phred quality score < 30) and ambiguous nucleotides were removed. The remaining high-quality reads, 193,477,064 (91.4%), were obtained for transcriptome assembly and analysis.

Of the 21,869 genes from yellow catfish transcriptome, 9,919 were found to be co-expressed in both XX and XY gonads, and 51 and 229 genes were detected to be expressed exclusively in XX and XY gonads in one year juveniles, respectively (As showed in Figure 1). For two year old adults, 7,911 genes were found to be co-expressed in both XX and XY gonads, and 40 and 1,188 genes were detected to be expressed exclusively in XX and XY gonads, respectively.The transcriptome of yellow catfish gonad was first sequenced, assembled and characterized; it provides lots of valuable genomic resource for better understanding of yellow catfish sex determination and differentiation issues. Several important sex-biased genes, which were candidate genes may involve in yellow catfish gonadogenesis, testicular/ovarian determination, and possibly sex determination.

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P2001 Culture condition is one determinant of the pluripotent state in porcine induced pluripotent stem cells. Wei Zhang, Yangli Pei, Liang Zhong and Jianyong Han (State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University)

The domestic pig is an ideal animal model for human disease because of its appropriate organ size, physiology and life span. Despite no robust porcine embryonic stem cell lines were established yet, porcine induced pluripotent stem (piPS) cells should offer an alternative resource for farm reproduction and clinical application. Since the first piPS cell line was established in 2009, the piPS cells produced in various laboratories exhibit different characteristics and quality. The aim of our experiment was to explore suitable medium for obtaining porcine naïve pluripotent stem cells. Here, We reproted that two types of piPS cell lines which we generated by four Yamanaka's factors transduction exhibit different pluripotent states maintaining in different culture conditions, one of which was similar to human embryonic stem cells (hpiPSCs) morphologically, and the other was much more closed to mouse ES cells (mpiPSCs). We analyzed the transcriptional profiling of the two types of cell lines using Affymetrix GeneChip Porcine Genome Array. We found that the TGF-beta signaling pathway was signifcantlly activated in both two piPS cell lines which indicated that the pluripotency network in pig was different from human and mouse. Some pluripotent markers, such as *GATA6*, which was expressed in the early inner cell mass, were upregulated in the mpiPSCs but not in hpiPSCs. By changing culture condition, the hpiPSCs can be converted into mouse ES cell-like state. We concluded that culture condition is one determinant of the pluripotent state of piPS cells. These evidences help us profoundly understand the pluripotency network in piPS cells and may faciliate derivation of the *bona fide* porcine embryonic stem cells.

P2002 The regulatory effects of H3K27me3 on bovine mastitis susceptibility and resistance to *Staphylococcus aureus*. Minyan Song (China Agricultural University) and Yanghua He (University of Maryland)

With unprecedented biotechnology development, epigenetic modification is considered to be a flexible and substantial regulatory factor to functional traits. Tri-methylation of lysine 27 on histone H3 (H3K27me3), an important epigenetic mark, normally represses gene expression in mammals. Bovine mastitis has cost tremendous economic losses in modern dairy industry. *Staphylococcus aureus* (*S. aureus*) is one of the most frequently isolated pathogens caused subclinical, clinical and chronic mastitis of dairy cows. Previously, we reported the blueprint of bovine H3K27me3 marks in Holstein lymphocytes and disclosed that H3K27me3 played significantly repression roles mainly around up 2Kb of the transcription start site of the genes. Here, we further analyzed and compared the genome-wide regulatory effects of H3K27me3 modification on genes expressions in lymphocytes between *S. aureus* mastitic cows and healthy controls. We found that the significantly differentially expressed genes are mainly associated with immune and disease-related processes in healthy controls, suggesting that these genes could be candidate resistant genes to *S. aureus* mastitis. Moreover, the expression of eight genes (*e.g. TPM*) was down-regulated by H3K27me3 in *S. aureus*mastitic cows which could be *S. aureus* susceptible-related genes. Overall, for the first time we studied H3K27me3 modification differences and its regulatory effects on genes expression between *S. aureus* mastitic resistant and susceptible cows, the down-regulated genes and their H3K27me3 modifications could be potential genetic and epigenetic markers resistant to *S. aureus* mastitis.

P2003 Searching for an epigenetic cause of sheep muscle hypertrophy. Christine Couldrey, Rudiger Brauning, Paul Maclean, Harold Henderson and John McEwan (AgResearch)

DNA methylation plays a central role in regulating many aspects of growth and development in mammals through regulation of gene expression. Next generation sequencing technologies now allow genome-wide, high resolution analysis of DNA methylation using methodology known as reduced representation bisulfite sequencing (RRBS). While RRBS has proven to be effective in understanding DNA methylation landscapes in humans, mice, and rats, to date, few studies have utilised this technology for investigating DNA methylation in agricultural animals. Here we describe the utilisation of RRBS to investigate DNA methylation in sheep *Longissimus dorsi* muscle. The Carwell phenotype, used as a proof of principle in these studies, is a desirable muscular hypertrophy inherited in an imprinted manner, for which, in spite of considerable resequencing efforts, the causative mutation has not been identified. RRBS analysis of *Longissimus dorsi* muscles provided data of suitably high quality for DNA methylation analysis at all levels of resolution from genome-wide to individual nucleotides. Combining RRBS data with mRNAseq data allowed the sheep *Longissimus dorsi* muscle methylome to be compared with methylomes from other species. Many similarities were observed between DNA methylation patterns in sheep and other species. Single nucleotide level analysis revealed significantly different DNA methylation levels at a small number of CpG sites in the region known to harbour the causative (epi)mutation of the Carwell pheontype. Differences in methylation were examined for their potential to be the causative epimutation. Taken together, the RRBS data presented here highlight the complexity of epigenetic regulation. However, similarities observed across species are promising, in that knowledge gained from studies in humans and mice may be applied, with caution, to agricultural species. Accurate measurement of DNA methylation in agricultural animals will contribute an additional layer of information to the genetic analyses currently being used to maximise production gains in these species.

P2004 The analysis of global methylation levels and DNA methltransferases experssion in chickens. Qian Zhang, Hao Zhang and Wenyu Gou (College of Animal Science and Technology, China Agricultural University)

Epigenetics is defined as the study of mitotically and/or meiotically heritable changes in gene function independent of changes of the DNA sequence. One way of adapting to hypoxia in is to regulate the expression of downstream genes through changing the epigenetic modifications. DNA methylation plays an important role in regulating genes expression. DNA methylation patterns can be established through the activities of DNA methytransferases(DNMTs).

The experiment eggs were collected from 2 popolations raising at highland, Tibetan chicken(TC) and Lhasa White chicken(LWC); and 2 populations raising at lowland, Chahua chicken(CC) and Tibetan chicken at Beijing(BTC). The eggs were incubated in hypoxia. Chicken chorioallantoic membranes(CAM) were collected on the 16th day of incubation(6 individuals each group) for preparation. DNA methylation levels were detected by Methylated DNA Quantification kit and mRNA expression of DNMTs were quantified with Q-PCR.

Significant higher levels of DNA methylation were observed in TC and LWC than lowland population ($P<0.05$). Increased levels of DNA methylation patterns have relationships with maintaining the stability of DNA structure in hypoxia and hypermethylation can conceal

related binding sites to down regulate expression of targeting genes and keep the balance of cellular enviroment.

Results of the Q-PCR indicated significantly increased expression of DNMT1 in TC compared with BTC($P<0.05$), and there were no distinctions among others($P>0.05$). Significant mRNA expression increase of DNMT3a was noticed in TC compared wih others($P<0.05$). TC had significantly lower DNMT3b than others($P<0.05$). And the mRNA expression of DNMT3b in BTC is significantly higher than CC($P<0.05$), but no significant differences was observed between BTC and LWC($P>0.05$). The results suggested the interaction between the two de no DNMTS(DNMT3a and DNMT3b) may play a role in establishing global hypermethylation in TC. The epigenetic modifications of BTC make great changes after migration, perhaps it can be ascribed to the loss of DNA methylation during adaptation to normoxia.

P2005 The differential expression analysis in Myostatin peptides transgenic and control mice. yuanxin miao (huazhong agricultural university)

Myostatin (MSTN) is a member of the transforming growth factor–β superfamily, which plays negative roles in muscle growth. Myostatin gene is specific expressed in the skeletal muscle tissue. Mutant or inhibition of MSTN can make a dramatic increase of muscle mass in many kinds of mammal species. By far, the molecular mechanism of this process is still not completely understood. In this study, we chose a muscle hypertrophy mouse as model, which was generated by transgenic of the N-terminal peptides of MSTN drived by the skeletal muscle specific MCK promoter. We compared analyzed the mRNA profiles of the transgenic mice and their littermates without gene modified using high-throughput RNA-sequencing method. According to our results, 120 genes were significant different expressed between the transgenic mouse and its control. Among them, 90 genes were up-regulated in the hypertrophy muscle tissue, and 30 genes were down-regulated. Functional analysis results indicated that the different expressed genes were related closely to the signaling pathways of Hypertrophic cardiomyopathy, Dilated cardiomypathy and Focal adhesion. Therefore, our results offered new data for elucidating the molecular mechanism of the muscle hypertrophy caused by inhibition of the MSTN protein.

P2006 A possible method of *Xist* gene regulation and its impact on early embryonic development of *bovine* SCNT. Liming Liu and Jindun Zhang (Inner Mongolia University), Wei Sun (Inner Mongolia University and Inner Mongolia Saikexing Reproductive Biotechnology Co.,Ltd), Lixia Zhao (Inner Mongolia Saikexing Reproductive Biotechnology Co.,Ltd) and Xihe Li (Inner Mongolia University and Inner Mongolia Saikexing Reproductive Biotechnology Co.,Ltd)

Xist is an X-linked gene that produces a noncoding RNA, and it is one of the first imprinted genes to be expressed in the early embryo with expression beginning at zygotic genome activation. This experiment investigates in feasibility of *Xist* suppressor vector TALER transfected into embryonic fibroblast of fetal *calf* to control genetic expression of *Xist* which mCherry is used as reporter gene of carrier transfection efficiency and also the impact of *Xist* gene on early development of cloned cattle embryo by appling this transgenic cells. The results are as follow: 1. The result of relative expression level of the treatment group has been reduced by 93.85% of *Xist* gene expression compared with that of control group, indicating the carrier transfection system designed in this experiment can effectively suppress genetic expression of *Xist*. 2. The positive of *Xist*

expression suppression are selected for SCNT, and untreated cell is used for comparison. The result of SCNT shows rates of 8-cells, morula and blastocyst of treatment group vs. control group are 78.8% vs 75.1% (P>0.05), 54.4% vs. 50.6% (P>0.05), 12.3% vs. 27.8% (P<0.01) and 0 vs. 26.6% (P<0.01). The above results show that TALER-mCherry vectors of *Xist* gene repressor in female fetal bovine fibroblast cell can suppression of *Xist*gene expression and also promote the 2-8 cell embryonic development of cloned embryo, but they does not effect morula/ blastocyst development following SCNT. Therefore, its mechanism of the regulation of *Xist* gene expression on early embryonic development of cloned embryos needs further investigation. Keywords: *Xist* gene, Taler plasmid, Embryonic fibroblast, Early embryonic development, SCNT

P2007 Novel and differentially expressed miRNAs in Staphyloccucus aureus infected mammary gland in Holstein cows. Jing An, Lingzhao Fang, Xiao Wang, Chao Liu, Yachun Wang, Shengli Zhang, Yuan Zhang and Ying Yu (Department of Animal Genetics and Breeding, China Agricultural University)

Novel and differentially expressed miRNAs in Staphyloccucus aureus infected mammary gland in Holstein cows Jing An#, Lingzhao Fang#, Xiao Wang, Chao Liu, Yachun Wang, Shengli Zhang, Yuan Zhang, Ying Yu*Department of Animal Genetics and Breeding, China Agricultural University, 100193, Beijing, China*Corresponding author: yuying@cau.edu.cn Subclinical mastitis is the most frequent and costly disease of dairy cattle because it causes long-term reduction in milk yield and is a hidden source of infection. Staphyloccucus aureus（S. aureus) is a most common source of subclinical mastitis and its treatment with antibiotics is often ineffective. MicroRNAs (miRNAs) are small non-coding RNAs (~22nt), which post-transcriptionally regulate gene expression and are known to be critical regulators of epithelial immune responses. However, the role of miRNAs in bovine responses to S. aureus is still largely unknown. To study the microRNAs involved in the mechanism of S. aureus caused mastitis, we identified novel and differentially expressed miRNAs in S. aureus infected mammary gland of Holsteins via Solexa sequencing and bioinformatics. The present study designed three groups including control group, the low concentration S. aureus infection group, and the high concentration one. In the three groups, 220, 467 and 495 conserved miRNAs were discovered, including 2, 8 and 13 novel miRNAs, respectively. Amongst the three groups, the common conserved miRNAs were 206, whereas, unique miRNAs were 4, 158 and 184, and the novels were none, 6 and 11, respectively. Total 209 unique miRNAs were found in low concentration group compared to the control group, whereas 212 miRNAs were revealed in high concentration group compare to the control group. Moreover, miRNAs annotation indicated that miRNA-155 (a regulator of immune cell maturation and innate immune response) and miRNA-181a (a regulator of lymphocyte differentiation) were found in the infected mammary gland tissue. In conclusion, the results imply that the miRNAs could play important roles in mastitis susceptibility to S. aureus.Founded by NSFC (31272420).

P2008 Promoter CpG methylation status in porcine *RTL1* is associated with its expression level. Mu Qiao, Huayu Wu and Shuqi Mei (Hubei Key Laboratory of Animal Embryo Engineering and Molecular Breeding, Institute of Animal Husbandry and Veterinary, Hubei Academy of Agricultural Sciences, Wuhan 430064, China)

RTL1 (retrotransposon-like 1) gene has been identified as imprinted gene in the human and mouse. It plays essential roles in maintenance of

the fetal capillaries, and it is required for muscle hypertrophy in callipyge sheep. The imprinting status and mechanisms that regulate the porcine *RTL1* transcription are poorly understood. Here, we isolated the porcine *RTL1* gene and detected the imprinting status in prenatal porcine tissues. The results indicate that *RTL1* was imprinted in all the tested tissues. Moreover, we first identified porcine *RTL1* gene core promoter region (-940/+3) using luciferase reporter assay system and found that promoter activities were significantly higher in the mouse myoblast C2C12 cells than that in the porcine fibroblast cells, implying that *RTL1*promoter could possess muscle-specific characteristics. Using quantitative real-time PCR (qRT-PCR), we detected the expression pattern of *RTL1* in porcine muscle tissues on two different developmental stages, the result showed that the expression level of porcine *RTL1* gene was significant higher in fetal 65 days than in fetal 30 days ($P < 0.01$). In addition, we analyzed the DNA methylation status of *RTL1* promoter using bisulfite sequencing polymerase chain reaction (BSP), and found that the DNA methylation status was significant lower in fetal 65 days than in fetal 30 days ($P < 0.01$). These results indicate that *RTL1* expression levels were negatively associated with the methylation status of the *RTL1* promoter. Our study identify the relation between epigenetic modifications and gene expression changes in pig, which possibly contributed to pig breeding and genetics.

P2009 X Chromosome Inactivation in Porcine Embryo Obtained *in Vitro*. huiying zou and dawei yu (China Agricultural University)

XIST plays an important role in triggering X-inactivation in eutherians. Impeding ectopic *XIST* expression from the active X chromosome can significantly improve mouse cloning efficiency, which may provide an efficient method for the production of SCNT agricultural animals. But the lack of data about *XIST* has limited our understanding of porcine XCI. Currently, we have successfully indentified the porcine *XIST* gene, encoding a 25,065-bp transcript consisting of 7 exons. Next we found an *XIST* SNP between two different breeds: Duroc and Rongchang, then we can distinguish the two *XIST* alleles from each other in female donor nucleus from an intercross. 25% female SCNT embryos(E7) analyzed showed biallelic *XIST* expression in porcine(100% in female mouse clones). It was further confirmed by Immunostaining for the nuclear H3K27me3 patch. Two Xi-like H3K27me3 domains appeared in 30% female SCNT embryos(E7). Although relative low *XIST* expression can be detected in most male porcine clones, only 20% of them colocalized one H3K27me3 domain. Then we investigated the H3K27me3 histone pattern during early development of porcine embryos obtained after in vitro Fertilization(IVF), SCNT and parthenogenetic embryos. No visible Xi-like H3K27me3 domains was displayed in any morula and early blastocysts(E4), indicated that XCI may not be initiated at this stage while XCI occurs in all cells of early mouse blastocysts. Even in post hatching female IVF and SCNT blastocysts(E8), blastomeres with strong punctate signals of H3K27me3 with a variable frequency from 40% to 85%, indicating the first round of XCI may not be completely established in porcine embryos until E9. The visible Xi-like H3K27me3 domains in all parthenogenetic blastocysts(E8)may suggest that an non-imprinted mechanism of Xi takes place in porcine embryos. Thus our finding suggested differences exist between porcine and mouse involved in XCI regulation, the mouse has its limitations as a model to understand early porcine XCI.

P2010 Histological analysis of testis development and spermatogenesis block in the mule (*Equus ferus* × *asinus*). Hongmei Han (Research Center for Animal Genetic Resources

of Mongolian Plateau, Collage of life Sciences, Inner Mongolia University)

Most mules, the hybrid of horse and donkey are infertile, only very few of their female were reported to be fertile. The main reason of the mule sterility is concerning that due to the odd number of chromosomes which result in the meiosis block and could not form normal gametogenesis, however, experimental evidence has not yet been found at present. In this study, testis development and spermatogenetic block were investigated in male mule which compared with male horse and donkey, following by HE and immunofluorescent staining technology in paraffin section. We hope these results could provide some basic information for the mechanism of reproductive regulation in mules.
HE staining results show that the mule testis has few blood vessels, a large number of white connective tissues, lower number of seminiferous tubules and germ cells compared with horse and donkey. Both of mules and horse or donkey have normal Sertoli cells in their testis. Immunofluorescent staining shows that the average number of spermatogonia and spermatocyte in per seminiferous tubule in the four mules is 13.5, while the number of donkey and horse are 50.6 and 43.5, respectively. In addition, the average number of SCP3-positive cells in seminiferous tubules in four mules is 4.9, significantly lower than 17.5 in horse and 12.5 in donkey. There is not any spermatozoon found in the seminiferous tubules in all the four mules which compared with 49.7 in horse and 47.4 in donkey. In summary, we hypothesize that the stage of spermatogenesis block in mules is meiosis division, however, very few spermatogonium of them can enter the first meiosis, but could not enter the second meiosis to form the spermatids and sperm. To understanding the mechanism of reproductive block in mules need further genetic analysis.

P2011 The derivation of trophoblastic cell lines from porcine early embryos. dongxia hou (The Key Laboratory of National Education Ministry for Mammalian Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot, Inner Mongolia, China), Rongfeng Li (The Center of Metabolic Disease Research Nanjing Medical University, Nanjing) and Xueling Li (The Key Laboratory of National Education Ministry for Mammalian Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot, Inner Mongolia, China)

The trophoblast is the first to differentiate during mammalian embryogenesis and play a pivotal role in the development of the placenta. The trophoblasts will differentiate into partial placental tissues during development; therefor these cells can be used to investigate trophectoderm differentiation and placental development *in vitro* or *in vivo*. The mouse and human trophoblast stem cells have been well studied, but the reports on porcine trophoblast stem cells or trophoblast cells is really rare. In this article, the putative porcine trophoblast cells were derived from pig parthenogenetic activation (PA) blastocysts or in vitro fertilization (IVF) blastocysts and cultured in the medium supplement with bFGF on STO feeder layers. The cells exhibited epithelium-like morphology with tight connection, contained abundant lipid droplets, and had an obvious boundary with the feeder cells. Multinuclear can be observed in some cells. The chromosome analysis showed that parthenogenetic trophoblast cells were diploid at the early stage of culture and doubled to tetraploid or became abnormal at later stage. The cells stained positive for alkaline phosphate by histochemical staining, and expressing of *SSEA1*, *CDX2*, *KRT7* and *KRT18* can be detected by immunofluorescence staining. RT-PCR analysis of the cells demonstrated expression of *TEAD4*, *CDX2*, *ELF5* and *HAND1*. Microarray analysis also showed the TSC and TR markers, such as *CYP11A1*, *PLAU*, *HAND1*, *PPARG*, *ID2*, *LEF1*, *IFNGR1* and *FGFR2*, highly expression in

trophoblast cells than the blastocysts. In conclusion, the porcine trophoblast cells can be isolated from either PA embryos or IVF embryos in bFGF supplemented medium. The cells exhibit characteristics similar with mTSCs and express several pluripotent markers and trophoblast specific makers.

P2012 Global Analysis of Epigenetic Differences Related to Porcine Obesity. Claus Jorgensen (University of Copenhagen)

M Jacobsen, S Pundhir, CM Junker, PM Sørensen, P Karlskov-Mortensen, CS Bruun, LJA Kogelman, S Cirera, C Anthon, J Gorodkin, S Pant, HN Kadarmideen, R Barres, M Fredholm and CB Jørgensen

Göttingen minipigs become obese when fed ad libitum, whereas production pigs stay lean, due to decades of effective selection for lean meat. We have generated a unique pig resource population designed with the purpose of studying the genetic and epigenetic component of obesity. This 3-generation inter-cross population consists of 563 animals generated by crossing minipigs with production pigs. All pigs in the pedigree were characterised with respect to 36 obesity traits (e.g. DEXA, slaughter values, growth, conformation traits), while one third were subjected to a glucose tolerance test. In addition 25 metabolic parameters have been measured on serum and plasma collected from the animals. Twenty-four pigs with extreme phenotypes (obese/lean and high and low glucose uptake) are currently undergoing a comprehensive investigation, where differences in methylation and gene expression levels (including expression of non-coding RNAs) will be related to the development of the obese and lean traits. Mature adipocytes and preadipocytes (CD31-CD34+ cells) were isolated from the visceral adipose tissue from the abdominal cavity. From the mature adipocytes both DNA and RNA were isolated and the global methylation pattern of the DNA was investigated by the two approaches; MethylMiner and mRRBS. Isolated RNA was subjected to RNA-seq and smallRNA-seq. Combined analysis of expression and methylation reveals differences in both methylation patterns and expression levels between the phenotypes, and some of these differences are coinciding in regions. These data will be presented.

P2013 The rules of cloned bovine embryos X chromosome inactivation and Xist expression. Zhengzhi Cui, Dawei Yu and Ning Li (China Agricultural University)

In mammals, one of the two X chromosomes is converted from an active euchromatic state into transcriptionally inert heterochromatin by a process known as X chromosome inactivation (XCI). The Xist gene is implicated in XCI. As we all know, cloning mammals by somatic cell nuclear transfer (SCNT) is highly inefficient. Most SCNT-generated embryos die after implantation because of unidentified, complex epigenetic errors in the process of postimplantation embryonic development. Previous studies have proved that Xist is ectopically expressed from the active X chromosome in cloned mouse preimplantation embryos of both sexs. Deletion of Xist on X active chromosome showed normal global gene expression and resulted in about an eight- to ninefold increase in cloning efficiency. Here we report that whether the same phenomenon occurs in cloned bovine preimplantation embryos. In this study, RNA FISH analysis revealed that Xist was only expressed in the female in vitro fertilization (IVF) embryos. Nevertheless, in male clones, Xist was abnormally activated. In addition, the expression level of Xist in female clones was much higher than IVF embryos. But only 25% female SCNT embryos (E8) analyzed showed biallelic XIST expression (100% in female mouse clones). Blastomeres with two signals of XIST with a frequency from 1% to 5%,

indicating much lesser extent of ectopical expression of XIST in bovine SCNT embryos than that in mouse SCNT embryos. The Immunostaining for the nuclear H3K27me3 patch did not show two Xi-like H3K27me3 domains in female bovine SCNT embryos at this stage. In female IVF embryos visible Xi-like H3K27me3 domains was not displayed untile E8, indicating the first round of XCI is just started at this stage. The different phenomenon between cloned bovine embryos and cloned mouse embryos may due to the different XCI pattern.

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P3001 Induction of *ovine* primary myoblasts differentiation and proliferation by *AAV*-mediated follistatin overexpression. mahmood nazari, Fatemeh Salabi, Li Zhang, Fuping Zhao, Caihong wei and Lixin Du (Institute of Animal Sciences, Chinese Academy of Agricultural Sciences)

Follistatin (FST) has been shown to bind some *TGF*-β family members and can function as a potent myostatin *(MSTN)* antagonist. Recently, studies have revealed that overe-xpression of *FST* by adeno-associated virus increase muscle growth in vivo. In the present study, to explore the effects of *FST* over-expression on ovine primary myoblasts *(OPM)* proliferation and differentiation, *FST* was over-expressed by adeno-associated virus serotype 2 *(AAV* 2) vector in growth media (GM) and differentiation media (DM). Real-time quantitative PCR analysis indicated that over-expression of FST resulted in a dramatic increase of *Akt I* and *CDK2* expression, and a decrease of *p21* expression but had no effect on *p57* mRNA expression in proliferating conditions. Also, over-expression of*FST* contributed to an increase of *Myogenin, Myo D*, *Myf5*, p57 and *p21* mRNA expression and had no effect on *MSTN* and *ActRIIB* mRNA expression in differentiation conditions. Moreover, cell cycle analysis confirmed that *FST down-regulated p21*, a *CDK*inhibitor, and increased the levels of *CDK2* expression in OPM cells. Hence, *FST* positively regulated the G1 to S progression. These results confirmed that *FST* induce proliferation through down-regulation of p21, because only *p21* levels are down-regulated as a result of *FST* over-expression in myoblasts, whereas no change was observed in the levels of the p57 expression. In conclusion, our results provided the first evidence that the *AAV* viral system can use to gene transfer in sheep. Moreover, the results showed that*AAV* vector can successfully express *FST* in OPM cells in vitro. These findings demonstrated that *FST* induces proliferation through down-regulation *of p21* gene under proliferating conditions in ovine primary myoblasts and promotes differentiation through up-regulation of *Myogenin, Myo D* and *p21* in differentiation conditions by blocking the *MSTN* signal.

P3002 Studying growth and fatness regulation through hypothalamic and hepatic transcriptome analyses in a porcine experimental backcross. Ana Fernández, Dafne Pérez-Montarelo, Ángel Martínez-Montes, Estefania Alves and Carmen Rodríguez (Instituto Nacional de Investigación y tecnología Agraria y Alimentaria), Jose Luis Noguera (IRTA), Josep Maria Folch (UAB, CRAG) and Almudena Fernández (Instituto Nacional de Investigación y tecnología Agraria y Alimentaria)

Growth and fatness are economically important traits for pork production. Furthermore, the pig is used as a biomedical model for studying human obesity, energy metabolism and diabetes. The aim of the current study was to identify genes and gene networks involved in porcine growth and fatness traits by analyzing the transcriptome with RNA-Seq technology of the main tissues responsible for growth, food intake and fat accumulation regulation, hypothalamus and liver. Divergent animals for growth and fatness traits were selected from an F1 (Iberian x Landrace) x Landrace backcross, using a principal component analysis. Four males of each extreme were selected from each divergent group. Hypothalamic and hepatic RNA samples were sequenced with Illumina Hi-Seq 2000 equipment, and analyzed using CLC Genomics Workbench software. Raw data was trimmed, filtered following standard criteria and mapped against the Sscrofa10.2 assembly. Differential expression analysis was performed assuming a Gaussian distribution of data. For each tissue, those genes with a mean expression > 0.5 FPKM, fold change between groups ≥ 1.5, and p-value and q-value ≤ 0.05 were considered as differentially expressed (DE). A total of 384 genes were identified as DE

in hypothalamus, 254 in liver, and four of them were common to both tissues. The most significant gene ontology enriched terms corresponded to hypoxia response in hypothalamus and carbohydrate metabolism in liver. In order to identify hypothalamus-liver gene networks, a co-expression analysis was conducted for the DE genes in both tissues, through correlation estimates. The results showed 246 significant correlations (q-value 0.97). Some interesting results corresponded to the co-expression between SOX12 transcription factor (DE in hypothalamus) and vitamin D receptor (DE in liver), involved in human obesity, and the CLK1 and CLK4 pre-mRNA processors (DE in hypothalamus) and the sugar transporter SLC37A3 (DE in liver), involved in tissue glucocorticoid production.

P3003 Myostatin knockout by zinc-finger nuclease mRNA stimulate ovine skeletal muscle satellite cells proliferation and differentiation. Fatemeh Salabi (Animal Science institute) and Mahmood Nazari and Wen G. Cao (animal science institute)

Myostatin *(MSTN)* has previously been shown to negatively regulate skeletal muscle satellite cell proliferation and differentiation. Satellite cells are quiescent muscle stem cells that promote muscle growth and repair. The aim of the current study was to investigate effect of *MSTN* knockout by using the zinc finger nucleases mRNA (*MSTN-KO ZFN* mRNA) on sheep primary satellite cells *(PSC)* differentiation and proliferation. *MSTN-KO ZFN* mRNA induced 5-bp deletion in *MSTN* gene, which leads to frame shift mutation. *ZFN* mRNA and donor vector were cotransfected into sheep *PSC* and obtained mutant-type cell clones by flow cytometry. To assess *MSTN* protein levels, Western blot was performed on Mutant and Wild-type *PSC* clones. Western blot results showed that *MSTN* protein expression was down-regulated in Mutant-type PSC clones. Real-time quantitative PCR results indicated that knock out of myostatin contributed to an increase of *CDK2, FST, IGF-I* and *Pax* 7, and a decrease of *p21* mRNA expression in proliferation condition. In addition, *MSTN* knockout increased *Myo D, Myf 5, Myogenin* and *p21* expression at the transcript level in differentiation condition. Cell proliferation assay showed that the proliferation of Mutant-type PSC clone was significantly greater than wild-type PSC cell clone ($P<0.01$). Giemsa staining revealed that Fusion percentage in Mutant-type *PSC* clone was 60.21%, which was significantly greater than the 43.05% observed with Wild-type PSC clone ($P<0.01$). These findings suggest that knockout of *MSTN* in Mutant PSC cultured resulted in more nuclei contributing to myotube formation than Wild PSC clone in differentiation condition. These results suggested that *MSTN* is a potent negative regulator of satellite cell differentiation and proliferation. Furthermore, *ZFNs* system inactivated *MSTN* gene in primary satellites could be used to generate transgenic sheep in future.

P3004 MiR-195/497 inhibit myoblast proliferation through targeting *Igf1r/Insr* and cyclin related genes and are negatively regulated by NK-κB. Wei Wei (Key Laboratory of Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University) and Jian-Bo Bai, Wei-Ya Zhang, Hai-Xin Zhang, Yuan-Yuan Zhao, Xin-Yun Li and Shu-Hong Zhao (Key Laboratory of Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan 430070, PR China)

In order to identity key miRNAs during porcine skeletal muscle development, microarray was performed to get the differentially expressed miRNAs. Expression of miR-195 and miR-497 were increased significantly in adult porcine longissimus muscle compared to 33- and 65-day fetuses. In this study, the specific role of

miR-195/497 in skeletal muscle development was investigated. Firstly, Q-PCR was used to confirm the increased expression in both aging mice and differentiating myotubes. Then miR-195 and miR-497 were transfected into C2C12 cells and flow cytometry was applied to analyze the cell cycle. The results showed that more cells were arrested in G0/G1 stage and the proliferation index was decreased, in another words, it indicated that miR-195/497 could inhibit myoblasts proliferation. After target prediction, Luciferase activity assay, and Western blot validation, cyclins protein Ccnd2 and Ccne1 were finally identified as the new targets of miR-195/497 in myoblasts. Besides, cell growth related genes, Insr and Igf1r, were also confirmed as their targets. Next, immunofluorescence and Q-PCR were applied to analyze the myotube formation and myogenic marker gene expression to uncover the role of miR-195/497 during C2C12 cells differentiation, but no significant difference was detected when miR-195/497 was over-expressed. Moreover, transcription factors of miR-195/497 were analyzed and 3 binding sites of p65 (subunit of NK-κB) were predicted. Luciferase activity, EMSA, and CHIP-PCR were performed to prove that p65 did bind the promoter region of miR-195/497. The miR-195/497 promoter activity and their expression were raised when p65 was silent by siRNA or inhibitor, and were decreased when p65 was over-expressed by vector or activator. In conclusion, our results indicated that miR-195/497 could inhibit myoblasts proliferation by targeting cyclins and Insr/Igf1r, and were transcriptionally negatively regulated by NK-κB.

P3005 The immune responses of bovine monocyte-derived macrophages infected with *Staphylococcus aureus in vitro*. Anna Lewandowska-Sabat (Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences), Bjørg Heringstad (Department of Animal and Aquacultural Sciences, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences), Trygve Solberg (Geno Breeding and A.I. Association), Anne Storset (Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences) and Ingrid Olsaker (Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences)

Mastitis is the infectious disease of the mammary gland considered to be the most frequent and costly disease in dairy production. Understanding mechanisms of host immune response to infecting pathogens will facilitate breeding strategies for improved disease resistance. In the mammary gland, local recruitment and action of macrophages is a key immunological defense mechanism against infection. The transcript levels of several immune responsive genes in blood monocyte-derived macrophages challenged *in vitro*with *Staphylococcus aureus* was analyzed in Norwegian Red sires with high and low breeding values for clinical mastitis. The genes selected for this analysis were identified based on previous genome-wide association mapping of mastitis resistance in Norwegian Red cattle. The results show that the proinflammatory cytokine response to *S. aureus* infection is increased in sires with high breeding values compared to sires with low breeding values for mastitis resistance. Moreover, our previous analysis of genome-wide transcript profiles of blood monocyte-derived macrophages from Norwegian Red heifers showed that *in vitro* infection with *S. aureus* induced both classical and alternative macrophage activation pathways. This suggests that alternative activation of macrophages may be a mechanism contributing to intracellular persistence of *S. aureus* in the course of inflammation, potentially leading to subclinical mastitis. Further studies examining immune

responses of bovine macrophages to other mastitis causing pathogens will be performed using animals carrying different haplotypes of mastitis resistance quantitative trait loci (QTL).

P3006 Endometrial gene expression profile from pregnant sows with extreme phenotypes for reproductive capacity. Sarai Córdoba, Ingrid Balcells and Anna Castelló (Centre For Research in Agricultural Genomics), Cristina Ovilo (Nacional de Investigación y Tecnología Agraria y Alimentaria (SGIT-INIA)), Jose Luis Noguera (Institut de Recerca i Tecnologia Agroalimentàries (UdL-IRTA)) and Oriol Timoneda (Centre For Research in Agricultural Genomics)

Prolificacy is one of the most important traits in swine industry because of its positive impact on productivity. Reproductive success can be influenced by multiple factors including ovulation rate, uterine capacity and foetal survival rates. The availability of a pig genome sequence and development of high-throughput techniques such as RNAseq have increased the discovery of genes and non-coding regulators involved in relevant biological processes. However, mechanisms involved in pig litter size variation remain unknown. The aim of this study was to identify key differences in gene expression associated to swine reproductive efficiency. We performed a whole transcriptome analysis in 12 sows at day 30-32 of its gestation, resulting from an Iberian x Meishan F2 population. Individuals were classified according to its estimated breeding value (EBV) as High (EBV>0) and Low (EBV<0) prolificacy. Uterine endometrium was collected and RNA sequenced to profile the mRNAome and miRNAome using an Ion 318™ Chip (Ion Torrent PGM) for each library and sample (High n=6; Low n=6). Computational analysis of mRNA libraries evinced 66 differentially expressed (DE) genes. Subsequent Gene Ontology analysis pointed out 5 candidate genes that were further validated by RT-qPCR on the whole extreme F2 population (High n=16; Low n=20): *PTHLH* (Mammary gland development - $p<0.05$*; H/L ratio=3.69), *MMP8* (Embryonic development - $p<0.05$*; H/L ratio=4.41), *SCNN1G* (Response to hypoxia - $p<0.05$*; H/L ratio=3.42),*PTGS2* (Placental Development - $p<0.05$*; H/L ratio=3.50) and *HPGD* (Pregnancy - $p=0.118$; H/L ratio=1.69). Same approach was used on miRNA libraries identifying 14 DE miRNAs. RT-qPCR analysis revealed no significant differences on their expression levels, nevertheless, we identified a robust negative correlation between these miRNA and validated target genes expression levels. These correlations were statistically significant for miR-133a ($p<0.01$) and miR-92a ($p<0.01$). The identified and validated differentially expressed RNAs provide a list of powerful candidate genes that contribute to a better understanding of the genetic architecture of prolificacy-related traits.

P3007 Interaction between adipogenic regulator genes and dietary omega-3 polyunsaturated fatty acids in adipose tissues in lambs. Olaia Urrutia, Beatriz Soret, Kizkitza Insausti, Jose Mendizabal and Ana Arana (Universidad Pública de Navarra)

Adipose tissue (AT) quantity and fatty acid (FA) composition are important factors in terms of meat quality. Omega-3 polyunsaturated fatty acids (PUFA) have potential health benefits therefore there is interest in increasing PUFA in meat using sources rich in those FA. This work aimed to study the effect of linseed (rich in 18:3n-3) or algae (rich in EPA and DHA) supplementation on the expression of the central transcriptional regulators of adipogenesis and lipogenesis *PPARγ*, *CEBPα* and *SREPB-1c* in intramuscular (IM) and subcutaneous (SC) AT. Its effect on fat deposition, G3PDH enzyme activity, cellularity, and FA profile was also studied. Thirty one male Navarra breed lambs were assigned to three groups (isoproteic diets):

control, C (barley and soya); L (barley, soya and 10% linseed) and LA (barley, soya, 5% linseed and 3.7% algae). The results showed that although the contents of 18:1t11, 18:3n-3, EPA and 22:5n-3 in IM AT of L and LA groups increased ($P < 0.01$) there were not significant differences in IM fat content, which is consistent with no variations in adipocyte diameter and number, G3PDH activity (involved in triglyceride esterification) or *PPARγ*, *CEBPα* and *SREBP-1c* expression. On the contrary, in SC AT the expression of *PPARγ* and *CEBPα* were higher in L and LA than in C ($P < 0.05$) although there was no difference in *SREBP-1c*. There was as well an increase in G3PDH activity and adipocyte diameter was higher in L and LA lambs ($P < 0.05$). These results suggest that higher FA fluxes reaching SC tissue (stated by an increase in 18:1t11, 18:3n-3 and 22:5n-3) could enhance the lipogenesis process and triglyceride synthesis mediated by *PPARγ* and *CEBPα* whilst in IM AT this would not occur. To conclude, SC and IM AT may show adipogenic tissue-specific response to omega-3 PUFA supplementation by regulating the main transcription factors.

P3008 Bovine polledness – RNAseq based gene expression during fetal development. Natalie Wiedemar (Institute of Genetics, University of Bern, Bern, Switzerland), Dominique Wiener (Institute of Animal Pathology, University of Bern, Bern, Switzerland) and Réný Bruggmann (Interfaculty Bioinformatics Unit, University of Bern, Bern, Switzerland)

Polledness (absence of horns) in cattle is an autosomal dominantly inherited trait with allelic heterogeneity. So far two independent underlying causal structural DNA mutations located on BTA 01 have been identified, a complicated insertion-deletion in beef cattle (PC) and a haplotype of variants including an 80 kb duplication in polled Holstein cattle (PF).
As the known mutations take place in intergenic segments without any functional candidate genes in the flanking regions, molecular consequences of these mutations on horn growth remain unclear. Therefore we performed gene expression analysis, starting with RNA-sequencing of biopsies from horn-bud tissue of a wildtype horned and a homozygous polled foetus ~150 days post fertilization. This analysis revealed a large number of differentially expressed genes across the genome including a single long non-coding RNA (*LOC100848215*) located close to the identified mutations on BTA 01 and known in cow and buffalo only. At the conference we will present results of a currently ongoing comprehensive RNA-Seq experiment. Therefore, biopsies of horn-bud tissue and neighbouring frontal skin were taken from 24 foetuses between 65 and 160 days post fertilization divided into eight groups. In parallel, we used the material for a histological analysis of the microscopically visible structural changes during horn bud development. Each of the age-matched groups consists of one polled (PC) and two wildtype horned foetuses. Therefore, 48 total-RNA libraries were prepared and sequenced on an illumina HiSeq2500. We hope to further gain new insights into the molecular understanding of horn development.

P3009 Identification of the crucial developmental stage and cis-regulatory motifs for large-scale myoblast fusion in sheep. Caihong wei and lixin du (Institute of Animal Science) and Hangxing ren (Chongqing Academy of Animal Sciences)

It is well known that most of myofibers are formed before birth in sheep, however, the crucial myogenic stage and the cellular and molecular mechanisms underpinning phenotypic variation of fetal muscle development remain to be ascertained. We examined the developmental characteristics of fetal muscle at 70, 85, 100, 120, and 135 days of gestation in sheep using histological, microarray, and qRT-PCR methods.

We showed that day 100 was an important checkpoint for change of muscle transcriptome and histomorphology in fetal sheep, and that the period of 85-100d was the vital developmental stage for large-scale myoblast fusion. During this special developmental stage, cis-regulatory motifs for E2F1 and MEF2A were significantly overrepresented in decreasingly and increasingly expressed genes in microarray respectively. Further analysis demonstrates that the mRNA and phosphorylated protein levels of E2F1 and MEF2A significantly declined with myogenic progression in vivo and in vitro. qRT-PCR analysis indicates that PI3K or FST signal pathways may be involved in myoblast differentiation and fusion under control of E2F1 respectively, and that down-regulation of MEF2A contributes to transition of myofiber types by differential regulation of the target genes involved at the stage of 85-100d. We for the first time clarify the threshold for increase in myofiber size and number during gestation in sheep, which would be beneficial to meat sheep production. This study also presents a repertoires of gene expression in muscle during large-scale myoblast fusion at transcriptome-wide level, which contributes to elucidate the regulatory network of myogenic differentiation.

P3010 Characterization of 3' untranslated region (3' UTR) of *CAST* gene and its putative functional effect in beef tenderness.

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The system calpain-calpastatin (*CAPN1-CAST*) regulates post-mortem proteolysis and affects beef tenderness. Some SNPs in *CAST* gene have been associated with meat tenderness, including a SNP in 3'UTR region described by Barendse et al. (2002) (BTA7:g.98579663A>G on bovine genome assembly UMD 3.0). However the functional effect of this polymorphism has not been studied. The aim of this study was to search for polymorphisms with functional consequences in the 3' UTR region of *CAST* gene. Genomic DNA of the 749 bp fragment of the 3' UTR region from 49 animals with extreme tenderness estimated values and alternative genotypes for g.98579663A>G of two Spanish beef breeds (Parda de Montaña and Pirenaica) was amplified by polymerase chain reaction (PCR) for direct cycle sequencing. Eight polymorphisms were found in this region in both breeds. The majority of polymorphisms occurred as multiSNP combinations for individual subjects. Haplotype analysis identified 3 main haplotypes. *In silico*analysis using Microinspector software showed that six of them modify putative target sites of three bovine miRNA (2 for bta-miR-542-5p and 1 for bta-miR-488). The SNP g.98579663A>G modified one putative target site for bta-miR-542-5p. The multiple potential binding sites of miRNAs in large target RNAs and the energetically most favourable hybridisation site were predicted using RNAhybrid. The three main haplotypes had different minimum free energy for the different miRNAs, except for one of the bta-miR-542-5p. The sequences of the three main haplotypes were cloned into a luciferase reporter construct to evaluate the functional effect of the three haplotypes. The sequence that contains our haplotype of interest were cloned into pmirGLO (Promega) vector and confirmed by sequencing. For luciferase reporter assays, C2C12 cells, widely used as a skeletal muscle model, were transfected using JetPEI™. *Renilla* luciferase

was used as a normalization control. Reporter activity was detected 48 h after transfection with the Dual-Glo Luciferase Assay System (Promega).

P3011 Generation of bi-transgenic pigs overexpressing human lactoferrin and lysozyme proteins in milk. Dan Cui, Jia Li and Linlin Zhang (China Agriculture University), Shen Liu (GenProtein Biotech Ltd) and Ning Li (China Agriculture University)

Intensive swine production industry uses antibiotics to treat diseases and improve pig growth. This can not only cause antibiotic resistance, but can also pollute the environment or eventually affect human public health. To date, human lactoferrin (hLF) and human lysozyme (hLZ) have been known as non-adaptive but interactive antimicrobial members and could act in concert against bacteria, which contribute to host defense. Therefore, their expression in pigs might be an alternative strategy for replacing antibiotics in the pig production industry. In our study, we produced hLF and hLZ bi-transgenic pigs and assessed the milk's antibacterial ability. Integration of both transgenes was confirmed by PCR and southern blot. Both the hLF and hLZ were expressed in the mammary gland of bi-transgenic pigs, as detected by western blotting. The expression amounts were 6.5 g/L for hLF and 1.1 mg/L for hLZ using ELISA. Interestingly, pig milk containing hLF and hLZ had synergistic antimicrobial activity and growth improvement effect. Our results suggest an alternative approach for avoiding the use of antibiotics in the pig industry, which would be of great benefit to the commercial swine production.

P3012 Genome-wide expression profiling in muscle of lambs in response to the intake of concentrate supplemented with vitamin E or alfalfa grazing. Laura González Calvo (Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)), Roberto Martin Hernández (IMDEA-Alimentación), Margarita Joy (Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)), Malena Serrano (INIA), Jose Ordovás (IMDEA-Alimentación/) and Jorge Calvo (Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA))

Feeding animals with either concentrates supplemented with vitamin E or alfalfa grazing has been proven to reduce the oxidative process that occurs in meat products. Indoor lambs were fed a commercial concentrate (n=7, C) or concentrate supplemented with 480 mg of dl-α-tocopheryl acetate/kg dry matter (DM) (n=7, VE) for 30 days before slaughtering at 22–24 kg of live weight. Simultaneously, 7 unweaned lambs grazed in alfalfa paddocks (ALF, 154 mg α-tocopherol/kg DM) with their dams. Using global transcriptomic data of *longissimus thoracis* muscle with the Affymetrix® Ovine Gene 1.1 microarray, 312 genes were identified as differentially expressed in VE compared to C (155 up- and 157 down- regulated), whereas 426 genes were found to be differentially expressed in ALF compared to C (206 up- and 220 down-regulated) ($p<0.01$). These differentially expressed genes were selected for a functional analysis by using GeneCodis, a web-based tool for the ontological analysis of large lists of genes. Some of the identified significant biological processes were proteasomal ubiquitin-dependent protein catabolic process, collagen fibril organization, cellular response to oxygen levels and skeletal muscle cell differentiation in VE vs. C comparison; whereas terms as regulation of actin filament polymerization, skeletal muscle tissue development and fatty acid metabolic process were significant in ALF vs. C comparison. The KEGG terms revealed that pathways in cancer, focal adhesion, propanoate metabolism, protein processing in endoplasmic reticulum and regulation of actin cytoskeleton pathways were significantly altered in VE vs. C, and ALF vs. C comparisons. Pathways related to

infectious diseases were also found in ALF vs. C comparison: pathogenic Escherichia coli infection, bacterial invasion of epithelial cells and shigellosis. Further exploration of the links between these pathways and vitamin E will lead to a better understanding of how vitamin E affects the oxidative process that occurs in meat products.

P3013 Single-base resolution DNA methylomes revealed differentially methylated regions in two genetically distinct inbred chicken lines following avian influenza virus infection. Jinxiu Li (The State Key Laboratory for Agro-biotechnology, China Agricultural University), Rujiao Li (Beijing Institute of Genomics, Chinese Academy of Sciences), Ying Wang (Department of Animal Science, University of California, Davis), Xiaoxiang Hu and Li Li (The State Key Laboratory for Agro-biotechnology, China Agricultural University), Susan Lamont (Department of Animal Science, Iowa State University), Blanca Lupiani and Sanjay Reddy (Department of Pathobiology, Texas A&M University, College Station,), Huaijun Zhou (Department of Animal Science, University of California, Davis) and Ning Li (The State Key Laboratory for Agro-biotechnology, China Agricultural University)

Avian influenza virus (AIV) infection can not only cause significant economic losses to the poultry industry, but also raises a great public health threat to humans. DNA cytosine methylation is one of the epigenetic modifications that play important roles in diverse biological processes. To elucidate the epigenetic regulatory mechanism underlying disease resistance to AIV infection in chicken, two genetically distinct, highly inbred chicken lines, Leghorn (susceptible to AIV infection) and Fayoumi (relatively resistant to AIV infection) were challenged with a low-path H5N3. DNA isolated from chicken lungs at 4 days post-infection was used to perform the single-base resolution DNA methylome using whole-genome bisulfite sequencing (MethylC-seq), and RNA from the same tissue was used for RNA-seq. A total of 278.54 gigabases (Gb) of sequence were generated from eight samples of four groups (infected Leghorn, non-infected Leghorn, infected Fayoumi and non-infected Fayoumi). The methylation profile showed that chicken genome has a similar global methylation pattern to mammals. The results suggest that promoter hypo-methylation was crucial for regulation of gene expression in chickens. In addition, there were positive correlations between DNA methylation level and mRNA expression in gene-body regions for moderately expressed genes. Furthermore, we identified 1,360 line-specific methylation regions (lDMRs) and 188 infection-induced differentially methylated regions (iDMRs). The number of iDMRs-associated genes was greater in Fayoumi than in Leghorn, and 13/188 iDMR-associated genes were shared between two chicken lines. Our results suggest that DNA methylation was altered by virus infection. This work established a comprehensive and precise DNA methylation pattern in chickens and laid a solid foundation for future studies on epigenetic modification involved in AIV infection in chickens.

P3014 The transient expression of miR-203 and its inhibiting effects on skeletal muscle cell proliferation and differentiation. Wen Luo and Xiquan Zhang (South China Agricultural University)

Previous studies have shown that miR-203 is a skin-specific microRNA (miRNA) with a profound role in skin cell differentiation. However, emerging microarray and deep sequencing data revealed that miR-203 is also expressed in embryonic skeletal muscle and myoblasts. From the results of northern blotting and Q-PCR (quantitative polymerase chain reaction), we found that miR-203 was transiently

up-regulated in chicken embryos on days 10 to 16 (E10-E16) and was sharply down-regulated and even not expressed after E16 in chicken embryonic skeletal muscle. Histological profiles and weight variations of embryo skeletal muscle revealed that miR-203 expression is correlated with muscle development. *In vitro* experiments showed that miR-203 exhibited down-regulated expression during myoblast differentiation into myotubes. By used EdU staining, CCK-8 assay and cell cycle analysis, we found that miR-203 overexpression inhibited myoblast proliferation. And the results of the fusion index analysis and the expression of differentiation-related genes show that miR-203 overexpression can also inhibited myoblast differentiation, particularly repressing late-stage myotube formation. During myogenesis, miR-203 inhibited *c-JUN* and *MEF2C* expression at the mRNA and protein levels, respectively. Overexpression of *c-JUN* significantly promoted myoblast proliferation. Conversely, knockdown of *c-JUN* by siRNA suppressed myoblast proliferation. In addition, knockdown of *MEF2C* by siRNA significantly inhibited late-stage myotube formation. Altogether, these data not only suggested that the expression of miR-203 is transitory during chicken skeletal muscle development but also showed a novel role of miR-203 in inhibiting skeletal muscle cell proliferation and differentiation by repressing the mRNA and protein levels of *c-JUN* and *MEF2C*, respectively.

P3015 Transcriptome wide investigation of parent-of-origin expressed genes in mule (horse x donkey) by next-generation semiconductor-based sequencing. Francesca Bertolini (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna), Enrico D'Alessandro (Department of Veterinary Medicine, Division of Animal Production, University of Messina), Giuseppina Schiavo (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna), Ida Greco, Concetta Scimone and Marco Ghionda (Department of Veterinary Medicine, Division of Animal Production, University of Messina), Pier Martelli and Rita Casadio (Biocomputing Group, University of Bologna), Vincenzo Chiofalo (Department of Veterinary Medicine, Division of Animal Production, University of Messina) and Luca Fontanesi (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna)

High throughput transcriptome sequencing of hybrids could be used to discovery potential imprinted transcription in animals. The approach is based on detecting differential gene expression by counting RNA-seq reads of alternative alleles identified by heterozygous single nucleotide polymorphisms in the hybrids. The parental origin of the two alleles can be easily identified if the parents are homozygous for alternative forms. The mule is a hybrid between a donkey father and a horse mother that can be analysed at the transcriptome level to identify the origin of the transcripts from the two donor species, taking advantages from the differences of their genomes. In this study we investigated the mule blood transcriptome by next generation semiconductor-based sequencing. Four mules, two full-sibs and two half-sibs, generated by two different donkey fathers and three different horse mothers, were investigated. Genomic DNA of the parental donkeys and horses was sequenced using the Ion Proton sequencer, obtaining a coverage of ~5X (horses) and ~10X (donkeys). Whole blood samples of the mules was used to obtain mRNA that was sequenced using the same sequencing technology. A total of ~34 Gbp of RNA-seq data was generated. All obtained sequencing reads were aligned with the horse reference genome (EquCab2). Duplicate reads were eliminated and common variants were filtered considering a minimum coverage of 4X for the donkey genome sequences and 50X for the mule transcriptome reads. More than one

variable position in the same transcript was used to determine the origin of the reads. Only concordant results from all mule transcriptomes were filtered to identify putative imprinted alleles. Results indicated that fifty genes were expressed only from one of the two original alleles. According to these first results, the mule might constitute an interesting model to investigate the mechanisms and the effects of the parent-of-origin gene expression.

P3016 A complex Genomic Structural Variation On Gallus gallus Chromsome 27 is Associated with Beard in Chickens. Ying Guo, Xiaorong Gu, Zheya Sheng and Yanqiang Wang (CAU), Chenglong Luo, Hao Qu and Dingming shu (Institute of Animal Science, Guangdong Academy of Agricultural Sciences) and Yiqiang Zhao, Xiaoxiang Hu and Ning Li (CAU)

Beard, a common phenotype among chickens, is a group of elongated feathers that bunch below a bird's beak. To study the genetic basis and molecular processes of the trait, we generated an F2 intercross line using Huiyang Beard chicken and Lingnan yellow-feather broiler (non-Beard). In this research, we showed that Beard is a autosomal dominant trait and is caused by a complex structural variation (SV) on Gallus gallus chr27 using GWAS, linkage analysis, array-CGH and Resequencing. Fine mapping showed that the SV involved three >15-kb-genomic-region duplicating and tandem inserting into the downstream of the first copy number variation region (CNVR). We have examined more than 23 chicken breeds including three other Beard chickens and confirmed that the SV was completely associated with Beard. Continuous expression of genes located in these regions during the critical period of Beard morphogenesis were demonstrated in both embryos and adults. We identified that two candidate genes, which come from the same gene family, were strongly associated genes with Beard. It is now widely accepted that SVs account for quite a number of domestic animal phenotypic variations. Thus, our results suggest additional evidence that complex genomic structural variation has a prominent phenotypic effect.

P3017 Maternal nutrition induces gene expression changes in fetal muscle and adipose tissues in sheep. Francisco Peñagaricano, Xin Wang, Guilherme Rosa, Amy Radunz and Hasan Khatib (University of Wisconsin - Madison)

Maternal nutrition during different stages of pregnancy can induce significant changes in fetal tissues, which in turn can have lifelong implications. The objective of this study was to evaluate the effect of different maternal diets on the transcriptome of fetal tissues in sheep. Ewes were bred to a single sire and from days 67±3 of gestation until necropsy (days 130±1), they were fed one of three diets: alfalfa haylage (HY; fiber), corn (CN; starch), or dried corn distiller's grains (DG; fiber plus protein plus fat). Longissimus dorsi, subcutaneous adipose depot, and perirenal adipose depot tissues from individual fetuses were pooled and then analyzed by RNA sequencing. Reads were mapped to the ovine reference genome using Tophat, and the resulting alignments were used to reconstruct transcript models using Cufflinks. Differential gene expression was evaluated using Cuffdiff. Additionally, gene set enrichment analysis was performed using a test of proportions based on the cumulative hypergeometric distribution. Remarkably, maternal diets induced significant gene expression changes in fetal tissues. In muscle, a total of 224 and 823 genes showed differential expression (FDR < 0.05) between DG vs. CN, and HY vs. CN, respectively. In subcutaneous and perirenal adipose tissues, 745 and 208 genes were differentially expressed (FDR < 0.05), respectively, between CN and DG diets. Notably, the expression of most genes was decreased in fetuses whose mothers were fed CN diet. Furthermore, pathway analysis revealed that

several GO terms, InterPro entries, and KEGG pathways were enriched (FDR < 0.05) with differentially expressed genes related to chromatin biology, tissue and organ development, and in particular in the adipose tissues, to fatty acid metabolism. Overall, our findings provide evidence that maternal diet affects the transcriptome of fetal tissues in sheep. Functional ramifications of the observed changes in gene expression, in terms of physiology of the offspring, warrant future investigation.

P3018 Isolation and cultivate of melanocytes from the dermis of fox (*Vulpes vulpes*). Jiarong Bao and Fuhe Yang (institute of special animal and plant sciences of chinese academy of agricultural sciences)

Melanocytes originate from neural crest cells, biosynthesis the pigment granules, which play a critical role in species trait, camouflage and mimicry for animals. Coat color is a phenotypic marker of fur animal species, which is determined by the pigment produced by melanocytes. To date, more than 300 genetic loci and more than 170 coat color associated genes have been identified, which influence pigmentation in various ways, the genetic pathways infulenceing coat coloration remain to be known. Color variation and color pattern can be made as a powerful model for studying the genetic mechanisms that determine phenotype.
In this study, we established a feasible method for isolation and culture melanocytes from fox (*Vulpes vulpes*) skin samples. First, skin biopsies were harvested from the dorsal region of adult foxes and enzyme digestion by Dispase II. Second, isolation the melanocytes from the dermis layer with forceps and cell sieves. Third, the primary cells were cultured by Keratinocyte serum-free medium (K-SFM) supplemented with EGF, bovine pituitary extract without phorbol-12-myristate-12-acetate (PMA) for three days. Then, replacing the medium to K-SFM with PMA. After a few such passage operations, it will yield highly pure populations of melanocytes. At last, the melanocytes were confirmed by detection of color genes expression, such as MC1R, TYR and MITF, using RT-PCR identification.
This study was to develop and validate a system for isolation, purification and passage culture of melanocytes from the dermis of fox, which is an efficient carrier for investigation the color gene function and unraveling the process of melanin biosynthesis.

P3019 Study on the developmental expression of the genes of *MyHC I, MyHC IIa, MyHCIIb, and MyHC IIx* in longissimus dorsi tissue in Mashen and Large White pigs. Guoqing Cao (college of Animal Science and Veterinary Medicine, Shanxi Agricultural University), Jingmin Jia, Pengfei Gao, Xiaofen Yang and Wei Li (College of Animal Science and Veterinary Medicine, Shanxi Agricultural University), Yanqing Zhang and Kai Xu (college of Animal Science and Veterinary Medicine, Shanxi Agricultural University), Jianzhong Shi (Datong Pig Breeding Farm) and Xiaohong Guo and Bugao Li (College of Animal Science and Veterinary Medicine, Shanxi Agricultural University)

Muscle fiber type composition is in relation to meat quality because it correlates with intramuscular fat and tenderness of muscle. Based on the isoforms of myosin heavy chain, there are four fiber types MyHC I, MyHC IIa, MyHC IIb, and MyHC IIx in adult mammalian skeletal muscles, which were encoded by a separate gene. Mashen pig exhibits lower growth rate, poorer feed efficiency, and lower lean meat content than Large White pig, but the meat sensory quality is superior. The aim of this study was to compare the developmental expressions of *MyHC* genes at mRNA level in longissimus dorsi tissue during 2-6 month old between Mashen and Large White pigs by qRT-PCR. The mRNA relative expressions of *MyHC I*, *MyHC IIa*,

MyHC IIx and *MyHC IIb* in Mashen were significant different from those in Large White pig (*P*<0.05). The expressions of *MyHC I* were increased with the age both in Mashen and Large White pigs, and Mashen's expressions were always significant greater than that of Large White's (*P*<0.01). The relative expressions of *MyHC IIa* were higher before 3-month old, and decreased at 4-month age, then increased again afterward both in Mashen and Large White pigs, and the expressions in Mashen pig were greater than that in Large White pig at all detected points. The relative expressions of *MyHC IIx* and *MyHC IIb* in Mashen pig were significant greater than that in Large White pig at 2-month age. After 3-month age, the expressions in Large White were increased gradually, greater than that in Mashen pig. The results showed that the proportion of *MyHC I* and *MyHC IIa* was higher in Mashen pig, Which was positively related to the color characteristics, better water-holding capacity and tenderness of meat.

P3020 The different regulation roles of MSTN in Muscle satellite cells and Preadipocytes. feng zhang (Huazhong Agricultural University), bing deng (Wuhan Institute of Animal Science and Veterinary Medicine, Wuhan Academy of Agricultural Science &Technology) and siwen jiang and kun chen (Huazhong Agricultural University)

Myostation (MSTN), which is a secreting protein of transforming growth factor-β (TGF-β) superfamily mainly expressed in skeletal muscle, also had some function in the mature process of adipocyte.Researches had found that it has different regulation roles in the adipogenesis of Muscle satellite cells and Preadipocytes, but the reason is not clear. In this study we had analyzed PPARγ and MyoD expression in the transcription, protein and methylation levels in the two kinds of cells, when they were treated by 100 ng/mL MSTN for 48 h. The results show that the transcription level of PPARγ was downregulated in 24 h time point and then upregulated in 48 h time point in Preadipocytes, while downregulated always in Muscle satellite cells when cells were treated by MSTN. The expression of MyoD was significantly increased in Preadipocytes and decreased in Muscle satellite cells,respectively.In protein level, the level of PPARγ and MyoD were upregulated in Preadipocytes and downregulated in Muscle satellite cells, respectively.The CpG methylation level of PPARγ and MyoD promoter were decreased in Preadipocytes and increased in Muscle satellite cells respectively. From these results above, we speculated that there are different regulation role for PPARγ and MyoD in Muscle satellite cells and Preadipocytes during treatingby MSTN.

P3021 A Splice Mutation in the *PHKG1* Gene Causes High Glycogen Content and Low Meat Quality in Pig Skeletal Muscle. Jie Yang, Lisheng Zhou, Jun Ren, Xianxian Liu, Bin Yang, Zhiyan Zhang, Huanban Ma, Xianhua Xie, Yuyun Xing, Yuanmei Guo, Junwu Ma and Lusheng Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, 330045, Nanchang, China)

Glycolytic potential (GP) in skeletal muscle is economically important in the pig industry because of its effect on pork processing yield. We have previously mapped a major quantitative trait loci (QTL) for GP on chromosome 3 in a White Duroc × Erhualian F2 intercross. We herein performed a systems genetic analysis to identify the causal variant underlying the phenotype QTL (pQTL). We first conducted genome-wide association analyses in the F2 intercross and an F19 Sutai pig population. The QTL was then refined to an 180-kb interval based on the 2-LOD drop method. We then performed expression QTL (eQTL) mapping using muscle transcriptome data from 497 F2 animals. Within the QTL interval, only one gene (*PHKG1*) has a

cis-eQTL that was colocolizated with pQTL peaked at the same SNP. The *PHKG1* gene encodes a catalytic subunit of the phosphorylase kinase (PhK), which functions in the cascade activation of glycogen breakdown. Moreover, a gene coexpression network centering on *PHKG1* appeared to be associated with glucose metabolism. Deep sequencing of *PHKG1* revealed a point mutation (C>A) in a splice acceptor site of intron 9, resulting in a 32-bp deletion in the open reading frame and generating a premature stop codon. The aberrant transcript induces nonsense-mediated decay, leading to lower protein level and weaker enzymatic activity in affected animals. The mutation causes an increase of 43% in GP and a decrease of > 20% in water-holding capacity of pork. These effects were consistent across the F2 and Sutai populations, as well as Duroc × (Landrace × Yorkshire) hybrid pigs. The unfavorable allele exists predominantly in Duroc-derived pigs. The findings provide new insights into understanding risk factors affecting glucose metabolism, and would greatly contribute to the genetic improvement of meat quality in Duroc related pigs.

P3022 Digital gene expression and proteomics analyses for hepatic lipid metabolism in chicken embryos. Wei Na, Yuanyuan Wu, Chunyan Wu, Yuxiang Wang, Ning Wang, Zhi-Qiang Du and Hui Li (Northeast Agricultural University)

As an enclosed and easy-to-manipulate system, chicken embryos contribute enormously to our understanding on vertebrate development. Avian embryonic growth and development rely mainly on yolk lipids. Therefore, hepatic lipid metabolism and lipogenesis have been studied extensively, and also used as a model for fatty liver diseases. However, knowledge on the molecular mechanism of hepatic lipid metabolism in chicken embryos is still very limited. We performed digital gene expression profiling and comparative proteomics on chicken livers at five important embryonic developmental stages (E7, E12, E14, E17 and H1), which were selected from chicken lines with significant differences in abdominal fat content. Each developmental stage had its unique gene expression pattern and stage-specific differentially expressed genes. Furthermore, the three rapid growth periods (E12, E14 and E17) had similar expression patterns. Between the two broiler lines, E17 had the largest number of significantly differentially expressed genes (979), specifically enriched in fatty acid metabolism and biosynthesis, PPAR signalling and glycolysis pathways, which was complemented by the identification of differentially expressed APOA-I gene through quantitative proteomics. Therefore, genome-wide gene expression and proteomics analyses recapitulated the developmental pattern of chicken embryos, and found the important molecular pathways for hepatic lipid metabolism. Stage-specific gene pathways could be involved in the hepatic lipid metabolism in chicken embryos divergently selected for abdominal fat content, which could also provide novel insights for embryonic liver development and diseases.

P3023 Identification of genes related to azoospermia in chickens on a genome-wide scale using digital gene expression profiling. Yanyan Sun, Zhuwei Wang, Yulin Bi, Hao Bai, Fuguang Xue, Ranran Liu and Jilan Chen (Institute of animal science, Chinese Academy of Agricultural Science)

Azoospermia is the medical condition of not having any measurable level of sperm in the semen. According to the observation in Beijing-You chickens, azoospermia affects 10% of the male population. It is significantly associated with the low fertility. Previous studies shed light on the genetic contribution on azoospermia. The objective of the present study was to identify the important genes and metabolic pathways related to azoospermia in

chickens. Digital gene expression (DGE) analysis, which is based on new generation of high-throughput sequencing technology, was performed on the azoospermia and normal testis collected from Beijing-You chicken, respectively to capture the global gene expression difference. In comparison with the normal testis, 16,378 differentially expressed genes (DEG) were identified in the azoospermia testis totally. Of these DEGs, 545 were up-regulated and 202 were down-regulated under the criteria of false discovery rate < 0.001 and differential folds > 2. Cytochrome c oxidase (Cox) and Cyclin F was the most up-and down regulated gene, respectively. qRT-PCR were performed and verified the DGE results independently. Gene ontology functional analysis showed that the DEGs functioned in acetyl-Coa hydrolase activity and prostaglandin-D synthase activity. It was revealed from the pathway analysis that, the DEGs were mainly linked to the pathways like nicotinate and nicotinamide metabolism and focal adhesion. Combining the analysis, five candidate genes (PTGDS, hPCDS, SPAG6, WNT2, Cox, and Cyclin F) were highlighted as candidate genes related to azoospermia in chickens. To the best of our knowledge, this is the first genome-wide study to investigate the transcriptional difference in the azoospermia and normal testis of chickens. The identified DEGs in the present study are worth of further functional characterization to better understand the molecular genetic mechanism of azoospermia.

P3024 Identification and functional verification of EEF1D associated with milk production traits in dairy cattle. Yan Xie, Shaohua Yang, Xiaogang Cui and Dongxiao Sun (China Agricultural University)

Our recent genome-wide association study (GWAS) has identified 105 genome-wide significant SNPs for milk production traits. Of these, one SNP (rs109661298) for milk fat percentage is located within the bovine EEF1D gene on BTA14.The mRNA expression patterns of EEF1D showed that gene were highly expressed in the mammary gland of lactating cows than in other 7 tiusses (cardiac muscle, uterus, kidney, liver, lung, ovary and small intestine). Thus the EEF1D gene was considered as a novel candidate in dairy cattle. With rapid amplification of 5' cDNA end (5' RACE), two novel alternatively spliced transcript variants EEF1Da and EEF1Db (GenBank: KC190039 and KC190038) were isolated. Tissue expression pattern showed that the mRNA expression of EEF1Da was significantly higher than that of EEF1Db and similar to the overall mRNA level of EEF1D in the mammary gland. Furthermore, based the complete mRNA sequence of EEF1Da, we designed 4 siRNA fragments for RNAi and transfected them into bovine fibroblasts. With real-time quantitative RT-PCR, we found that 3 out of 4 siRNA can significantly reduced the mRNA expression level of the EEF1D gene by 70% down of normal. Two siRNAs were synthesized corresponding to the DNA sequences of EEF1D to construct the lentiviral shRNA expression vectors LV3-E1357 and LV3-E1893. By using the packaging system: pGag/Pol, pRev and pVSV-G in 297T cells, the lentiviral particles were successfully obtained and transected into the mammary epithelial cells (BMECs) developed from lactating Holstein cows, so that positive cells with stably expression of shRNA survived for resistances of puromycin were got. To investigate whether the reducted expression of the EEF1D gene affect milk related traits, genome-wide gene expression patterns of normal BMECs and BMECs with lower expressed EEF1Da were analyzed with bovine 4x44k microarray expression profiling (Agilent). Such findings will lay the foundation for inferring biological functions of EEF1D gene on milk production traits.

P3025 MicroRNA-27b regulates porcine skeletal muscle satllite cells proliferation and differentiation by targeting *MDFI*. Jun Duan

(South China Agricultural University)

MiR-27b was proved involving in skeletal muscle development. In this study, we detected that *miR-27b* was down-regulated in *Notch1*-overexpression postnatal porcine skeletal muscle satellite cells (PSCs). *MyoD* Family Inhibitor, *MDFI*, was predicted as one of the target genes of *miR-27b* by Targetscan. Luciferase resporter assays showed that co-transfection of *miR-27b* with the *MDFI* 3'-UTR resulted in a significant decrease in luciferase activity. To better understand the regulation of *miR-27b* responsible for PSCs, *miR-27b* and *MDFI* were enhanced or interfered respectively in PSCs. The results showed *miR-27b* restricted proliferation and promoted differentiation, whereas *MDFI* had the opposite effect by EdU proliferation assay and *MyHC*-immunocytochemistry. Furthermore, after enhancement of *miR-27b*, the expression of *MDFI* and *cyclinD1* were significantly decreased while *MyoD*, *myogenin* and *P21* were significantly increased by qRT-PCR and Western blot. After interfering *miR-27b*, the expression of *MDFI* and *cyclinD1* were significantly increased while *MyoD*, *myogenin* and *P21* were significantly decreased. Meanwhile, after over-expression of *MDFI*, the expression of *MyoD*, *myogenin* and *P21* were significantly decreased while *cyclinD1* was significantly increased; after interfering *MDFI*, the expression of *MyoD*, *myogenin* and *P21* were significantly increased while *cyclinD1* was significantly decreased. Taken together, these findings suggested that *MDFI* was the target gene of *miR-27b* and *MDFI* promoted proliferation and suppressed differentiation; *miR-27b* could regulate PSCs proliferation and differentiation by targeting *MDFI*. This work was supported by National Natural Science Foundation (31072008) and National High Technology Research and Development Program of China (2013AA102502).

P3026 Discovery of microRNAs regulated by notch signaling pathway in porcine skeletal muscle satellite cells. Jian Xu (College of Animal Science, South China Agricultural University)

Porcine skeletal muscle satellite cells (PSCs) are critical important for the postnatal muscle growth and regeneration. It has been revealed that Notch signaling pathway and some microRNAs play decisive roles in regulating the process of myogenesis. However, it has not yet been clarified whether there are microRNAs which interact with notch signaling to affect the development of satellite cells. We constructed a PSCs model which constitutively expressed *Notch1 intracellular domain* (*NICD*), and sought the differential expressed microRNAs by HiSeq 2500. Firstly, we isolated the fresh PSCs and transfected the cells with pEGFP-NICD (tested group) and pEGFP-N1 (control group) separately, and got the cell clones by G418 screening. Secondly, we tested the expression of *NICD* by qRT-PCR, Western blot and immunocytochemistry. The expression of*NICD* was increased significantly in pEGFP-NICD treated cells ($P<0.01$). Thirdly, the proliferation of the PSCs was detected by Edu assay, and the tested group had a higher ration of Edu+ than the control group. Finally, the microRNAs were separated and sequenced by HiSeq 2500. There were 69 microRNAs up-regulated and 115 microRNAs down-regulated ($|\text{fold change (log2)}|\geq 1$; $P<0.01$) in the tested group. These microRNAs might be involved in the notch signaling pathway to regulate the PSCs, and the results might provide a new insight into the mechanism mediated by notch signaling underlying the porcine muscle development. This work was supported by National Natural Science Foundation (31072008) and National High Technology Research and Development Program of China (2013AA102502)

P3027 The Expression of Coat Colour Genes

in Jinhua Pig (*Sus Scrofa*). Junsheng Zhao, Haiguang Mao, Yifei Shen and Ningying Xu (Zhejiang university)

Microphthalmia-associated transcription factor (*MITF*), *tyrosine kinase receptor KIT*,*tyrosinase* (*TYR*), *tyrosinase-related protein-1* (*TYRP1*) and *tyrosinase-related protein-2* (*TYRP2*) are melanin biosynthesis pathway genes. Jinhua pig is a Chinese indigenous pig breed with two-end-black coat colour. The expression of those genes has been investigated in the white and black skin of Jinhua pig in this study. The skin samples were collected from the black region of the head and tail, as well as the white region in the belt of Jinhua pig. The isolation of total RNA was performed using the miRNeasy Mini Kit (Qiagen) following the manufacturer's instructions. The expression of *TYR*, *TYRP1*, *TYRP2*, *MITF* and *KIT* were carried out by qPCR, and 18S rRNA was used as a reference control. The analysis was performed by the 2-ΔΔCt method. The result showed that *TYR* and *TYRP1* genes had no expression in white coat region, even they normally expressed in black region. The *TYRP2* gene had expressed in all the regions, and over 5 fold higher than that in white region. Finally, there was no significant expression difference in different colour regions for the *MITF* and *KIT* genes. The transcription of *TYR*, *TYRP1* and *TYRP2* is regulated by MITF. However, in this study, *MITF* shows inconsistent expression pattern with *TYR*, *TYRP1* and *TYRP2*. It could be speculated that there are other transcriptional regulation factor(s) determining the two-end-black trait in Jinhua pig. Acknowledgement: This work was supported by the National High Technology Research and Development Program of China (863 Program) (No. 2011AA100302).

P3028 Detection of miRNAs targeting porcine *PNPLA2* gene. Ruhai Xu, Xiaohong Chu and Fuzeng Lu (Zhejiang Academy of Agricultural Sciences)

Patatin-like phospholipase domain-containing 2 (PNPLA2) was described to predominantly perform the initial step in triglyceride hydrolysis and identified as an important triglyceride hydrolase in lipid droplets/adiposome turnover in mammalian cells. To analyze the possible miRNA targeting sites in porcine *PNPLA2* gene, a *PNPLA2* 3'-UTR-luciferase reporter vector was constructed and the effect of miRNAs on its activity were evaluated in 293T cell line. Methods: The miRNAs targeting *PNPLA2* were predicted by bioinformatics analysis. 3'UTR of *PNPLA2* was amplified from porcine genomic DNA and cloned into pMIR-report luciferase vector (pMIR-luc-3'UTR). Then pMIR-luc-3'UTR was transiently co-transfected with candidate miRNA mimics into 293T cells using lipofectamine 2000 transfection reagent. The dual-luciferase reporter assay system was used to quantitate the reporter activity. Results: A 450 bp 3'-UTR fragment of porcine *PNPLA2* gene was successfully cloned into the pMIR-report luciferase vector. The predicted miRNAs targeting *PNPLA2* 3'-UTR included ssc-miR-769-3p, ssc-miR-1343, ssc-miR-7137-3p, ssc-miR-671-5p, ssc-miR-339-5p, ssc-miR-149, ssc-miR-1249 and ssc-miR-1296-5p. Compared with the control group, the luciferase activity of reporter construct treated with ssc-miRNA-1343 mimic, ssc-miR-769-3P mimic, ssc-miR-7137-3P mimic or ssc-miR-671-5P mimic was decreased respectively，especially for the ssc-miRNA-1343 mimic with a decrease of 50%. Conclusion: Ssc-miRc343，ssc-miR-769-3P, ssc-miR-7137-3P and ssc-miR-671-5P could suppress the luciferase activity of *PNPLA2* 3'-UTR-luciferase reporter, and might play important roles on the expression of porcine *PNPLA2* gene. Acknowledgment: The work was supported by the National Natural Science Foundation of China (31201783, 31072006) and Natural Science Foundation of Zhejiang Province (Y3100569).

P3029 The regulation of PR domain of porcine PRDM16 in preadipolytes differentiation. Feng Jiang (South China Agricultural University)

PRDM16 (*PR* domain containing 16) is a zinc finger protein that regulates muscle and fat metabolism. Previous studies suggested mouse *PRDM16* is a histone methy- ltransferase and methylates lysine 9 in histone H3, its activity has been mapped to the *PR*domain, and H3K9 correlates with transcriptional repression. However, it is not clear whether the *PR* domain of porcine *PRDM16* regulates the process of preadipolytes differentiation. In this study, His-tagged *PR* fusion protein was constructed and purificated, and the activity of histone methyltransferase was detected by western blotting with modification-specific antibodies for H3K9.The result showed that *PR* domain has histone methyltransferase activity. *PR* domain over-expression vector was successfully constructed with eukaryoric expression vector pcDNA3.1(+) and the vector was transfected into the preadipocyte. The differentiation of adipocyte was detected by oil red staining and GPO-POD method. Over-expression of *PR* domain can significantly suppress the formation of lipid drops and the accumulation of triglyceride after 8 days inducing differentiation. The mRNA expression of *C/EBP*α was significantly down-regulated (*P*<0.01), *LPL* and *AP2* also down-regulated (*P*<0.05), *PPAR*γ and *C/EBP*β were reduced to some extent but it did not reach significant level by qRT-PCR. The results showed that *PR* domain down-regulated lipogenesis-related genes and can suppress adipocyte differentiation. It suggested porcine *PRDM16* as a histone methyltransferase plays an important role in fat deposition. This study was supported by the Science and Technology Planning Project of Guangdong Province (2010A020102003), the Key Program for Scientific and Technological Innovations of Higher Education Institutes in Guangdong Province (cxzd117).

P3030 Validation of two novel loci identified in GWAS showing strong association with milk production traits in Chinese Holstein. Shaohua Yang, Dongxiao Sun, Tian Dong and Xiaojun BI (China Agricultural University)

Our previous genome-wide association study (GWAS) has identified 38 high confident significant SNPs for milk production traits in Chinese Holstein. In this study, totally 26 annotated genes were mined to contain or be near to at least one significant SNP. Of them, EEF1D and PDE9A were highly expressed in mammary gland of lactating cows (p<0.01), such that they were considered as two novel candidates. Through re-sequencing the entire coding regions and 5′, 3′- regulatory regions, a total of 9 and 11 SNPs were discovered for EEF1D and PDE9A, respectively. Association analysis exploited the haplotype, composed of rs137492431 and rs136760996 (r2=1, D'=1) in the 5′- regulatory region of EEF1D, was associated with milk yield (p=0.0004 and 0.0026), fat percentage (p=0.0135 and 0.0080), protein yield (p=0.0119 and 0.0253) and protein percentage (p=0.0199), and rs383169619 with fat yield (p<0.0001) and fat percentage (p=0.0033 and 0.0077). These EEF1D SNPs were subsequently confirmed having low linkage disequilibrium (LD) with the well-known DGAT1 K232A mutation (r2=0.037~0.008; D'=0.371~0.365), indicating their independent effects on milk production traits. For PDE9A, one haplotype showed associations with three yield traits (p<0.0001~=0.0013) and fat percentage (p=0.0001 and 0.0138). The other haplotype was associated with all the 5 traits (p<0.0001~=0.0276) except protein percentage in first lactation. Additionally, rs137492431, rs210080144 and rs135748250 were predicted to change the binding sites of transcript factors CREB, AML-la and USF, respectively. Correspondingly, it was found the mRNA

expression of EEF1D with GG at rs137492431 and PDE9A with GG at rs210080144 and AA at rs135748250 were higher than the other genotypes in lactating mammary gland ($p<0.05$, $p<0.01$), respectively. In conclusion, our findings provide confirmatory evidences for our previous GWAS and strongly suggest EEF1D and PDE9A represent two key candidates for milk production traits for further biological function validation.

P3031 Gene expression profiles of bovine mammary gland and association with milk composition using RNA-seq and miRNA-seq. Xiaogang Cui (China Agriculturel University) and Dongxiao Sun, Shengli Zhang, Qin Zhang, Yan Xie and Shaohua Yang (China Agricultural University)

In this study, we analyzed the whole expression patterns of transcriptome and miRNAs of the mammary glands of four lactating Holstein cows with extremely high and low milk protein and fat percentage by using RNA-seq and miRNA-seq. As for mammary gland transcriptome, with HiSeqTM 2000, about 200 million uniquely mapped reads were obtained which represented 15549 mRNA transcripts, across the four mammary gland samples. Among them, 31 differentially expressed genes were revealed between high and low groups of cows. Integrated analysis of differential gene expression, previously reported QTL and genome-wide association study (GWAS) discoveries and biological functions showed that *TRIB3*, *VEGFA*, *PTHLH*, *SAA1*, *M-SAA3.2* and *SAA3* could be the promising candidates for milk fat and protein traits. Also, we sequenced the small RNA libraries of the same four mammary epithelium samples with HiSeqTM 2000. Small RNA tags, which could be mapped to bovine genome UMD3.1.66, were aligned to the miRNA precursor of bovine from miRBase19.0 to obtain the miRNA count as well as base bias on the first position of identified miRNAs with certain length and on each position of all identified miRNAs, respectively. As a result, 497 known miRNA and 49 novel miRNAs were identified with the softwares miREvo and mirdeep2. Among them, by using the DESeq R package (1.8.3), 74 differential miRNAs were identified ($p<0.05$, FDR $q<0.01$). Especially, 28 of 31 differential expressed genes identified by RNA-seq in this study, including *TRIB3*, *VEGFA*, *PTHLH*,*RPL23A*, were predicted to be the targets of such 74 differentially expressed miRNAs. Our findings indicated that the differentially expressed gens and miRNAs could be related to milk protein and fat traits and need to be further investigated in future.

P3032 Transcriptomic response of Atlantic salmon against *Piscirickettsia salmonis* in head kidney and liver, showing differential susceptibility to the disease.. Phillip Dettleff and V ́ctor Mart ́nez (University of Chile)

Despite the existing measures to control the disease, Piscericketsiosis (SRS), caused by *Piscirickettsia salmonis*, remains as a major problem on the Chilean salmon industry. We use Illumina high-throughput sequencing of individuals bearing different genetic resistance backgrounds in order to characterize the differential expression of genes involved in the pathogenesis in head-kidney and liver. The transcriptome of both tissues was *de novo* assembled using Trinity; reassembled with CAP3 to remove redundancy; blasted with blastx and annotated with Blast2GO. Reads were mapped to the *de novo* assembly and differential expression analysis was performed with CLC Genomics Workbench. In head-kidney a total of 200 genes were differentially expressed between the susceptible and resistant group, while in liver 414 genes were differentially expressed, with 40 genes differentially expressed in both tissues simultaneously (fold change > 2.0 and FDR < 0.01). As expected, immune genes appear to be differentially expressed between the groups (e.g. MHC class I antigen, IGHM, IL-1, TNFR, CC

chemokin, complement C1Q, complement C3, PD-L1) together with apoptosis and inflammation related genes (e.g. caspace, NFKB2 and MMPs). Additionally, transferrin (gene that controls the level of free iron) was differentially expressed. These results show the complexity of the interaction of the host and the pathogen and how susceptible/resistant hosts triggered a differential immune response against this disease. Therefore, modulating the side effects of an excessive inflammatory response, minimizing the tissue damage, together with regulating the available levels of free iron for the bacterium. This study gives valuable information about the components explaining the resistance of this disease and the host-pathogen interaction in liver and head kidney from a transcriptomic point of view. Funding: FONDECYT 1120608, CONICYT scholarship for PhD.

P3033 Spatiotemporal Expression of *FOXL2* Gene and Its Function in Prehierarchal Follicular Development of the Hen Ovaries. Rifu Xu, Yingying Zhang, Xiancong Fan, Ning Qin, Liu Qiang, Manli Wei and Zhichao Lv (Jilin Agricultural University)

The Forkhead transcription factor FoxL2 has been shown to be an essential transcription factor for ovary development and plays a significant role in the postnatal ovary and follicle maintenance. However, the expression characteristics and potential role of*FOXL2* gene remain unexplored in hen ovarian development. Lohmann brown commercial laying hens (n = 20) sampled for this experiment were randomly selected and sacrificed at 21 weeks of age. At post mortem, prehierarchal follicles (PF) (1-8mm) and whole ovaries were collected for the follow-up experiments of quantitative real-time PCR, *in situ* hybridization (ISH), immunohistochemistry (IHC) and culture of PF (6-8 mm) under treatment with FoxL2, FSH, FST, activin A (actA) and inhibin A (inhA). we initially found that *FOXL2* mRNA is expressed at highest level in the ovarian stroma and the prehierarchal follicles (1-6 mm diameter) and is at lowest in the larger preovulatory (F2-F1) follicles, and *FOXL2* transcript is predominantly located in oocytes and granulosa layer cells from the primary follicles (30-90μm in diameter) and the undifferentiated PF follicles of 60μm-8mm in diameter, of which signals of *FOXL2* mRNA are much stronger in small (FOXL2 mRNA to FSH, FST, actA and inhA, there is a 4-fold increase in *FOXL2* mRNA from 5-7mm to 7-8mm in diameter of the PF follicles treated for 12h, and the number of granulose layer cells within the PF follicles (6-8 mm) significantly increased under the treatment of FoxL2, FSH and/or actA for 24h. Collectively, the present result indicated that *FOXL2* gene is involved in dominant follicle selection of ovary in the hen by a complicated regulating mechanism. ACKNOWLEDGEMENTS: This work was supported by the National Natural Science Funds (No.31272431), the National High Technology Research and Development Program of China (863 Program) (No.2011AA100305), the China Agriculture Research System (No.CARS-42), and the Key Project of Education Department of Jilin Province (No.2013-47).

P3034 Identification of different metabolic pathways for breast muscle development between fast- and slow-growing chickens. Hongyang Wang, Ranran Liu, Guiping Zhao, Maiqing Zheng, Jilan Chen and Jie Wen (Institute of Animal Science, Chinese Academy of Agricultural Sciences)

Muscle development influences the efficiency of poultry meat production. The incubation period is of critical importance because muscle fiber formation is complete and the muscle fiber number is determined around the time of hatch. With the aim of clarifying the mechanisms underlying muscle development, protein expression profiles were examined in the breast muscle of both Beijing-You (BJY, slow-growing)

and Cobb chickens (CB, fast-growing) at mid-hatching to early growth period by iTRAQ techniques (Isobaric tags for relative and absolute quantitation). The protein which expression differed more than 1.5 fold and the P value less than 0.05 between each pair-wise comparison (Embryonic day 12 vs Ed 17; Ed 17 vs d 1; d 1 vs d 14) was defined as differentially expressed protein (DE). Of more than 2000 proteins identified, 246 proteins were expressed differentially in Cobb and 270 proteins in BJY. Based on KEGG analysis of DE proteins from each pair-wise comparison within breed, enriched pathways in each period were identified. Through further comparison between two breeds, striking differentiation of metabolic pathways was discovered. For the stage where embryonic muscle is growing rapidly (Ed 12 to Ed 17), the oxidative phosphorylation pathway was more active in CB than in BJY and the expression of ATP synthase subunit e, ATP synthase subunit beta, cytochrome b-c1 complex subunit and NADH-ubiquinone oxidoreductase 75 kDa subunit involved were increased more than three folds in CB. For the stage where postnatal muscle is growing rapidly (d 1 to d 14), the pyruvate metabolism pathway was more active in Cobb than in BJY and the rate-limiting enzyme, pyruvate kinase muscle isozyme, increased eight fold in Cobb. There was no difference detected on the expression of those proteins in BJY.

P3035 Developmental changes and effect on intramuscular fat content of H-FABP mRNA expression in Chinese ringed-neck pheasants（Phasianus colchicus）. Qiong Wu, Fuhe Yang and Xiumei xing (The State Key Laboratory of Special Economic Animal Molecular Bioloy)

H-FABP is thought to be cytosolic protein with important role in the deposition of lipid in muscles. Research studies have shown that expression of H-FABP was induced during adipogenic differentiation of stromal-vascular adipocytes. Therefore, H-FABP gene may play an important role in the development of intramuscular adipocytes. H-FABP gene is considered as candidates for intramuscular fat (IMF) accretion. At present, more in-depth studies on H-FABP gene sequence and function are available for humans, chickens, pigs, cattles, grass carps, and African clawed frogs. The Chinese ring-necked pheasant is raised by commercial farms in most parts of China, because of special fleshy flavor, such as good fecundity, good adaptability, small ferity and so on. However information on H-FABP gene and its expressions in domestic Chinese ring-necked pheasant still remains very scarce. The aim of the present study was to assess the expression of H-FABP gene, and to evaluate the association between H-FABP gene expression of different tissues and IMF content in Chinese ringed-neck pheasants. The expression pattern of pheasant H-FABP mRNA was examined in this study by quantitative RT-PCR. The results showed that pheasant H-FABP gene was expressed in many tissues, including heart, lung, kidney, muscle, ovary, brain, intestine, stomach and adipocyte tissues, and highly expressed in heart. H-FABP gene also was expressed in different growth stage (1, 7, 30, 60, 90, 100 days) , and reached a peak at 60 days. A significantly negative correlation was just found between the H-FABP mRNA expression level in lung tissue and breast muscle IMF content ($P<0.05$). These results suggest that the expression of the H-FABP gene are associated with IMF content in Chinese ringed-neck pheasants.

P3036 Possible effect of melanin on immune organs development in Silkie chicken. Deping Han, Shuxiang Wang, Yuanyuan Zhang and Xuemei Deng (China Agricultural University)

Silkie chicken (Silkie) is famous for hyperpigmentation in inner organs. In this study, accurately melanocytes distribution was distinguished and the effects of melanin on immunity were discussed. White Leghorn (WL)

and Silkie, aged at 1day, 2, 4, 6, 10, 15 and 23 weeks were obtained for immunohistochemistry analysis. Indexes of spleen, thymus and bursa were measured. Ultrastructure features of melanocytes in thymus and bursa were observed by transmission electron microscope. Expression of apoptosis associated proteins BAX and BCL-2 were detected by TUNEL and immunohistochemistry. The results showed that before 6 weeks old, spleen, thymus and bursa developed slower in Silkie than those in WL, but no significant difference was detected in the later stages. Melanocytes were observed in the parenchyma of thymus and bursa with co-localization with mast cells and lymphoctyes. Numbers of CD4+and CD8+ T cells in thymus and spleen were significantly fewer in Silkie before 6 weeks old. Numbers of B cells in Silkie were significantly fewer than those in WL before 6 weeks old, but more cells in Silkie at 23 weeks old. Additionally, fewer apoptotic cells and lower expression of pro-apoptotic protein BAX were found in thymus and bursa of Silkie at 23 weeks old. In conclusion, our results indicated that adoptive immunity was inhibited in Silkie before 6 weeks old, which might be associated with the effect of melanin on proliferation and maturation of lymphocytes in the organs. Melanin seems to be able to prohibit lymphocytes apoptosis by reducing BAX protein expression in the process of bursa degeneration.

P3037 Fine mapping of a major QTL for carcass traits on pig chromosome 7. Lisheng Zhou, Jun Ren, Lin Li, Huashui Ai, Zhiyan Zhang, Bing Yang, Yuanmei Guo, Shijun Xiao, Yin Fan, Junwu Ma and Lusheng Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, 330045, Nanchang, China)

Carcass traits are economically important in the industry. We have previously identified a major QTL for carcass length (CL) and backfat thickness (BFT) on pig chromosome 7 (SSC7) by genome scan in a White Duroc × Erhualian F2 cross. To fine map the SSC7 loci, we performed a series of follow-up studies including genome-wide association study (GWAS), identical-by-decent (IBD) analysis, linkage and linkage disequilibrium (LDLA) analysis and recombination breakpoint analysis in the F2 pedigree. The IBD analysis allowed us to map the QTL within a 1.7 Mb segment. LDLA mapping defined the QTL to an interval of 1.3 Mb. A recombination breakpoint analysis further map the QTL interval to a 600-kb region that contains *HMGA1* and other 6 annoted genes. We identified a total of 134 variants in the 600-kb region and found out a *HMGA1* SNP shows the strongest association with CL and BFT in the F2 population. We further performed GWAS in an Erhualian purebred population and identified significant locus ($P < 10\text{-}12$) for CL around the 600-kb region on SSC7. However, this locus had no significant effect on BFT, supporting that the causal variants for CL and BFT at the SSC7 locus are likely different.

P3038 The effect of follicle stimulating hormone on abdominal fat and intramuscular fat deposition *in vivo*. Ranran Liu, Yingying Li, Guiping Zhao, Maiqing Zheng, Peng Li, Li Liu and Jie Wen (Institute of animal sciences in Chinese academy of agricultural sciences)

Fat distribution is the process of differential accumulation of fat in different parts of the body. Abdominal fat and intramuscular fat are important economic traits in broiler chickens. Effects of the gonadotrophic hormone, follicle stimulating hormone (FSH), on fat metabolism have been found recently; FSH significantly promotes lipid deposition in preadipocytes. This study aims to verify the effect of FSH on abdominal fat and intramuscular fat deposition *in vivo*, and to understand the mechanisms. In males, results show that there were no differences in abdominal fat percentage and the triglyceride

(TG) content in breast muscle between controls and chicken with FSH treatment (4 mIU/day, subcutaneous, days 7–13). In females, the abdominal fat percentage and the TG content in breast muscle in FSH treatment chickens were significantly higher than in controls, while the serum TG content was significantly lower. There were no differences in the serum contents of estrogen, luteinizing hormone, low density lipoprotein and high density lipoprotein except the increased FSH in treatment groups. Treatment with FSH also significantly increased the relative expression of diacylglycerol acyl transferase 2 (*DGAT2)*, fatty acid synthase, heart type fatty acid binding protein *(H-FA*BP), uncoupling proteins*(UCP3)* and *PPARγ* in breast muscle and the relative expression of *DGAT2*, *H-FABP*, *UCP3* and *SREBP*-1c in abdominal fat tissue. There were no significant effects of FSH on the expression of these genes in liver. The observed changes indicate that FSH appears to increase the intramuscular fat content in breast muscle and the accumulation of abdominal fat in females by enhancing fatty acid synthesis and lipid transport; none of these effects were found in liver.

P3039 Genome-wide analysis reveals artificial selection on coat colour and reproductive traits in Chinese domestic pigs. Chao Wang, Hongyang Wang, Yu Zhang and Bang Liu (Huazhong Agricultural University)

Pigs from Asian and European were independently domesticated from ~9000 years ago. During this period, strong artificial selection has led to dramatic phenotypic changes in domestic pigs. To determine the genetic basis underlying these morphological and behavioural adaptations, we performed genome-wide sequencing and combined both Fst and Hp analysis to search for regions with selective sweep signals in the indigenous Chinese breed, Tongcheng pigs. Genes located in the selected regions were significantly associated with lipid metabolism, melanocyte differentiation, neural development, and other biological processes, which coincided with the evolutionary phenotypic changes in this breed. Two selected genes, *MITF* and *EDNRB*, are crucial for melanocyte development and are most likely associated with the two-end black colour of Tongcheng pigs. Additionally, studies of other white spotted pigs from China revealed coordinated signatures at both loci, supporting *MITF* and *EDNRB* as candidate genes for white spotting patterns in Chinese pigs. Moreover, a silent mutation c669T>C of *ESR1* co-localised with a major quantitative trait locus of litter size, and the variant G allele exhibited high allelic frequency in most Chinese pig populations. In addition, high haplotype similarity was found in *PRM1, PRM2, TNP2 and JMJD1C* genes associated with reproductive traits among Chinese pig populations. This study reveals strong candidate genes underlying the genetic basis for phenotypic changes in Chinese domestic pigs and will be helpful for elucidating the genotypic/phenotypic relationship in future functional studies.

P3040 Detection of TSH mRNA and Thyroxin Level during Embryonic and Post-Hatch Development in Duck. Chi Song, Weitao Song, Chao Di, Chunhong Zhu, Zhiyun Tao, Wenjuan Xu, Wenqi Zhu and Huifang Li (jiangsu lnstitute of Poultry Science)

Thyroid axis plays an important role in animal growth and development. The hypothalamus releases thyrotropin-releasing hormone (TRH) which stimulates the pituitary to produce thyroid-stimulating hormone (TSH). TSH stimulates the thyroid to produce thyroxine, and then deiodinases convert tetraiodothyronine （T4） to the active hormone triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body. In this study, the mRNA expression of TSH gene and thyroid hormone concentrations were detected in Gaoyou duck

and Jinding duck (two famous Chinese native duck breeds) during embryonic stage and 1 week after patch. The result indicated that the TSH gene expressed in the pituitary gland of Gaoyou and Jinding duck during early embryonic development, and the expression patterns of TSH gene in the two breeds were similar. Only development stage effect (P<0.05) existed in this study. There was no significant difference of TSH mRNA level between the two breeds. The TSH expression reached a peak level at embryonic day 27 and then decreased. Thyroid hormones (T3, T4 and FT3) were detected in this study, and the results showed hormone levels decreased rapidly with the increase of developmental time. The T4 concentration in Gaoyou duck at embryonic day 13, 21 and 27 were significantly or very significantly higher than that in Jinding duck (*P*P<0.01). Thyroid hormones (T3, T4 and FT3) in duck embryo were probably mainly from egg-laying duck. And these results indicated that TSH gene and thyroid hormone were involved in the embryo development, hypothalamus-pituitary-thyroid gland axis plays an important role in the regulation of duck embryo development.

P3041 Investigation on the relationship between genetic variation of KRT83 and curly fleece of Tan sheep (*Ovis aries*). Yufang Liu, Xiaolong Kang, Chengkun Liu and Meiying Fang

Tan sheep (*Ovis aries*), a Chinese indigenous breed, has special curly fleece after birth, especially at one month old. However, this unique phenotype disappears gradually with age and the underlying reasons of trait evolvement are still unknown. In our previous study, *KRT83* gene was detected to be related with the formation of curly fleece in Chinese Tan sheep by screening the suppression subtractive hybridization library, which was constructed using Tan sheep at different physiological stage. In order to investigate the relationship between *KRT83* and molecular mechanism of curly fleece in Tan sheep, we firstly cloned the full-length cDNA sequence of *KRT83* by RACE technology. The full length of *KRT83* gene consists of 1795bp, which encodes a 493-amino-acid protein. The genetic variation analysis among different sheep populations detected several mutations located in *KRT83* coding region. However, all of them were synonymous mutations. Multiple alignments of *KRT83* cDNA with other species indicated that they were 80% identities. In particular, the cDNA sequence was found to be 97.23% identical to the cattle sequence. *KRT83* expression profile was investigated and it showed that *KRT83* was widely expressed in all tissues, especial highly expressed in skin. Above results might indicate that *KRT83* gene plays a crucial role in the process of hair growth in Tan sheep. Further q-PCR data from skin showed that the expression of *KRT83* gene at one-month-old was significantly higher than that at 48-month-old. Future studies will be focus on the validation of *KRT83* biological function at two different developmental stages. This study might provide some clues for elucidating the relationship between *KRT83* and curly fleece in Chinese Tan sheep, as well as supplying some potential values for understanding the relationship between *KRT83* and human hair disorder and texture changes.

P3042 A 3-bp indel mutation in the promoter of *TRIB3* gene identified by RNA-seq is association with milk production traits in Chinese Holstein. Ruobing Liang, Shaohua Yang, Xiaogang Cui, Yan Xie, Shengli Zhang, Qin Zhang and Dongxiao Sun (China Agricultural University)

Our recent RNA sequencing (RNA-seq) has identified 31 differentially expressed genes were revealed between the mammary glands of Holstein cows with high and low protein and fat percentage. Combing with the reported quantitative trait loci and genome-wide

association study data, *TRIB3* (Tribbles homolog 3) gene was considered as a novel promising candidate affecting milk protein and fat percentage in dairy cattle. *TRIB3* is a protein involved in multiple signaling pathways, including MAPK, TGF-beta and the PI3K pathway, which short-term physiological regulation is assessed in adipose tissues as well as its ability to modulate glucose transport.
In this study, using clone sequencing approach, a three-nucleotide insertion (-1792 3N ins) variant in the promoter region of *TRIB3* was identified. Forward primer was end-labelled with tamra was synthesized and Fluorescent dye-labelled PCR products were separated using an ABI 377 DNA sequencer. With GeneMapper4.0, the individual genotypes of the 3-bp indel of 717 Chinese Holstein cows were determined, which were from 12 sire families in the Beijing Sanyuanlvhe Dairy Farming Center. The EBV values and pedigrees of the 717 cows were provided by the Dairy Data Center of China. Associations of such mutation with the 5 milk production traits were evaluated with the mixed animal model using SAS 9.1.3. It was shown the 3-bp indel polymorphism was significantly associated with protein yield (p=0.0278) and fat yield (p=0.0465), in which the 3-bp insertion genotype was advantageous compared with the deletion one. Correspondingly, significant additive effects and substitution effects of the indel mutation on protein yield (p<0.05) and fat yield (p<0.05) were also found. While, no significant dominant effects were revealed. Based on the sequence pattern, the 3-bp indel mutation might be a potential enhancer. The regulation of such mutation on *TRIB3* gene and milk traits formation will be further investigated in depth.

P3043 MicroRNA-21 regulates skeletal muscle development by target TGFβI in pigs. Zhonglin Tang, Lijing Bai and Kui Li (Institute of Animal Science,Chinese Academy of Agricultural Science)

MicroRNA（miRNA）, a short (22~24 base pairs) non-coding RNA, plays a critical role in skeletal muscle development. Using Solexa deep sequencing, we detected 229 and 209 known miRNAs in swine skeletal muscle at 90 days prenatal (E90) and 100 days postnatal (D100), respectively. We identified 138 miRNAs that were up-regulated in muscle at E90 and 31 miRNAs up-regulated at D100. Of these, 9 miRNAs were selected to validate the small RNA libraries by quantitative RT-PCR (QRT-PCR). Interestingly, we found that the miRNA-21 was significantly down-regulated in skeletal muscle at D100 with a 17-fold change ($p < 0.001$). Bioinformatics analysis suggested that the transforming growth factor beta induced (TGFβI) was a potential target for miRNA-21. Subsequently, we carried out dual luciferase reporter assays and western blot analysis in PIEC cells. The both results documented that the TGFβI was the target of miRNA-21. Co-expression analysis revealed that the expression of TGFβI and miRNA-21was negatively correlated (r=-0.421, p value=0.026) at mRNA level during skeletal muscle development at 28 stages. Our result firstly suggested that miRNA-21 is a myogenic miRNA that regulated skeletal muscle development by targeting the TGFβI of the TGF-β signaling pathway. This study is helpful to understand the role of miRNA-21 in skeletal muscle development and identify the candidate gene of production meat traits in pigs.

P3044 Discovering Key Transcripts of Regulating Feed Conversion and Breast Muscle Growth using RNA-Seq. Feng Zhu, ZhenHe Zhang, JinFeng Zhang, JianMing Yuan and ZhuoChen Hou (China Agricultural University)

Breast muscle growth and feed conversion are received much attention in duck breeding. Understanding the role of specific transcripts in muscle and intestine is conducive to study

interrelated biological processes. However analysis of duck transcriptome is tough as a result of low-quality reference genome. In this study, we took jejunum and breast muscle from two-tailed population of Peking duck and Maple-Leaf duck sorted by feed conversion rate (FCR) and breast muscle rate (BMR). Libraries were made from the pooled samples and were sequenced using Hiseq2000 platform. Here for improving follow-up analysis, a reference transcriptome was generated by *de novo* assembly and we applied reciprocal-best blast algorithm to annotate contigs with chicken transcripts information. Final ref-transcript database has 16,663 contigs with N50 length of 1530bp after eliminating low-quality sequences and redundancies. Totally, 11960 (72%) contigs had a one-to-one relationship with chicken transcripts and the rest (28%) were denoted as uncertain. Most uncertain sequences (4498, 27%) had no coding potentiality except only one. Other 167 (1%) sequence were not detected reading framework. After mapping and annotation, we found a great amount of differentially expressed transcripts were enriched in biological process about cell differentiation, transmembrane transportation, muscle development, immunization and glycoprotein metabolic process. Especially, the most significant enriched genes were showed in the nuclear receptor peroxisome proliferator-activated receptor β (*PPAR*β signal pathway) which was the master regulator of fat-cell formation. Our results showed fat metabolism related genes have an important role in breast muscle growth and feed conversion of duck. We provided new candidate genes for improving feed conversion and breast muscle.

P3045 Dissecting the molecular basis of a major QTL for fatty acid composition in *longissimus dorsi* on porcine chromosome 14. Wanchang Zhang, Bin Yang, Yin Fan, Junjie Zhang, Leilei Cui, Junwu Ma, Lusheng Huang and Jun Ren (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University)

Fatty acid composition is a crucial index relevant to nutrition, taste and processing of pork. Stearic acid (C18:0) is an important fatty acid which accounts for about 12% of total fatty acids in *longissimus dorsi* muscle. In this study, we investigated the responsible genes and casual variants underlying a significant loci ($P < 10\text{-}14$) for muscle fatty acid content on chromosome 14. By applying GWAS and LDLA analyses on two experimental populations including a White Duroc × Erhualian F2 cross and a Sutai pig population, we narrowed the critical region to a 500 Kb (120.5 Mb-121.0 Mb) interval that contain 10 candidate genes. We next examined correlation between expression levels of all 10 genes and C18:0 content in 580 samples. Of the 10 genes, *SCD* showed the strongest correlation with C18:0 content. This gene encodes a rate-limiting enzyme for catalysis of C18:0 and C16:0 to C18:1 and C16:1. We re-sequenced the entire *SCD* gene and its 10 Kb surrounding region in 15 parental individuals with known QTL genotypes in the two experimental populations, and identified 70 SNPs and 2 InDels. Of them, 11 SNPs and 1 InDel show complete concordance between variation genotypes and QTL genotypes. Through a series of bioinformatics analyses, we identified two SNPs at the promoter region of *SCD* as the most plausible causal variants. The association between the two SNPs and muscle C18:0 content were further confirmed in a Duroc × (Landrace × Yorkshire) hybrid population (N = 610). The two variants showed significantly pleiotropic effects on C16:0, C16:1, C18:0 and C18:1 across F2, Sutai and DLY hybrid populations. A survey of allele frequency of two SNPs in 2782 pigs representing diverse breeds showed that the two SNPs are only segregated in Duroc or Duroc-derived pigs. Our finding is of considerable importance and has an immediate

translation into breeding practice for improving pork quality.

P3046 Host lack of *Atg5* gene influences gut microbiome in mice. Wenjing Zhao (Department of Animal Science, School of Agriculture and Biology, Shanghai JiaoTong University), Chao Liu (State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences), Shuyun Liu and Zhengxiao Zhai (Department of Animal Science, School of Agriculture and Biology, Shanghai JiaoTong University), Wei Li (State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences) and He Meng (Department of Animal Science, School of Agriculture and Biology, Shanghai JiaoTong University)

Although the homeostasis of commensal microbiota is vital to host physiological and immunological functions, the role of host genetics affect the intestinal microbiome in order to benefit for host protection remains obscure. Autophagy, existed in every organ, gives a major push to programmed cell death. Besides 'self' degradation, it also involves with immune regulation through the phagocytosis of pathogenic microorganism. We investagated how *Atg5*, a essential gene in autophagy metabolic pathways influenced gut microbiome along host intestinal tract. 21 mice including 13 *Atg5*+/+(WT), 8 *Atg5*-/-(KO) were used in this study and microbiota genome DNA were extracted from contents of duodenum(D), jejunum-ileum(J), cecum(E), colon(O), feces(F) samples, respectively. 16S rRNA V4 region were amplified by PCR and sequenced using Miseq platform. The sequences of taxonomy were classified by MGRAST pipeline. The abundance of species level were selected for significant difference analysis by metastats and to calculate relative contribution. The distribution of gut microbiota were changed obviously in different intestinal positions between WT and KO mice. Significantly different species with high relative contribution were involved in host intestinal disease of Crohn's disease, colon cancer such as Lachnospiraceae bacterium A4, Alistipes finegoldii. And Alistipes putredinis, Lachnospira multipara were pertinent with host metabolism of cellulose and pectin degradation. Our results suggest *Atg5* gene plays a causal role in the changes of gut microbiota across intestinal tract, demonstrate that the genetic background of host have considerable effect, at least in part, on gut microbiota.

P3047 Enhanced effects of porcine *WFIKKN2* on muscle cell development are associated withdown-regulated *myostatin* function.. Hong Wang, Ling Sun and Xue Xu (Huazhong Agricultural University), Jinzeng Yang (University of Hawaii) and Bang Liu (Huazhong Agricultural University)

WFIKKN2 is a large secreted protein consisting of a whey acidic protein domain, a follistatin domain, an immunoglobulin domain, two kunitz domains and a netrin domain. *WFIKKN2* inhibits the function of *myostatin*, which is well known as a negative regulator of skeletal muscle mass. The gene of *WFIKKN2* in pigs has not been studied. This study reported porcine *WFIKKN2* gene and characterized two variant transcripts named *WFIKKN2TV1* and *WFIKKN2TV2*. Spatial expressions of these two transcripts in two pig breeds were analyzed and compared along with *myostatin* expressions. In the skeletal muscle tissues from different developmental phases, two transcripts of *WFIKKN2* mRNA obviously showed an opposite expression patterns in compared with *myostatin*mRNA levels. In C2C12 cell culture, the over expressions of both *WFIKKN2* transcripts revealed that only *WFIKKN2TV1* significantly up-regulated the endogeneous expression of *WFIKKN2*, and the up-regulation can be recoved by *myostatin* co-expression. When we used C2C12 as a model to detect porcine *WFIKKN2*

function in muscle and found that *WFIKKN2* promote the differentiation of C2C12 by effecting *myostatin* signal activity. *In vivo*, *WFIKKN2* gene expression was significant higher in *myostatin*+/- pigs than in WT pigs. In *Myostatin*-propeptide transgene mice with enhanced muscle mass and depressed *myostatin* function, *WFIKKN2* gene expression was obviously higher in transgenic mice than that in WT mice. In conclusion, the results from animal samples and C2C12 cell culture studies reveal that the enhanced effects of *porcine WFIKKN2* on promoting muscle cell development are associated with down-regulation of *myostatin* function.

P3048 Establishment of the swine gut microbiome during early life and impact on host phenotypes. Nuria Mach (INRA, UMR 1313 Génétique Animale et Biologie Intégrative , Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative), Mustapha Berri (UMR1282 ISP, INRA, Nouzilly, France), Jordi Estellé (INRA, UMR 1313 Génétique Animale et Biologie Intégrative , Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative; CEA, DSV-IRCM-LREG, Jouy-en-Josas, France), Florence Levenez (INRA, UMR1319 MICALIS, Jouy-en-Josas, France; AgroParisTech, UMR1319 MICALIS, Jouy-en-Josas, France), Gaetan Lemonnier and Catherine Denis (INRA, UMR 1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative; CEA, DSV-IRCM-LREG, Jouy-en-Josas, France), Claire Chevaleyre and François Meurens (UMR1282 ISP, INRA, Nouzilly, France; UMR1282 ISP, Université de Tours, Tours, France), Jean-Jacques Leplat (INRA, UMR 1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative; CEA, DSV-IRCM-LREG, Jouy-en-Josas, France), Joel Dore (INRA, UMR1319 MICALIS, Jouy-en-Josas, France; AgroParisTech, UMR1319 MICALIS, Jouy-en-Josas, France), Claire Rogel-Gaillard (INRA, UMR 1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative; CEA, DSV-IRCM-LREG, Jouy-en-Josas, France) and Patricia Lepage (INRA, UMR1319 MICALIS, Jouy-en-Josas, France; AgroParisTech, UMR1319 MICALIS, Jouy-en-Josas, France)

The gastrointestinal tract of pigs contains a complex and dynamic microbiome that influences numerous aspects of health, metabolism and performance. Composition, diversity, and dynamics of microbial communities of 31 healthy pigs were studied across 5 age strata (days d14, d36, d48, d60 and d70 after birth). Luminal secretory Immunoglobulin A (IgA) concentration was analysed at d70, and body weight was recorded over the 70 days following birth. Fecal DNA was extracted at all time points and DNA amplicons of the V3-V4 hypervariable region of the 16S rRNA gene were pyrosequenced. Generated sequences were quality filtered, normalized, and organized by phylogeny and into operational taxonomic units (OTU). On average, over 1,927 sequences per sample were obtained. Bacteroidetes,Firmicutes, and Proteaobacteria were the predominant bacterial phyla present at each age. All pigs were weaned at d28 and a shift in microbial composition was observed between d14 and d36. Overall diversity and richness were low at d14, showing dominance of *Bacteroides*, *Oscillibacter*, unclassified *Ruminococcaceae*, *Eubacterium* and *Clostridium* cluster IV genera. Bacterial composition and diversity in piglets older than 48 days showed high homogeneity relative to the first days of life, with dominance of *Prevotella* and unclassified *Porphyromonadaceae* genera. Temporal trajectory of bacterial communities from d14 up to d70, showed the presence of two main groups,

primarily distinguished by levels of *Bacteroides* and unclassified *Ruminococcaceae* (group 1) and *Prevotella* (group2). Dominance of *Prevotella* was significantly correlated to increased concentrations of luminal IgA and decreased body weight at d21 and d28 after birth. We conclude that the establishment of microbiome during early life impacts piglet phenotypes and might have long-term consequences on the adult health and performances.These findings may have a potential implication for the development of dietary strategies aimed at improving animal health and performance during the weaning period.

P3049 Integrating miRNA and mRNA expression profiling uncovers miRNAs underlying fat deposition in sheep. Guangxian Zhou, Xiaolong Wang, Chao Yuan and Xiaochun Xu (Northwest A&F University), Jiping Zhou (QingHai University), Rongqing Geng (YanCheng Teachers University) and Yuxin Yang, Zhaoxia Yang and Yulin Chen (Northwest A&F University)

MicroRNAs (miRNAs) are endogenous non-coding RNAs that regulate various biological processes including adipogenesis and fat metabolism. The aim of this study was to identify and characterize novel and conserved miRNAs, involved in fat deposition, in the adipose tissues from a fat-tailed (Kazak sheep) and a thin-tailed (Tibetan sheep) sheep breeds. We also evaluated the correlative relationship between miRNA and mRNA expression data using statistical enrichment approaches. By comparing sequencing data of Kazak sheep and Tibetan sheep, 815 mature miRNAs were identified. Of these, only five miRNAs are listed in the database of sheep miRNAs, and the other 239 miRNAs have not been previously described in this species. 539 miRNAs were expressed in the both breeds, whereas 179 and 97 miRNAs were uniquely expressed in KS and TS, respectively. Integration of miRNA and mRNA expression profiling identified candidate miRNAs target genes that are down-regulated at the transcriptional level and show an inverse correlation with the miRNA expression in the same corresponding tissues. We also provided evidences that miRNAs play roles in fat deposition through their ability to regulate fundamental pathways including cellular growth and proliferation, cellular movement and migration, Extra Cellular Matrix degradation. Our results define miRNA expression signatures that contribute to the fat deposition and lipid metabolism in sheep and other livestock for meat production.

P3050 Proliferin promotes the proliferation of murine myoblast cells. Jiawei He (Huazhong Agricultural University)

Proliferin (PLF), also known as mitogen-regulated protein (MRP), is a part of the prolactin gene family. Proliferin is an angiogenic placental hormone that involved in angiogenesis of the placenta and expressed in the placenta, hair follicles of skin and the place of wound healing. Previous studies have focused on the research of the placenta and angiogenesis, but little was known whether it promotes the proliferation of the murine myoblast cells or not. In this study, we investigated the impact of murine Proliferin on proliferation using C2C12 cells. The results showed that: 1) The expression of Proliferin was high in the proliferative phase, while reduced in the differentiated phase, which was consistent with the RNA sequencing results of myoblast cells. 2) After Proliferin interference, flow cytometry was used to analysis the rate of replicated cells. Consistent with the expectation, the rate was decreased. Besides, Proliferin detection by EDU also demonstrated a reduction in the number of replicated cells. While Xcelligence gave an intuitive cell growth curve, which showed that the proliferation was damaged in comparison to the control group. 3) No obvious change was found in the expression level

of differentiated markers after interfering or transfecting Proliferin in the differentiated phase, indicating little function for cell differentiation. 4) RNA sequencing showed that many genes involved in mitosis and cell migration were down-regulated after Proliferin interference, which explained the reason why the cell proliferation was damaged. In conclusion, our results identified that Proliferin plays a significant role in promoting cell proliferation, while has little influence on differentiation. This founding may shed light on the mechanism of muscle growth and development.

P3051 Whole-genome pooling re-sequencing uncovers selective loci among goat populations. Xiaolong Wang, Guangxian Zhou, Yu Jiang and Chao Yuan (Northwest A&F University), Hailong Yan (Yulin University), Xianyong Lan and Xiaopeng An (Northwest A&F University), Jiuzhou Song (University Maryland) and Yuxin Yang and Yulin Chen (Northwest A&F University)

Goats (*Capra hircus*) were one of the first domesticated animals for around 10,000 years. The evolution and distribution of goats are likely to strengthen our understanding of the origin and localizations of agriculture and early human civilizations. Based on their producing proposes, goats could be traditionally divided into versatile types, such as milk, meat, fibre, and skin types. Next generation sequencing using a pooled DNA samples containing multiple individuals from the same population is an effective method to investigate population variability and differentiation. Here, we conducted whole genome re-sequencing of domestic goats to identify genetic signatures of economically important traits among different types of goats. The genome sequencing yielded over 200 Gb data, and generated an average of 10-fold coverages of the goat genome using pools of genomic DNA from eight goat breeds for meat, dairy and fibre producing purposes. We have discovered more than 10,000,000 single nucleotide polymorphisms (SNPs), and around 1,000,000 insertion-deletion variations (INDELs). A number of genomic regions were identified with genome-wide significant differences in haplotype frequencies, which were consistent with selective sweeps. We further ascertained strong signals of selection and putative candidates that probably target for the contributions of coat colour, high altitude adaptation, body size, and the diameters of fibres in goats. Our results provide new insights into understanding of genetic components controlling agriculturally important traits during goat domestication, evolution and selection based on whole genome pooling re-sequencing approach in multiple populations.

P3052 Genome wide association study identifies 20 novel promising genes associated with milk fatty acid traits in Chinese Holstein. Cong Li, Dongxiao Sun, Shengli Zhang, Sheng Wang and Qin Zhang (College of Animal Science and Technology, China Agricultural University)

Detecting genes associated with milk fat composition could provide valuable insights into the complex genetic networks of genes underling variation in fatty acids synthesis and point towards opportunities for changing milk fat composition via selective breeding. In this study, we conducted a genome-wide association study (GWAS) for 22 milk fatty acids in 784 Chinese Holstein cows with the PLINK software. Genotypes were obtained with the Illumina BovineSNP50 Bead chip and a total of 40,604 informative, high-quality single nucleotide polymorphisms (SNPs) were used. Totally, 83 genome-wide significant SNPs and 314 suggestive significant SNPs associated with 18 milk fatty acid traits were detected. Chromosome regions that affect milk fatty acid traits were mainly observed on BTA1, 2, 5, 6, 7, 9, 13, 14, 18, 19, 20, 21, 23, 26 and 27. Of these, 146 SNPs were associated with more than one milk fatty

acid traits; Most of studied fatty acid traits were significant associated with multiple SNPs, especially C18:0 (105 SNPs), C18 index (93 SNPs) and C14 index (84 SNPs); Several SNPs are close to or within the *DGAT1*, *SCD1* and *FASN* genes which are well-known to affect milk composition traits of dairy cattle. Combined with the previously reported QTL regions and the biological functions of the genes, 20 novel promising candidates for C10:0, C12:0, C14:0, C14:1, C14 index, C18:0, C18:1n9c, C18 index, SFA, UFA and SFA/UFA were found, which composed of *HTR1B, CPM, PRKG1, MINPP1, LIPJ, LIPK, EHHADH, MOGAT1, ECHS1, STAT1, SORBS1, NFKB2, AGPAT3, CHUK, OSBPL8, PRLR, IGF1R, ACSL3, GHR* and *OXCT1*. Our findings provide the groundwork for unraveling the key genes and causal mutations affecting milk fatty acid traits in dairy cattle.

P3053 Noninvasive visualization of circadian metabolic rhythm in single cell level *in vivo* by Fluorescence Lifetime Microscopy. Hong Wang (State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing), Chiara Stringari (Laboratory of Fluorescence Dynamics, Biomedical Engineering Department, University of California, Irvine), Mikhail Geyfman (Department of Medicine, University of California, Irvine), Viera Crosignani (Laboratory of Fluorescence Dynamics, Biomedical Engineering Department, University of California, Irvine), Vivek Kumar and Joseph Takahashi (Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas), Enrico Gratton (Laboratory of Fluorescence Dynamics, Biomedical Engineering Department, University of California, Irvine) and Bogi Andersen (Department of Medicine, University of California, Irvine.)

Many biological and cellular processes oscillate in a circadian manner (with periods of around 24 hours), which are regulated by circadian clock. Metabolism is one of such processes and is shown to interplay with circadian clock, epigenetic regulations, cell proliferation and immunity. However, a noninvasive method to monitor the circadian oscillation of metabolism *in vivo* is still in need. Here, we have employed Fluorescence Lifetime Microscopy (FLIM) to visualize the circadian metabolic rhythm of basal epidermal stem/progenitor cells in single cell level. NADH was selected as a label-free intrinsic marker and it's lifetime, which elongates at protein-bound state, was determined. As the free/bound NADH ratio is associated with the NADH/NAD+ redox ratio, the lifetime imaging can be transformed to show the oxidative/reductive state of the cells. We show that the metabolic state of the cells are more reductive during the night and oxidative during the day. As the cells go through S-phase mainly during the night, we speculate that the circadian regulation temporally separates oxidative metabolism and S-phase to minimize ROS-induced DNA damage. We also show that the circadian redox rhythm is dependent on the core clock gene *Bmal1*. These findings have implications for health and productive performace of animals. The method can be imployed to big animals as well.

P3054 The transcriptome analysis of pig muscle tissues for growth and meat quality traits. Zhi Wang (China Agricultural University), Qing Li (Anhui Academy of Agricultural Sciences) and Peng Shang, Bo Zhang, Dong Ban and Hao Zhang (China Agricultural University)

The transcriptome research established in high-throughput sequencing has become the mainstream method for screening differential expression genes (DEGs) in whole-genomic wide. Chinese indigenous pig breeds, Diannan Small-ear pig (DSP) and Tibetan pig (TP), have genetic characteristics of lower growth rate, more lipid deposition and better meat quality than introduced pig breeds, Landrace(LL) and

Yorkshire (YY). In present study, the tissues of longissimus dorsi muscle from the four pig breeds were performed RNA-seq and miRNA-seq using Hiseq 2000 to compare the transcriptome profiles between the two Chinese pigs and the two introduced pigs for identifying the functional genes related growth and lipid deposition. We obtained 27.18 G clean data through RNA-seq and detected that 18208 genes were positive expression and 14633 of them were co-expressed in the muscle tissues of the four groups. According to the standard of fold-change (FC≥2) and difference significant (P<0.01, Fisher-test) 233 DEGs were found between the Chinese pig groups (DSP and TP) and the introduced pig groups (LL and YY), and 198 of which were enriched in function categories and pathways of David database and gathered 77 clusters. The top score of the clusters was associated with muscle fiber contraction. Base on functional clusters we identified 156 DEGs related growth traits and 90 DEGs related lipid deposition. On miRNA-seq experiment we obtained 25.65 M reads and 311 positive miRNAs, including 271 known pig miRNAs and 49 novel miRNAs. Though statistical analysis 20 miRNAs were higher expression and 10 lower in groups of DSP and TP than in groups of LL and YY. Combining the DGEs and differential miRNA, we predicted 76 DEGs related growth traits were targets of 15 differential miRNAs and 51 DEGs related lipid deposition were the targets of 18 differential miRNAs. We constructed gene and miRNA regulatory networks to elaborate molecular mechanisms of the muscle growth and lipid deposition in pigs, which provided candidate genes and pathways for further research.

P3055 A significant polymorphism in EEF1D gene affects promoter activity. Xuan Liu (China agricultural university)

Our previous genome-wide association study identified EEF1D is a potential candidate functional gene, which has a significant SNP in intron associated with fat percentage trait with P value of 3.56E-06. We then carried out next generation sequencing technology to assess the candidate target regions of GWAS, and also found two SNPs in the promoter region of EEF1D significantly associated with milk yield, fat percentage and protein percentage traits in dairy cattle. Here we performed Real-time PCR and western blot to analyze mRNA and protein expression levels of EEF1D in different tissues, it showed that EEF1D was much higher expressed in mammary gland compared with other tissues in both mRNA and protein level. For there is no polymorphism in the coding region of EEF1D, we focused on three linkaged SNPs in the promoter region, which included one significant SNP in our association study. Two EEF1D promoter-luciferase reporter constructs were generated including GGC and AAT genotypes. The reporter constructs were transfected with 293T Cells in 24-well plate. Luciferase activities were measured 40 hours after transfection as an indicator of EEF1D promoter activity. Our results suggested that there was significant difference between two different haplotypes(P=4.04E-07), which the wild-type (GGC) showed higher promoter activity than the mutant-type. We further analyzed the transcription-factor binding sites of the three SNPs and found that the mutation at significant SNP (C to T) in our association study could lead to PHO4 and c-Myb binding sites increase. In conclusion, EEF1D was considered as a novel candidate functional gene and the significant SNP in the promoter region of EEF1D might be a promising functional variant.

P3056 Transcriptome-based analysis of tissue-specific expression profiles in Guanling cattle. wei chen (Guizhou university)

Background:We investigated the gene expression profiles in the longissimus dorsi,adipose,small intestine,liver,heart ,and hind shin tissues of 18-month-old Guanling cattle using the

Affymetrix Bovine Genome Array.Actively transcribed and tissue-specifically expressed genes were identified in the various tissues that reflected the biological functions of the tissues at the molecular level.The significance Analysis of Microarrays(SAM) software was used to identify the differentially expressed genes,and gene ontology and pathway analyses were conducted using the web-based Molecular Annotation System,version 3.0.

Result:A total of 24 128 genes were shown to be actively transcribed in the six tissue types,and the SAM analysis showed that a total of 598 genes were differentially expressed in the various tissues examined.These genes were predominantly involved in cell-cell adhesion(cell adhesion molecules),collagen fibril organization and synthesis, immune responses,and cell-matrix adhesion(extracellular-matrix receptors).The tissue-specific expression of 11 genes identified in the microarray analysis was quantified using reverse transcription and polymerase chain reaction.

Conclusion:The comparative analysis of the tissue transcriptomes revealed important information regarding the function of the differentially expressed genes that can serve as the basis for future experiments aimed at elucidating the associated molecular mechanisms that may be related to skeletal muscle growth and meat quality traits in cattle.

P3057 Differential expression of *Fsp27* gene in subcutaneous adipose of Mashen pigs and Yarkshire pigs. Pengfei Gao, Qingchun Yang, Zeyi Wang, Zhongde Pu, Xiaopei Zhao, Xiaojing Wang, Hong Liu, Xiaohong Guo, Guoqing Cao and Bugao Li (College of Animal Science and Veterinary Medicine, Shanxi Agricultural University)

The increasing of lipid droplet plays a crucial role in fat deposition and metabolism. Fat specific protein 27 (Fsp27) is one of the important regulatory proteins in lipid droplets formation, which effect the selective deposition and metabolism of triglyceride greatly. The expression profiles of *Fsp27* gene in subcutaneous adipose was analyzed by quantitative real-time PCR and Western blotting from Mashen and Yarkshire pigs at different developmental phases. The results revealed that the expression profile was similar between Mashen and Yarkshire pigs, except for the various abundance at the different time points. *Fsp27* mRNA expression level was very weak at first then increased, and reached its highest at 6-month-old, which was significantly higher than other stages($P<0.01$). The expression level of *Fsp27* in Yarkshire pigs was higher than that of Mashen pigs at 4-month-old ($P<0.01$), but lower in other months ($P<0.05$). Western blotting results showed that the highest level of Fsp27 protein expression of Yarkshire pigs occurred at 5-month-old, which was significantly higher than that of the other time points ($P<0.05$). The protein expression of Mashen pigs was higher at early development stagesand reduced to its minimum at 5-month-old, which was significantly lower than that of the other time points ($P<0.01$). The results suggested that the expression of Fsp27 are different between Yarkshire and Mashen pigs, which provide theoretical basis for further study the regulatory mechanism of lipid metabolism.

P3058 Profiling of microRNAs coded by mitochondrion in different equine tissues. Nuria Mach, Xavier Mata, Marine Beinat, Anne Vaiman, Marco Moroldo, Jerome Lecardonnel, Rachel Legendre and Sylvain Marthey (INRA, UMR 1313 Génétique Animale et Biologie Intégrative , Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative), Sean Kennedy (INRA, Micalis, UMR1319, Jouy-en-Josas, France), Laurent Schibler (INRA, UMR 1313 Génétique Animale et Biologie Intégrative , Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative) and Eric Barrey

(INRA, UMR 1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France; AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative; Inserm, UBIAE U902, France)

Mitochondrial microRNAs (miRNAs) are emerging as important mediators of post-transcriptional gene regulation in many cellular processes beyond energy metabolism and apoptosis. In order to identify mitochondrial miRNAs and assess their expression patterns and functions, we sequenced small RNA libraries from 6 different equine tissues (*platysma*, *gluteus medius* and masseter muscles, as well as heart, liver and cartilage). Reads in the range of 17-27 bp were retained and mapped to the reference horse genome and to known pre-miRNAs deposited in miRBase19. The analysis showed a total of 17 putative novel miRNA that were uniquely found in the mitochondrial genome. Seven of these novel miRNAs mapped to the 16S rRNA, 6 to different tRNAs, 3 to genes from subunits of complex IV, and 1 to the DLOOP locus. Only one of the predicted pre-miRNA sequences was significantly folded into a duplex structure and presented reads that corresponded to both 3' and 5' arms of the putative pre-miRNA hairpin. After data normalization, further analysis showed that the expression levels of 5 putative novel miRNAs were significantly different between tissues, suggesting that their expression may be specific of cell type and functions. Most of the novel miRNAs were preferentially expressed in *masseter* and *gluteus medius* muscles. A subset of 8 putative novel miRNA was selected and further confirmed by using a custom Agilent equine miRNA microarray. *In silico* analysis based on the combination of miRNA-mRNA interactions are in progress. Because we observed that most of the putative novel miRNAs in mitochondria did not derive from hairpin-forming precursor, functional experiments will be required to characterize miRNA generation and processing in the mitochondria. This study expands the body of putative miRNAs known to be related to the mitochondrion.

P3059 CAPN1 gene associate with water-holding capacity of skeletal muscle in chicken. Yaqiong Ge and Xuemei Deng (China Agricultural University)

As the main proteolytic enzyme in cytoplasm, CAPN1 plays an important role in myofibrillar degradation. In this experiment, six pure lines of Recessive White Plymouth Rock, Shouguang, Tibetan, CAU Brown, White Leghorn chickens were employed. Genomic DNA was extracted from vein blood for Sequenom Mass-ARRAY genotyping of the SNPs in CAPN1 gene. Four SNPs were found significantly associate with drop loss of breast muscle and two of them associate with abdominal fat weight. Three haplotypes were built according to the four SNPs: H1H1, H1H2 and H2H2. We found H1H1 has higher drip loss than H1H2 ($p<0.01$) and has higher abdominal fat than H1H2 and H2H2. Expression of CAPN1 in breast muscle shows that H1H1 expressed lower than H1H2 ($p<0.01$).

P3060 Comparative Transcriptome Analysis between Fast and Slow Twitch Skeletal Muscle in Yorkshire pigs. Lu Jing (Huazhong Agricultural University)

The fiber type composition of a given muscle contributed significantly to its meat quality. Red muscles, such as soleus and semitenderness, contain much more slow oxidative muscle fibers (type I and type IIA) and are rich in myoglobin and oxidative enzymes, whereas white muscles, such as extensor digitorum longus and longissimus dorsi, contain more fast glycolytic fibers (type IIX and type IIB) with abundant mitochondria and glycolytic enzymes. In this study, we analyzed the myofiber composition of soleus (slow-twitch muscle) and extensor longus digitorum (fast-twitch muscle) from three

10-week-old male Yorkshire pigs by immunohistochemistry and qRT-PCR assay, revealing an extreme difference of the ratio of type IIb fibers. With Affymetrix GeneChip, we identified 395 differentially expressed (DE) genes with at least 1.5 fold change at $p < 0.05$ level, including 190 transcripts highly expressed in slow muscle and 205 transcripts highly expressed in fast muscle. Quantitative real-time PCR analysis confirmed the expression pattern of ALDH2, PGM1, ACSDL1, FBP2, ACAA2, XIRP1 and CSRP3. We found that 152 DE genes were distributed in 123 myofiber-related QTL regions, of which 51 genes showed more than 2-fold change. The GO biological process analysis revealed ten significant pathways, including glycolysis/gluconeogenesis, TGF-beta signaling pathway, insulin signaling pathway, fatty acid metabolism, starch and sucrose metabolism, focal adhesion, etc. These results provide new insight in identification of functional candidate genes involved in muscle fiber type determination. We have identified a number of differentially expressed candidate genes including some members in Insulin signaling pathway, Fatty acid metabolism, Glycolysis / Gluconeogenesis 8 significant pathways were which might play an important role in different type of skeletal muscle fiber and lead to their distinct physiologic and metabolic characteristics. These results provide potential to identify new functional genes regulate fiber type determination.

P3061 A novel alternative splicing molecule of Wnt10b and the expression in sheep fetal fibroblast cells. Zu Yang (China Agricultural University)

China Wnt10b is known as a strong candidate for the “First Epithelial Signal” operating in hair follicle morphogenesis and self-renewal of hair follicles. To investigate the molecular effect of Wnt10b in Merino sheep skin, we built vectors with 5’-deletion fragments and CDS region of the gene and performed transient expression in sheep fetal fibroblast cells (SFFCs). The expression of 5'-deletion fragments revealed that Wnt10b gene is driven by a TATA-less promoter. While, the sequence from +1bp to +548bp (taking ATG as +1 nt) inhibit gene expression. We identified Wnt10b-AS, a novel splice variant lacking of exon 4, containing frame shift mutation leading to a putative shorter protein, in Merino sheep. We analyzed dynamic subcellular localization process of fusion proteins and found that the Wnt10b-EGFP firstly appeared in the cytoplasm, which then moved across the plasma membrane, and finally presented in extracellular matrix. This process was similar to the localization patterns occurred in human and mouse. However, Wnt10b-AS-EGFP was not observed in any of the process indicating no active Wnt10b-AS protein produced. RT-PCR analysis was used to identify the Wnt signaling pathway and keratin (Keratin 2.12 and K2.11 were used as the active growth phase markers of hair follicle) response to Wnt10b and Wnt10b-AS over expression in SFFCs. The results showed that Wnt10b can active Wnt signaling pathway and keratin expression in vitro. However, the Wnt10b-AS was highly efficient in Wnt signaling pathway but inefficient in keratin expression. We predict that Wnt10b-AS might exist at transcription level, having similar functions with Wnt10b but via different molecular mechanism.

P3062 The Screening of Transcription Factor Binding Sites of Myod Ⅰ gene promoter in Guanling cattle. Wen Zhang (Guizhou University)

【Objective】This study is to screen out transcription factor binding sites of Myod I gene promoter in Guanling cattle, which Provide theoretical basis for the subsequent experiment and basis for the regulation mechanism of Myod I gene promoter in Guanling cattle.【Method】This study using Promoter - Binding TF Profiling

Assay (I), nuclear extracts of muscle tissue from Guanling cattle and recycled and purified the purpose segments of Myod I gene Promoter to screen out transcription factor binding sites of Myod I gene promoter in Guanling cattle. At the same time, synthesizing the predicting result of transcription factor binding sites of Myod I gene promoter with online software to eventually screen out the transcription factor binding sites of Myod I gene promoter in Guanling cattle. 【Results】I preliminary screen out transcription factor binding sites of Myod I gene promoter in Guanling cattle. They are SRF, YY1, CDP, FAST - 1, NF - 1, MEF2.

P3063 Functional analysis of candidate interactions between miRNAs encoded by the pseudorabies virus (PrV) and pig genes. Nada Mahjoub and Sophie Dhorne-Pollet (Institut National de la Recherche Agronomique, AgroParisTech, UMR1313 Animal Genetics and Integrative Biology.), Gail Scherba (Department of Pathobiology, College of Veterinary Medicine, University of Illinois), Thomas Mettenleitter (Friedrich Loeffler Institut, Institute of Molecular Biology) and Elisabetta Giuffra (Institut National de la Recherche Agronomique, AgroParisTech, UMR1313 Animal Genetics and Integrative Biology.)

MicroRNAs (miRNAs) post-transcriptionally repress gene expression and are active against a large proportion of the transcriptome. Herpesviruses produce latent infections and are completely dependent upon latency as mode of persistence. Most herpesviruses encode miRNAs with targets in their own genomes and that of their host. Among alpha-herpesviruses, the Pseudorabies (PrV) genome contains 11 miRNA genes clustered in the Large Latency Transcript (LLT) region.
We use the pig-PrV system as a model to study and validate the biological role of viral miRNAs. Previously, a PrV BAC clone was depleted of a cluster (2.5 Kb) of nine miRNA genes. An in vivo experimental infection was carried out to compare the ability of a mutant clone to establish latency in porcine trigeminal ganglia. The Pence of miRNAs did not hamper the establishment of latency. However, animals infected by the mutant virus carried an increased number of viral particles in ganglia compared to those infected with the wild type virus, and the ganglia displayed an altered expression pattern of host cell mediated immune response. Some differentially expressed genes carried multiple targets for most PrV's miRNAs, suggesting that they could be modulated by these miRNAs during the dynamics of PrV's latency (N. Mahjoub, S. Dhorne-Pollet, W. Fuchs, M-L. Endale Ahanda, A. Arya, J. Loveland, F. Lefevre, T. Mettenleiter, E. Giuffra. To be submitted).
The OBGF400 (porcine neuroblast) and PK15 (porcine epithelial) cell lines allow exploring miRNA-mRNA interactions in two distinct transcriptomic backgrounds. We are using RISC-IP (Immunoprecipitation of the RNA-Induced Silencing Complex) to figure out the functionality of PrV's miRNAs and the co-enrichment in RISC of previously identified candidate host gene targets. Preliminary results indicated that LCT4 (leukotriene C4 synthase) is enriched 3.7 time in the RISC of OBGF400 cells upon PrV-mit-LLT1 mimics. Luciferase assays are being performed to support the most relevant results

P3064 MicroRNA-128 promotes myoblast differentiation in myogenesis. Lei Shi, Pinghua Li, Han Wang, Huizhi Li, Lingling Fu, Lichun He, Lei Tang, Ruihua Huang and Bo Zhou (Institute of Swine Science, Nanjing Agricultural University)

MicroRNAs play critical roles in skeletal muscle development. In order to elucidate the biology function and potential molecular mechanism of miR-128 in myogenesis, we constructed a plasmid which contains the target region of *Myostatin* (*MSTN*) bound by miR-128,

determined the expression profiles of miR-128 during myoblasts differentiation, introduced the precursor, mimics and inhibitor of miR-128 into C2C12 cells, and induced the C2C12 myoblasts differentiation. The mRNA and protein expression levels of myogenic markers (*Myf5*, *MyoG*, *MyHC*, *MSTN* and *Pax3/7*) were determined by RT-qPCR and Western blot, respectively. Protein of myogenic markers *MyHC* and *MSTN* was detected using immunofluorescence. Meanwhile, the dual luciferase reporter gene assay was carried out to validate putative target gene (*MSTN*) of miR-128.The results show that miR-128 was increased from the day 0, 1, 3, to 5 during myoblasts differentiation. Overexpression of miR-128 increased the expression of myogenic markers (*Myf5*, *MyoG*, *MyHC*, and *Pax3/7*) in mRNA levels ($P<0.05$), as well as the number of *MHC*-positive cells when transfected with mimics and precursor of miR-128. We also found that the protein expression of *MSTN* was decreased ($P<0.05$), while there was no remarkable change in the mRNA levels ($P>0.05$). Blocking the function of miR-128 by an antisense oligonucleotide inhibitor led a down-regulation of myogenic markers (*Myf5*, *MyoG*,) in mRNA levels. But mRNA levels of myogenic markers (*MyHC*, *MSTN* and *Pax3/7*) and the number of *MHC*-positive cells did not show notable change. In addition, the fluorescence activity of double fluorescence report plasmid was reduced ($P<0.05$) after co-transfection with mimics of miR-128.Taken together, these data suggest that miR-128 promotes myogenic differentiation of C2C12 cells and might target *MSTN*.

P3065 Transcriptome expression profiles and their association with muscle phenotypic traits in Texel fetal sheep carrying *g. +6723A* of *myostatin*. li LI (Sichuan Agricultural University)

Mutations in myostatin result in the dramatically muscle accumulation through increase of both myofiber number (hyperplasia) and myofiber size (hypertrophy). Currently MSTN is widely studied in postnatal animal, but myofiber number is generally set prenatally. Hence specialized analysis to systematically uncover the signaling mechanisms through which myostatin promotes skeletal muscle prenatally remain required.Using 16 Texel sheep fetuses harvested at five stages between day 70 and 135 of gestation, body weight, body length, and m. longissimus dorsi (LD) weight was measured directly; myofiber size and number were assayed via H.E. staining. We also genotyped the polymorphism of g. +6723G>A locus using sequencing and RFLP, examined the transcriptome in LD using microarray and validated with qPCR, identified the differentially expressed (DE) probes and subsequently clustered DE probes and annotated their enriched function by employing rMutilExperiment Viewer, STEM, and DAVID respectively.Three out of 16 fetus were AG genotype, others were AA at g. +6723G>A of myostatin. Their phenotypic traits valued increasingly with age except for myofiber density. Total 1 758 DE probes were identified and clustered into ten expression profiles, of which four containing 80% (1 391/1 758) were positively associated with phenotypic traits excluding myofiber density, reversed associations were found in the remaining. The most enriched functions in Group 1 were mitochondrion structure and metabolic process, while those in Group 2 included DNA replication and cell cycle. Noteworthy, some genes, like PDGFB and TGFB3 in Group 1, ARNT2 in Group 2, tethered GO terms on response of estrogen with others including muscle development and apoptosis. Conclusively, myofiber hypertrophy and hyperplasia are simultaneously governed by distinct gene cascade during the late gestation likely under the regulation of estrogen, with the dominance in hypertrophy involved four fifth DE probes. These results will contribute to the understanding of the muscle development and

myopathy.

P3066 Chronic administration of clenbuterol causes liver fibrosis besides its energy redistribution effect. Suyun Fang and yiqiang zhao, ning li and xiaoxiang hu (State Key Laboratories for Agrobiotechnology, College of Biological Sciences, China Agricultural University)

11 cloned barrows aged 200±1 days were administered continuously for 34 days with 5mg/d/pig Clenbuterol (CLB), a β2 specific adrenergic agonist widely used to promote livestock growth .Pigs were weighed every week. CLB increased the muscle gain by 8.35% and reduced the fat deposition by 8.36% with little impact on dressing percentage. The adipose tissue was utilized to perform paraffin section and the adipocyte cell size was verified to be decreased. We were surprised to find that 3 of the 6 treated pigs were accumulated by fat droplets in liver tissue through frozen section and oil O staining. We then conducted RNASeq with this 3 liver tissues and their 3 corresponding controls. Finally we found 72 different expressed isoforms, 61 of which were corresponding to known genes. 14 ones were involved in Synthesis and metabolism of fat. we also found the keratin genes were different expressed. This suggests CLB may affect the liver function and even cause liver fibrosis besides its energy redistribution effect.

P3067 Applying interleukin 6 gene promoter in overexpression toll-like receptor 4 transgenic sheep specifically regulate inflammatory responses in lipopolysaccharide-activated lymphocytes. Tongtong KAN (Beijing Key laboratory of Animal Genetic improvement, College of Animal Science and Technology, China Agricultural University)

Background: Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria which often cause diseases by elicit strong immune responses in sheep and lead to great losses. Toll-like receptor 4 (TLR4) can be bind by LPS and then activate the inflammatory responses. Two eukaryotic expression vectors (pCMV-TLR4 & pIL6-TLR4) were constructed into the production of TLR4 overexpression transgenic sheep. Methodology: Experimental group: pIL6-TLR4, control group: pCMV-TLR4 and wild-type. Transient transfected pEGFP-N1, pCMV-TLR4 and pIL6-TLR4 plasmid into sheep fetal fibroblasts. Microinjected the exogenous gene structures into fertilized eggs. Stimulated the fetal fibroblasts and peripheral-blood lymphocyte isolated from the 3 groups of sheep with 1000ng/mL LPS. Determined the expression of TLR4 mRNA and protein of the fibriblasts and lymphocyte. After that several important cytokines (TNF-α, IL-6, IL-8, IL-10, IFN-γ) in lymphocyte that have different functions in inflammatory responses were detected. Then detected several oxidative stress factors (NOS, NO, GSH, GSSG, ROS, MDA) that can be act as indicators of oxidative damages. Principal Findings: Overexpression of TLR4 gene was observed in transgenic sheep. The expression peak of TLR4 in fetal fibroblasts and lymphocyte with IL-6 promoter were at 4h ($p<0.01$) earlier than the other two groups. The expression of TLR4 with IL-6 promoter had the same variation with IL-6 mRNA expression. The expression of pro-inflammatory factors, chemotactic factors and anti-inflammatory factors of experimental group were all had a relatively high level after 4h ($p<0.05$). The generation of oxidative stress factors of pIL6-TLR4 transgenic sheep kept a middle level between wild-type sheep and pCMV-TLR4 transgenic sheep. Conclusions: The use of IL-6 gene promoter in overexpression TLR4 transgenic sheep can regulate the expression time and quantity of relative cytokines through its regulation of TLR4 gene expression in inflammatory responses, which suggested that it

can specifically regulate the inflammatory responses.

P3068 High definition epigenomic mapping and transcriptome analysis of butyrate-induced epigenomic landscape modification and dynamic genomic activities. Congjun Li (ARS, USDA)

Volatile short-chain fatty acids are nutrients especially critical to ruminants. VFAs, especially butyrate, affect cell differentiation, proliferation, and motility. Butyrate is a potent inducer of histone hyper-acetylation in cells and provides an excellent in vitro/in vivo model to study the epigenomic regulation of gene expression through its inhibition on histone deacetylases (HDACs). We analyzed in vitro and in vivo differential expression of genes induced by butyrate and the effect of butyrate on alternative splicing induced by butyrate in the bovine epithelial cell using deep RNA-sequencing technology (RNA-seq). In addition, the in vivo transcriptional effect of butyrate on the transcriptome of the rumen epithelium was quantified via serial biopsy sampling using high-throughput RNA-seq technology and bioinformatic tools. 216 differentially expressed transcript isoforms regulated by butyrate were detected. Utilizing next-generation sequencing technology, combined with chromatin immunoprecipitation (ChIP-seq) technology, we constructed a high-definition map of the epigenomic landscape with normal histone H3, H4, and their variants in bovine cells at the whole genome scale. We analyzed the enriched binding regions in the proximal promoter (within 5 kb upstream or at the 5'untranslateded region (UTR) from the transcriptional start site (TSS), and the exon, intron, and intergenic regions (defined by regions 25 kb upstream and 10 Kb downstream from the TSS. A de novo search for the binding motif of the 10 ChIP-seq datasets discovered numerous motifs from each of the ChIP-seq data sets. These consensus sequences indicated that histone modification at different locations changes the histone H3 and H4 binding preferences. A high degree of conservation in histone binding was also presented in these motifs. This first extensive epigenomic landscape mapping in bovine cells offers a new framework and a great resource for testing the role of epigenome in cell function and transcriptomic regulation.

P3069 Regulatory network of *IRX3* gene inferred from muscle transcriptome data in Iberian pigs. Cristina Ovilo, Almudena Fernández, Rita Benítez, Yolanda Núñez, Ana Fernández and Luis Silió (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (SGIT-INIA))

The *IRX3* gene (Iroquos Homeobox 3) codes for a transcription factor which has been recently discovered as a major genetic regulator of tissue development, body composition and obesity in human. Additionally, a *l.dorsi* transcriptomic analysis in pure Iberian and crossbred Duroc x Iberian piglets suggested a role for *IRX3* as regulator of growth and adiposity in pig muscle. Nevertheless, there is still little knowledge about its function in different species and tissues. Our aim was to investigate how *IRX3* coexpress with genes and molecules which it may regulate and identify its potential biological pathways and downstream targets in pigs. Using global transcriptomic data of *l.dorsi* muscle of 28 pure and crossbred Iberian piglets we identified 284 probes, corresponding to 258 unique known genes, which expression is significantly dependent on *IRX3* expression (FDR<0.05). Also, the expression values of 224 out of these 258 genes were significantly correlated with those of *IRX3* gene (FDR<0.05). Functional classification shows a net of pathways involved in developmental processes, response to stimulus, insulin signaling, immune and inflammatory response, regulation of gene expression and regulation of biosynthetic processes. We

identified transcription factor binding sites (TFBS) for *IRX3* in 46% of the gene promoters. The number of TFBS detected was significantly higher than expected by chance (*P*=7x10-11), suggesting that an important proportion of the detected genes could be downstream targets of *IRX3* regulation. Even more, 10 out of the identified genes with TFBS for *IRX3* were found to be transcriptional regulators. Differential coexpression between genetic types was studied for the pairs of 258 identified genes. Significantly different correlations (FDR<0.05) between genetic types were observed for 125 pairs, involving 104 genes. These include some key molecules and transcription factors with known roles in growth, adipogenesis and lipid metabolism, as *RXRG*, *CEBPD*, *GHR*, *MAX* or *MXI1*.

P3070 Efficient generation of pig iPS cells by the non- integrated episomal system. Shuangyu Ma (China Agricultural University)

Efficient generation of pig iPS cells by the non-integrated episomal system Shuangyu Ma, Xuguang Du, Sen Wu, Ning Li State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China Cell reprogramming is a process which differentiated cells can be reversed to a pluripotent or totipotent stage by nuclear transfer, cell fusion, or induced pluripotent stem cells(iPSCs). Because iPSCs are very similar to mES cells in many features, including expression of pluripotency markers, reactivation of both X chromosomes, the ability to generate chimeras and tetraploid complementation, moreover, they avoided many problems of the ethics and morality. So, they are important for both basic research and cell therapy. Induced pluripotent stem cells (iPSCs) can be generated by defined transcription factors. Remarkably, using OSKM transcription factors can successfully induced both mice and human iPS cells. Since pigs are very similar to human with the physiological characteristics and organizational structures. Thus it is necessary to generate the pig or other large animals iPSCs. Traditional retrovirus and lentiviral vector, carrying a large number of copies , have the risk of tumor or interfering normal gene expression, on the other side, more safe methods including protein and mRNA are very low efficiency. Therefore, non- integrated and efficient strategy is very important. Episome, a stable extrachromosomal replication vector, can inherit via cell division, during the passaging, it will be gradually lost, about 5% lost per generation. We have already successful induced pig iPSCs(piPSCs) by episomal system. The piPSCs had dense and smooth surface, correct karyotype, immunofluorescence analysis also showed pluripotent markers. These cell lines could be differentiated into the three germ layers in teratoma. In conclusion, our experiment aims to obtain efficient and safe piPSCs by non-integrated episome system. It will be great significance on the establishment of human disease and xenotransplantation pig models.

P3071 Whole-genome sequence of half-smooth tongue sole (*Cynoglossus semilaevis*) reveals the origin of ZW sex chromosome evolution and mechanism for sex reversal. Songlin Chen (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences), Guojie Zhang (BGI-Shenzhen), Changwei Shao (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences), Quanfei Huang and Geng Liu (BGI-Shenzhen), Wentao Song (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences), Na An (BGI-Shenzhen), Zhenxia Sha, Na Wang and Qiaomu hu (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences), Manfred Schartl (Physiologische Chemie I, University of Würzburg), Qisheng Tang (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences) and Jun Wang (BGI-Shenzhen)

In this study, we sequenced and assembled the genome of a flatfish, half-smooth tongue sole. Based on the difference of sequencing depth of Z/W linked-scaffolds between female and male, together with the high-resolution genetic map constructed by SSR and SNP, we assembled the Z and W chromosome of tongue sole. Using the Z-W homologous genes, we predicted that the age of the tongue sole sex chromosome pair is relatively young (~30 million years), which suggests a rather fast evolution of the tongue sole sex chromosomes.To ascertain the evolutionary trajectory of the tongue sole genome, we addressed the phylogenetic branching and divergence time by global alignment of tongue sole, tetraodon, medaka, and zebrafish, using human and chicken as outgroups. We found that the sex chromosomes of tongue sole are derived from the same ancester as chicken, but not as any known fishes. Notably, the sex chromosome of tongue sole and chicken showed convergent evolution, and Z chromosome of tongue sole exhibited partial dosage compensation in female as that of chicken. Moreover, one Z chromosome-linked gene, *dmrt1*, was found to be specifically expressed in male and required for testis development, showing the features of male-determining gene.Female grows 2-4 times faster than male. By comparing female/male genome, some sex-linked SSR markers were found and used to develop molecular technique for genetic sex identification of ZZ male, ZW female and WW super-female. We found that phenotypic female accounts for only 10-30% in cultured populations. After the analyses of genetic and phenotypic sex ratios in different families, we found more than 90% of pseudo-male offspring sex-reverted into a pseudo-male. Whole-genome methylation sequencing revealed that all second-generation pseudomales had inherited the Z chromosome from their sex-reversed fathers and retained the paternal methylation pattern, implying that trans-generational inheritance of DNA methylation status is particularly important for the inheritance of sex reversal.

P3072 Molecular characterization and developmental potential of mammary gland derived cells in goat (*Capra hircus*). Peter Dovc, Sonja Prpar Mihevc and Jernej Ogorevc (University of Ljubljana, Biotechnical Faculty)

The primary culture of mammary gland derived cells originating from goat (*Capra hircus*) females in different stages of lactation has been established and cells were grown on a thin layer of basement membrane matrix resembling *in vivo* conditions. The cell culture was examined morphologically, using immunohistochemistry and at transcriptional level using RNA sequencing and RT-PCR approach. The most prominent cell types in the primary culture were luminal, myopeithelial and mesenchymal cells. However, a smaller part of the cell population represented different types of precursor cells with developmental potential. The luminal cells were characterized by expression of *CK6A, CK18, CK19, Muc1* and *CSN2* genes whereas the myoepithelial cell fraction expressed*CK14, CK5, CK17* and *p63*. The precursor cells with stem cell character expressed *CD24, CD29, CD49f* and *EpCAM*. In addition, the stromal cells in the primary culture were characterized by expression of *smooth muscle actin, vimentin, catenin beta-1, SPARC, fibronectin 1, NOV, TGFBR2, ADAM, MMP* and *SFRP1*. RNA sequencing was employed to determine expression of lineage-specific markers. The highest level of expression was observed for markers typical for luminal, myoepithelial and mesenchymal cells. In addition, expression of markers associated with stem/progenitor character was confirmed. Based on our characterization we can conclude that established primary culture is composed mainly of epithelial (luminal and myoepithelial) and stromal cells. In the *in vivo* transplantation experiment, the regenerative potential of epithelial precursor cells was demonstrated. The enriched precursor cell population was

transplanted under the kidney capsule of the NOD-SCID mice, where alveolar structures producing milk proteins were formed. Based on typical cobblestone morphology, formation of typical cytoplasmic network of cytokeratin fibers and expression of typical molecular markers we can conclude that the established goat mammary gland derived cell line represents a good *in vitro* model for studying mammary gland development, differentiation and lactation.

P3073 Metabolic profiling of the prefrontal cortex and serum of cattle with divergent temperament types. Bodo Brand, Bettina Brandt and Frieder Hadlich (Leibniz Institute for Farm Animal Biology), Dirk Repsilber (Örebro universitet), Nicolas Schauer (Metabolomic Discoveries GmbH) and Siriluk Ponsuksili and Manfred Schwerin (Leibniz Institute for Farm Animal Biology)

Cattle temperament has been shown to affect handling, performance, health and reproduction. Calmer cattle are easier to handle and habituate, show higher growth rates and have a more robust immune response to pathogens in comparison to more excitable cattle. Although the number of studies investigating temperament in farm animals has increased greatly in the past decade, molecular pathways underlying temperament and molecular pathways linking temperament to production traits, health and reproduction have yet to be studied in detail. In this study a metabolic profiling of the prefrontal cortex and serum of cattle of extreme temperament types was performed to gain further insights into the molecular divergence between cattle temperament types. Therefore, 25 cows were selected from a total of 184 cows of the F2 generation of a German Holstein X Charolaise cross based on their temperament type assigned in accordance to their behavior in a novel object and a novel human test. In the prefrontal cortex and serum of these animals untargeted comprehensive metabolite profiling identified 627 and 1097 metabolite features comprising 235 and 328 putatively known metabolites, respectively. Fifty-one and fifty-four of these metabolite features were identified to have a high relevance in the classification of temperament types using a sparse partial least square discriminant analysis. A clear discrimination between "fearful/neophobic – alert", "interested – stressed", "subdued/uninterested – calm", and "outgoing/neophilic – alert" temperament types could be observed based on the abundance of the relevant metabolites in both the prefrontal cortex and serum. Putatively known metabolites with high relevance in the classification of temperament types revealed that the main differences between temperament types were related to the abundance of glycerophospholipids, fatty acyls and sterol lipids overall indicating differences in the stress and fear responsiveness of the animals.

P3074 Multiple lines of transgenic mice shed new light on the molecular mechanisms underlying the callipyge phenomenon.. Haruko Takeda (University of Liège), Xuewen Xu (Huazhong Agricultural University), Dimitri Pirottin (University of Liège), Huijun Cheng (Huazhong Agricultural University), Noelle Cockett (Utah State University) and Carole Charlier and Michel Georges (University of Liège)

The callipyge phenotype is a muscular hypertrophy of sheep that is characterized by polar overdominance: only heterozygous animals inheriting the *CLPG* mutation from their sire express the phenotype. The *CLPG* mutation inactivates a silencer that normally down-regulates genes from the imprinted *DLK1-GTL2* domain in *cis* in postnatal skeletal muscle. Consequently *+/CLPG* animals overexpress the paternally expressed *DLK1* and *PEG11* in muscle at the mRNA/protein levels, and this is thought to cause the phenotype. In *CLPG/CLPG* animals, *DLK1* and *PEG11* are

presumably *trans*-inhibited by maternally expressed non-coding RNAs, accounting for their wild-type phenotype. Transgenic mice expressing ovine DLK1 in muscle have a muscular hypertrophy supporting a role for DLK1 in determining the callipyge phenotype. In *CLPG/CLPG* animals, *PEG11* was shown to be sliced by miRNAs processed from anti-*PEG11* confirming the non-coding RNA-mediated trans-inhibition.

To further characterize the phenomenology we generated additional transgenic mouse lines. We first knocked the *CLPG* mutation in the mouse genome. We show that the *cis*-effects of the mutation observed in sheep are recapitulated on the distal (i.e. on *PEG11*when paternally inherited, on *GTL2*, anti-*PEG11*, *RIAN* and *MIRG* when maternally inherited) but not proximal side (on *DLK1* when paternally inherited), that the *trans*-effect of the mutation is not observed for *DLK1*, and that *+/CLPG* mice are phenotypically normal. We then generated ovine PEG11 transgenic mice. These expressed a muscular hypertrophy of similar magnitude as the DLK1 transgenic mice. There was no evidence for a synergistic effect in double DLK1/PEG11 transgenic mice.

These results functionally confirm the causality of the *CLPG* mutation, and suggest that ectopic expression of *PEG11* contributes to the muscular hypertrophy of callipyge sheep. That *+/CLPG* mice are phenotypically wild-type is attributed to the lower expression of PEG11 in these mice when compared to callipyge sheep and PEG11 transgenic mice.

P3075 Identification of inflammatory cytokine gene in osthole-treated peripheral blood mononuclear cells from Hanwoo (Bos taurus coreanae). Bong-Hwan Choi (National Institute of Animal Science, RDA, South Korea)

Osthole, a natural coumarin compound used in traditional Chinese medicines, exerts an anti-inflammatory effect, but its effects in cows remain known. In this study, the effect of osthole on lipopolysaccharide (LPS)- or concanavalin-A (Con-A)-stimulated peripheral blood mononuclear cells (PBMCs) was assessed. Jugular venous blood was collected from Korean calves, and PBMCs were isolated. They were then used to study the immune response of PBMCs to treatment with osthole and LPS or Con-A for 72 h by measuring inflammatory cytokines including tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in gene and protein levels. Osthole significantly inhibited the mRNA secretion of TNF-α and INF-γ in a dose-dependent manner. Therefore, osthole inhibited LPS- or Con-A-induced TNF-α and Con-A-induced IFN-γ production significantly in dose-dependent manner. These results clearly suggest that osthole inhibited the LPS- or Con-A-stimulated upregulation of pro-inflammatory cytokines in a dose-dependent manner, without causing obvious cytotoxic effects. Osthole could also protect cows from LPS- or Con-A-induced endotoxin shock, possibly by inhibiting the production of pro-inhibitory cytokines, which suggests that osthole might be a novel therapeutic agent for the prevention of inflammatory diseases. These results should increase our knowledge of bovine immunity.

P3076 Study on the *Gnαq* expression in different color skin of mouse. Zhihong Yin (Shanxi Agricultural University)

In recent years, people had begun to pay attention to the G protein signal transduction pathways, which involved in the color of hair. *Gnαq* is a subunit of heterotrimeric G proteins, which plays an important role to the endothelin B receptor in melanoytes . Studies have shown that transgenic expression of the endothelin receptor B ligand results in hyper-pigmentation in human skin and hair, and makes the hair darken.

In order to investigate the role of the *Gnαq* in the formation of mammalian skin color, this

experiment analyzed the quality of the *Gnαq* in different color of mouse. 1. We examined the mRNA relative quantity of the *Gnαq* in diffenert color skin tissues by QRT-PCR, which that the relative expression of the *Gnαq* mRNA in black skin tissues was 2.73 times that in white skin tisses. We hypothesize that the *Gnαq* is connected to the hair color. 2. The *Gnαq* protein was analyzed in different hair color of skin tissues by Western blotting. The data revealed that the *Gnαq* is expressed in both types of skin tissues. Furthermore, the expression level of the *Gnαq* in black skin tissues was higher than in white skin tissues.
In conclusion, the *Gnαq* is present in both white and black skin tissues, and there is definite difference. Thus, We can conclude that the *Gnαq* is connected to pigment formation in different hair color.

P3077 Functional analyses of gene regulation co-expression networks reveal the biology of muscle expression. Siriluck Ponsuksili, Puntita Siengdee, Yang Du, NARES TRAKOOLJUL, EDUARD MURANI, MANFRED SCHWERIN and Klaus Wimmers (Leibniz Institute for Farm Animal Biology)

Understanding the genetic contributions behind skeletal muscle composition and metabolism is of great interest in medicine and agriculture. Weighted gene co-expression network analysis (WGCNA) groups genes into modules based on patterns of co-expression. Network hub genes and regulators of genes in the modules are expected to play an important role in biology. The top hub gene of previously identified porcine muscle co-expression networks, Slc19a2, that correlated to post mortem phenotypes was analysed and validated by functional studies by using siRNA in the murine muscle cell line C2C12 and subsequent quantitative RT-PCR. Network genes, which play a key role in carbohydrate metabolism, showed alterations of the expression levels. Additional, we performed association mapping of co-expression network transcript abundance and identified several thousand expression quantitative traits loci (eQTL) including those corresponding to genes of modules, so called mQTL. We identified mQTL hot spots especially of genes of the co-expression network of module orange and dark-turquoise. By using siRNA knockdown, Cxcr7 was identified and validated as a regulator of the genes in module orange which are involved in response to wounding in muscle cells. Zfp36l2 was found to be a partial regulator in module dark-turquoise which plays a significant role in cell death or apoptosis. The integration of eQTL in module network (mQTL) enabled us to interpret the differentially-regulated genes from a systems perspective. We have identified a physiologically relevant gene network and used it to discover novel genes and regulatory mechanisms involved in the function of muscle cells. Genetic variation integration with co-expression networks can be used to gain insight into the function of muscle cells and the resultant phenotypes.

P3078 Investigation of gene expression response in the intestine of *Ascaridia galli* infested and non-infested village chickens of South Africa. Dikeledi Petunia Malatji (Agricultural Research Council), Farai Catherine Muchadeyi Muchadeyi (Agricultural Research council), Este Van Marle-Koster (University of Pretoria) and Ana Tsotetsi (Agricultural Research Council)

Parasitism is a problem particularly in scavenging chickens raised under village farming systems. Genetic control strategies are a more sustainable disease management strategy particularly for smallholder farmers that have limited resources. Very little information is available on the genetic resistance to gastrointestinal parasites in village chickens in South Africa. This study is aimed at investigating the gene expression profiles in chickens infected

by the *Ascaridia galli* parasite. Total RNA was isolated from segments of small intestines (anterior, middle and posterior) that were collected from three village chickens (C1, C2 and C3) sampled from the Limpopo provinces of South Africa. Intestines from C2 and C3 were sampled from chickens artificially infected with *A. galli* parasite while C1 served as a negative control. All chickens did not show any presence of parasite and the small intestines analysed did not display any detectable histological changes. High-quality RNA of the small intestine was processed using an Illumina RNA-sequence sample preparation kit following the manufacturer's instruction and sequenced using Genome Analyzer IIx (Illumina, Inc) and generated between 21,404,282 and 62,129,634 reads. Adapter sequences and sequences with suboptimal read quality score were trimmed using CLC-Bio workbench version 6.5. Reads that passed the quality control were mapped to the reference genome of *gallus gallus.gal gal4.74* using a high accuracy mapper with TopHat. A mapping percentage ranging from 58.8% to 74.3% was observed. Cuffdiff was then used to analyze differentially expressed genes. A total of 51280 unigenes were differentially expressed between any two-way comparisons of all the three chickens. Of these 1392 isogenes were significantly expressed between C1 and C2, 1722 between C1 and C3 and 265 between C2 and C3. The study forms the basis for understanding the genetics of parasite resistance and developing genetic improvement programs in village chicken production systems.

P3079 Analyzing the muscle transcriptome of pigs with divergent lipid phenotypes through RNA-Seq. Angela Cánovas (Center for Research in Agricultural Genomics (CRAG), Universitat Autònoma de Barcelona), Oriol Canela (Institute for Research & Technology in Food & Agriculture (IRTA)), Rayner González-Prendes and Marcel Amills (Center for Research in Agricultural Genomics (CRAG), Universitat Autònoma de Barcelona) and Raquel Quintanilla (Institute for Research & Technology in Food & Agriculture (IRTA))

We have used a RNA-Seq approach to analyse the transcriptome of commercial Duroc pigs with divergent phenotypes (HIGH and LOW) for 13 fatness traits. Before that, RNA from 105 gluteus medius (GM) muscle samples was individually hybridized on GeneChip Porcine Genomic arrays (Affymetrix, Inc., Santa Clara). Expression levels from microarray data were used to perform principal component analysis to select individuals displaying extreme transcriptomic profiles for lipid-related genes. In this regard, loci displaying differential expression (HIGH *vs* LOW groups) were related with processes such as lipogenesis (ACACA, FASN, DGAT2), fatty acid β-oxidation (ADIPOQ, PPARD), fatty acid synthesis and elongation (FABP4, ELOV5, ELOV6) and cell growth and development (TGFB2, ITGAV). The coding transcriptomes of 56 selected GM samples from HIGH (n=28) and LOW (n=28) pigs were sequenced with a HiSeq 2000 platform. An average of 70 million sequence reads were obtained from each sample and mapped to the pig reference genome. In all samples, 80% of the reads were categorized as successfully mapped. Expression levels from microarray and RNA-Seq platforms were compared and a substantial correlation between both data sets was found (r=0.7). Besides, SNP discovery analysis allowed us to identify several transcripts polymorphisms that have been subsequently associated with lipid traits such as palmitoleic content and serum cholesterol and triglyceride concentrations.

P3080 Validation of reference genes for multi-tissue gene expression studies in chicken. Sandra Bagés, Joan Estany, Marc Tor and Ramona Pena (University of Lleida-Agrotecnio Center)

Real-time quantitative PCR (qPCR) is a

well-established technique for estimating the amounts of mRNA expression. To standardise the amount of the starting material, it is critical to assay in parallel two or more reference genes as internal reference. Reference genes should ideally be present at a constant level in all samples in the experiment, at approximately equal concentration and amplifying with similar efficiency to the target genes. The expression of reference genes must not be affected by the experimental conditions. Validation of suitable reference genes has been conducted in livestock species for the most relevant target tissues. However, the few studies published to date in chickens have focused on lymphatic organs in response to viral and bacterial infections. The present study addresses a reference gene validation study in chickens in four tissues related to growth, fatness and product quality: the *pectoralis major* (breast), the *biceps femoris* (thigh) muscles, abdominal fat and liver. The animal material consisted of 32 laying hens. Five potential reference genes (*B2M*, *RPL32*, *SDHA*, *TBP* and *YWHAZ*) were selected based on studies published in other livestock species. Their stability of expression was studied by four different methods (geNorm, NormFinder, BestKeeper and DeltaCt) and genes were ranked according to the stability values. Although the final ranking varied slightly in each analysis, the four algorithms gave very similar results. There were distinct differences between tissues in the stability rankings. In general, *SDHA*gave the worst results (least stably expressed gene) in all tissues while *TBP* and *YWHAZ* tended to be on the top of the ranking (most stable expression). Conjoint analysis of the four tissues indicated that *RPL32* and *YWHAZ* were the genes of preference to be used in the normalisation process. These results can be directly applied to the gene expression studies in growth- and fat-related experiments in chickens.

P3081 Variation of muscle gene expression between high and low RFI Angus bulls by RNA-seq. Yizhou Chen (NSW Department of Primary Industries Elizabeth Macarthur Agricultural Institute)

Residual feed intake (RFI) is a measure of feed efficiency in beef cattle and is the difference between an animal's actual feed intake recorded over a test period and the expected feed intake based on the animal's size and growth rate. Several physiological systems related to basal metabolic rate and the homeostatic control of body mass have previously found to explain a large proportion of variation in RFI in Angus steers divergently selected for RFI. Skeletal muscle metabolism is a major determinant of energy expenditure. To study metabolic differences at gene expression level we sequenced the muscle transcriptomes of 48 young bulls selected for high and low RFI. We generated 1,339,474,108 high quality paired reads. In total 611,214,932 sequence reads were mapped to know bovine transcripts (UMD3.1). We identified 86 genes marginally differentially expressed between high and low RFI animals. These differentially expressed genes included protein coding genes, non coding genes and mitochondria expressed genes. A quarter of those differentially expressed genes related extracellular matrix activity and is up-regulated in low RFI animals. The extracellular matrix (ECM) and ECM proteins are important in developmental patterning, stem cell niches, cancer, and genetic diseases. In particular they interact with growth factors (GFs) to regulate their distribution, activation, and presentation to cells.

P3082 Genetic Polymorphism of Exon1 of PROP1 Gene and Its Correlation with Milk Production Traits in Xinjiang Brown Cattle. xixia huang (XinjiangAgriculturalUniversity)

Genetic Polymorphism of Exon1 of PROP1 Gene and Its Correlation with Milk ProductionTraits in Xinjiang Brown Cattle PROP1 (prophet of Pit-1)

has both DNA-binding and transcriptional activation ability. Its expression leads to ontogenesis of pituitary gonadotropes, as well as somatotropes, lactotropes, and caudomedial thyrotropes. In the present research, the genetic polymorphisms of exon1 of PROP1 gene of the Xinjiang Brown cattle were detected by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) the relationship between genotypes and average daily milk yield, Milk composition and SCS, at DNA level theoretical basis for the improved development and utilization and sequencing analysis, and the relationship between genotypes and average milk production traits were analyzed using the SAS8.1 statistic software with 277 cows data. The results showed that there were three genotypes named GG, GA and AA of exon1 of PROP1 gene in Xinjiang Brown cattle. Genotype frequencies were 0.563, 0.245 and 0.192 for GG，GA and AA, respectively. Allele frequencies were 0.686 and 0.314 for G and A which were at the Hardy-Weinberg equilibrium. The results of the least squares analysis showed GG cows were of higher milk protein percentage than GA and AA individuals（$P<0.01$）, and there was no significant difference in average daily milk yield, milk fat percentage and SCS($P>0.05$). DNA sequence analysis revealed that one mutation occurred in 209G→A, and was one silent mutation. Mutation detected in this study did not cause a change in the amino acid coding, but still caused a change in cow milk protein percentage, which may be the site is closely linked with another lactation performance-related functional gene.

P3083 Molecular signaling mechanism of striated muscle function and growth based on global gene expression analysis in *Rbm20-/-* rats (*Rattus norvegicus*). Wei Guo (Department of Animal Science, University of Wyoming), Huojun Cao (Institute of Biosciences & Technology,Texas A&M Health Science Center) and Marion Greaser (Animal Science, University of Wisconsin-Madison)

Striated muscle (heart and skeletal) function and growth have been associated with coordination of many extracellular and intracellular genes under pathophysiological conditions or stress. The *Rbm20-/-* rats have been linked to dilated cardiomyopathy (DCM) by regulating isoform transition of TITIN that plays an important role in muscle contraction. This study was to examine which signaling pathways are regulated through the *Rbm20* deficiency in rat heart tissues.

The Affymetrix Rat Expression Array 230 2.0 was used for global gene expression profiling by comparing *Rbm20-/-* rat heart tissue (KO) to wild type control (WT). Because TITIN undergoes developmental change, this study also included developmental stages: day1 (D1), day20 (D20) and day49 (D49) from both KO and WT animals. Total 202 genes were differentially expressed based on the two-way ANOVA analysis ($P<0.01$ and fold change>1.5). The number of differentially expressed genes increases with development, and the differentially expressed genes from D20 and 49 contain most of these from D1. D20 and 49 have more similar expression pattern than D1, which is consistent with TITIN developmental alteration. Gene Ontology and GSEA analysis suggested that down-regulated genes from all three ages are associated with mitochondrial activity, but they are also related to lipid metabolic at only d20 and 49; up-regulated genes from three ages are involved in cardiac function and GTPase activity, however, they are related to cytoskeleton binding, cell cycle, DNA metabolic and apoptosis at only d20 and d49. Genes involving in these pathways were selected and confirmed in cardiac and skeletal muscle by western blot and QPCR.

These results demonstrated that striated muscle function and growth affected by *Rbm20*-mediated TITIN isoform transition are likely regulated via PI3K/AKT pathway mediated cell cycle, apoptosis, protein synthesis and G-protein coupled signaling. Further study will focus on

these *Rbm20*-regulated signaling pathways and their role in cardiac function and skeletal muscle growth.

P3084 Proactive coping is associated with PBMC expression profiles promoting immune defense and recovery. Klaus Wimmers, Michael Oster, Marcel Adler, Mathias Scheel, Manuela Zebunke, Birger Puppe, Eduard Murani and Siriluck Ponsuksili (Leibniz-Institute for Farm Animal Biology)

Selection for production traits is presumed to have led to impairments concerning behaviour, animal welfare and immune function. However, knowledge of the molecular mechanism related to the suspected interactions is scarce. We address the relationships among traits related to performance, animal health and immunity, as well as behaviour and stress response. We used Tetanus toxoid (TT) vaccination as a model to evaluate the transcriptional response of peripheral blood mononuclear cells (PBMC) to immune stimuli by Affymetrix microarray analyses in German Landrace pigs. We previously showed that TT initiated a Th1 and Th2 immune response and provoked an immediate transcriptional response of immune and metabolic pathways within 2h. The reaction after initial and booster vaccinations cumulated to some 5000 genes that were differentially expressed at 12 time points over a period of four weeks. In order to link behaviour and immune response we assigned piglets to groups of animals with either proactive or reactive coping style as assessed based on repeated backtests. Individual coping characteristics were reflected as transcriptional differences at naïve state (week 5) and in response to the immune challenge (weeks 7 and 9) as well as at slaughter age (week 20). Prior to TT-vaccination both groups showed different expression of genes in cell communication, angiogenesis, pro-inflammation, and wound healing. Due to immune challenge these coping-specific expression signatures observed at naïve state were temporarily blurred (day 14) but subsequently restored and intensified (day 28). Accordingly, cluster analyses revealed a dominant influence of coping style on transcript levels at early stages. It appears that pigs coping with challenges in an active way might favor molecular pathways enabling an effective strategy for defense and recovery. The PBMC could be a suitable tissue to obtain molecular markers for distinct coping styles. In contrast to previous assumptions, coping-specific immunity in pigs lacks inherited shifts between Th1 and Th2 immune responses.

P3085 Chicken recombinant pluripotency transcriptional factor Nanog promotes the proliferation of chicken embryonic stem cell.. Yenan Wu, Jianshu Li, Zhengxing Lian and Hongbing Han (Beijing Key laboratory of Animal Genetic improvement)

Many reports have identified that overexpression of Nanog helped maintain the pluripotency of human and murine embryonic stem cells. However, we don't have enough research on the effect of Nanog on the cell proliferation especially in the chicken embryonic stem cells （cESC）. In this study, we have optimized the initial feeder-free cESC culture system by adding 6*His-Tag into the initial eukaryotic expression vector. The culture system contained hLif, mbFGF and four homologous reprogramming transcriptional factor: cLin28, cPouv, cNanog, cSox2. Based on this culture system, we explored the effect of the cNanog on the cESC proliferation . The pluripotency and the ability of self-renewal of the cultured cESC were identified by the AKP activity assess ,CCK-8 ,Nanog staining and EDU test. We divided into three distinct experimental group. During the first three passages, All the groups were cultured as usual. After that, we changed the concentration of the Nanog , group A 、B、C were treated without cNanog、 with 1*cNanog、with 2* cNanog respectively. Group B、C could maintain the

pluripotency and the ablility of self-renewal for over 8 passages, while group C just stopped at passage 6. After the cells were passaged 5 times, By High Content Analysis, we have indentified that the proliferation rates of positive cells in group A(without cNanog)、 group B(1*cNanog) and groupC(2*cNanog)were10.78%、12.78%、16.89% respectively(P<0.01). Also we have found that as the concentration of cNanog increased, the cell viability increased (P<0.05). Taken together, we demonstrated that cNanog promotes the proliferation of cESC, and the effect is dose-dependent.

P3086 Genetic Polymorphism of Exon1 of PROP1 Gene and Its Correlation with Milk Production Traits in Xinjiang Brown Cattle. xixia huang (xinjiang agricultural university)

PROP1 (prophet of Pit-1) has both DNA-binding and transcriptional activation ability. Its expression leads to ontogenesis of pituitary gonadotropes, as well as somatotropes, lactotropes, and caudomedial thyrotropes. In the present research, the genetic polymorphisms of exon1 of PROP1 gene of the Xinjiang Brown cattle were detected by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) the relationship between genotypes and average daily milk yield, Milk composition and SCS, at DNA level theoretical basis for the improved development and utilization and sequencing analysis, and the relationship between genotypes and average milk production traits were analyzed using the SAS8.1 statistic software with 277 cows data. The results showed that there were three genotypes named GG, GA and AA of exon1 of PROP1 gene in Xinjiang Brown cattle. Genotype frequencies were 0.563, 0.245 and 0.192 for GG，GA and AA, respectively. Allele frequencies were 0.686 and 0.314 for G and A which were at the Hardy-Weinberg equilibrium. The results of the least squares analysis showed GG cows were of higher milk protein percentage than GA and AA individuals （ P<0.01 ） , and there was no significant difference in average daily milk yield, milk fat percentage and SCS(P>0.05). DNA sequence analysis revealed that one mutation occurred in 209G→A, and was one silent mutation. Mutation detected in this study did not cause a change in the amino acid coding, but still caused a change in cow milk protein percentage, which may be the site is closely linked with another lactation performance-related functional gene.

P3087 A functional variant in the promoter region of ovine *stearoyl-CoA desaturase* gene (*SCD*) affects gene expression and fatty acid profile in muscle. Laura González-Calvo and Laura Iguacel (CITA), Alfonso Bolado-Carrancio (Universidad de Cantabria), Elda Dervishi (University of Alberta), Mª Magdalena Serrano (INIA), Guillermo Ripoll, Francisco Molino, Mireia Blanco and Margarita Joy (CITA), José Rodriguez-Rey (Universidad de Cantabria) and Jorge Calvo (Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) and ARAID)

This experiment was conducted to study the effect of the g.31A>C (GenBank acc. Number FJ513370) SNP, located in the promoter region of ovine SCD, on the transcription rate of this gene and fatty acid profile in two different muscles. Fifty Rasa Aragonesa male lambs were fed commercial concentrate. Lambs were slaughtered at 22–24 kg live-weight, and a piece of Semitendinosus and L. thoracis muscles were obtained for functional characterization, genotyping and a steak to determine fatty acid composition. Only palmitoleic acid (C16:1) and trans-octadecadienoic acid (C18:2 n-6tt) contents in Semitendinosus and L. thoracis, respectively, were affected by the SNP. To elucidate the functionality of this SNP, transcriptional activity and protein binding of the SCD were evaluated. The g.31A>C SNP was found to be associated with SCD gene expression in both muscles.

Genotypes carrying C-allele showed a lower expression rate compare to genotypes carrying A-allele. Because of the lower expression of the genotypes carrying the C-allele, methylation analysis of a 199 bp region of the SCD promoter, which include the g.31A>C SNP, was performed to verify if the C-allele could be methylated. Sequence analysis of the bisulfite converted DNA confirmed that the amplified region was not methylated. EMSA suggested the presence of a specific binding and allelic differences in the interaction with nuclear proteins. The experiments showed that the C-allele oligonucleotide had higher binding than the A-allele. The in silico analysis of transcription factor binding sites suggested that the nucleotide change could alter the affinity of the SP-1 (g.31A>C), AP-2 alpha (g.31C), WT1 (g.31C), NF-1 (g.31C) and C/EBP alpha (g.31A) nuclear proteins for the sequence including the g.31A>C polymorphism. However, EMSA supershift assay with anti-Sp1 revealed that SP1 nuclear protein was not interacting with this region of the SCD gene.

P3088 Identification of transcription profile of pig backfat and characterization of gene network influencing fat deposition by next generation sequencing. Paolo Zambonelli (Department of Agricultural and-Food Sciences (DISTAL), University of Bologna), Enrico Gaffo and Stefania Bortoluzzi (Department of Biology, University of Padova) and Roberta Davoli (Department of Agricultural and-Food Sciences (DISTAL), University of Bologna)

High throughput sequencing of transcripts allows the study of gene expression and regulation and is possible to analyze the transcription profile of specialized tissues, like adipose tissue, in order to discover gene networks influencing their metabolism and physiology.
To this aim we analyzed the transcripts of porcine backfat tissue to identify expressed short RNAs (sRNAs) and long RNAs (longRNAs) and to identify differentially expressed (DE) genes between pigs different for backfat thickness. Illumina NGS was utilized to study the transcriptome of 20 Italian Large White pigs extreme and divergent for the genetic index Backfat Thickness, calculated by the Italian National Association of Pig Breeders (ANAS) within the Sib-test genetic evaluation. The sRNAs were sequenced by 50nt single reads and the longRNAs by 100nt paired-end approaches. Bioinformatic analyses to filter the raw reads, to map the genes on the pig genome, and to detect DE genes were performed using publicly available software and custom made pipelines.
For the sRNAs (mainly miRNAs) we detected about 300 different mature types of which about 100 are currently to be considered as novel in pigs. Porcine transcripts targeted by the most expressed miRNAs were predicted, showing that they may impact on Wnt, insulin signaling, and axon guidance pathways. 26 DE miRNAs were detected between fat and lean pigs.
The porcine backfat transcription profile of longRNAs was characterized by the expression of about 11000 known genes. About 180 DE genes were detected between fat and lean pigs. Current work is focusing on annotation and functional characterization of the DE longRNAs.
Furthermore, is under way the validation of DE genes by qRT-PCR and the detection of interrelationship between sRNAs and longRNAs to predict the regulatory networks involved in the different aptitude for backfat deposition between fat and lean pigs.
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P3089 Isolation of miRNAs in swine (*Sus Scrofa*) ovaries and identification of differentially expressed miRNAs in follicles of Meishan and Duroc sows at mid-to-late follicular stage. Tao Huang, Lijuan Liu and Tengjiao Zhai (Shihezi University), Fei Yang (Sihezi University), Huiwen Lu and Mengsi Xu (Shihezi University) and Xiaoyan Wang and

Chengyi Song (Yangzhou University)

Ovulation rate is the first restrictive factor of litter size in pig. The difference in ovulation rate between breeds with high and low ovulation rate originate from the differences during mid- to later follicular phase in dynamics of follicular growth. In high ovulation rate sows, the follicle selection phase is prolonged and more preovulatory follicles emerge. The dynamic process involving selection and maturation of follicles is regulated and controled by a highly synchronized and exquisitely timed cascade of gene expression. Since miRNAs regulate gene expression extensively, and play an important role in the development of ovarian and female reproductive cells, the difference in ovulation rate between Chinese Meishan and Duroc sows maybe affected by the different expression of miRNAs in their ovarian follicles.Ovarian tissues of Meishan and Duroc at day 4 of follicular phase were collected, miRNA libraries were constructed and next-generation sequencing was conducted. In ovaries of Duroc and Meishan, a total of 272 and 275 pig miRNAs were detected, 125 and 155 predicted miRNAs sharing high homologies with known miRNA of other species were found, 49 and 57 novel miRNAs were detected, respectively. And some of the new miRNAs were verified.miRNA chips were used to screen the miRNAs differentially expressed in medium Follicles (with a diameter of diameter 5.0mm-6.9mm) between Meishan and Duroc sows. A total of 158 pig miRNAs were detected, and ssc-miR-30a, ssc-miR-708, ssc-miR-187, ssc-miR-486 shown significant difference in expression level between Meishan and Duroc, and 31 mammalian miRNAs unreported in pig shown significant difference as well. And the expression difference of some miRNAs were conformed. The expression profiles of several differentially expressed miRNAs in various tissues and follicles at different stages were investigated. The research provide an insight for better understanding the genetic basis and molecular mechanism of follicle development and high prolificacy affected by miRNAs in pigs.

P3090 Understanding the complex interaction between pseudorabies virus (PrV) and its natural host using RIP-Chip enrichment analysis.. Sophie Dhorne-Pollet, Jérome Lecardonnel, Florence Jaffrézic, Marco Moroldo and Elisabetta Giuffra (Institut National de la Recherche Agronomique, AgroParisTech, UMR1313 Animal Genetics and Integrative Biology.)

MicroRNAs modulate cellular pathways by acting at the post-transcriptional level, through molecular interactions with target mRNAs via the RNA-induced silencing complex (RISC). During a viral infection, complex interactions take place between the virus and its host which involve, among other factors, microRNAs. Some host microRNAs are crucial for immune responses, and a few of them target viral mRNAs and inhibit viral replication. Moreover, microRNAs encoded by some viruses (mostly herpesviruses) can modulate the transition from latent to lytic replication and attenuate the host antiviral immune responses.
An interesting model to study these complex interactions is the pseudorabies virus (an alpha-herpesvirus, PrV) responsible for Aujeszky's disease in pigs, its natural host. The intron of the large latency transcript of the PrV genome has been shown to encode a cluster of 11 microRNAs. To understand the biological role of viral microRNA during PrV infection, we previously compared a mutant PrV clone depleted of a cluster of microRNAs and a parental clone for their ability to establish latency *in vivo* in trigeminal ganglia.
Here we present a functional genomics approach based on the RIP-Chip assay to better understand the intricate interactions between the miRNAs found expressed by PrV during latency and the host mRNAs. We first optimized the experimental procedures in terms of cell lines,

microRNA transfection and immunoprecipitation: miRNA concentrations, transfection reagent, ribonucleoprotein antibody. Briefly: i) we transfected microRNAs in PK15 cells, ii) we immunoprecipitated the RISC complex, iii) we extracted the RISC-bound mRNAs and iv) we used microarrays to identify the mRNAs. Finally, we identified specific PrV microRNA targets using RIP-Chip enrichment analysis.

P3091 IGF-1 promotes the proliferation of skeletal myoblast cells through PI3K/Akt pathways in chicken. Minli Yu and Dongfeng Li (Nanjing Agricultural University)

During embryonic development, skeletal muscles undergo a process of structural and functional modeling. Insulin-like growth factor (IGF)-I plays an important role as a positive regulator of skeletal muscle growth and differentiation. However, the mechanism by which IGF1 regulates myoblasts proliferation remains largely unclear in chicken embryo. In order to reveal the molecular mechanisms regulating myoblast cells proliferation, we examined the stimulating effect of IGF-1 on myoblasts proliferation in chicken during embryonic stage. The results showed that IGF-1 significantly induced the proliferation of the cultured myoblasts by increased the phosphorylation of Akt. The promotion of myoblast cell proliferation by IGF-1 was inhibited by PI3K inhibitor LY294002 or AKT inhibitor KP372-1. Additionally, we found a significant increase in cell cycle-dependent genes (cyclin D1 and E, cyclin-dependent kinases 6 and 2) mRNA levels in proliferated myoblasts stimulated with IGF-1. Moreover, we examined the stimulation of skeletal myoblasts proliferation with IGF-1 in vivo. The results suggested that IGF-1 significantly promoted the incorporation of BrdU in a dose-dependent manner in chicken embryos. Collectively, these findings offer novel insights into the dynamic mechanism of IGF-1 action on myoblasts proliferation and suggest that PI3K/Akt signaling pathway plays an important role in regulating embryonic skeletal myoblast cells development.

P3092 MiR-22 promotes hair catagenesis by targeting multiple differentiation regulator genes. Zhengquan Yu and Shukai Yuan (China Agricultural University)

Hair growth undergoes recurrent cycling of controlled growth (anagen), regression (catagen), and relative quiescence (telogen), allowing the turnover of old and new hair. However, the regulational mechanism of hair cycle is still not fully understood. Our preliminary data revealed that expression level of hair miR-22 is highly associated with hair cycling，and become much higher in catagen stage compared to anagen, prompting us to examine miR-22 function on hair development. To investigate the role of miR-22, we take advantage of an inducible miR-22 overexpressing transgenic mice and miR-22 knockout mice. Our data showed that miR-22 ovexpression lead to hair loss by promoting the transition of hair follicle from anagen phase to catagen phase. In contrast, miR-22 deficiency resulted in the delay of catagenesis. We found that miR-22 inhibit cell proliferation and hair stem cell expansion. Further, Dlx3、Foxn1、Hoxc13 and Sostdc1 were identified as direct targets in hair follicle development. In summary, our findings strongly demonstrated that miR-22 plays a critical role in hair catagenesis.

P3093 MiRNAs expression profiling in transgenic and wild type littermate mice by Solexa deep sequencing. Ruheena Javed , Jing Lu , Miao Yuanxin , Jinzeng Yang , Xinyun Li and Jianhua Cao and Shuhong Zhao

MiRNAs are a class of short, non-coding RNA molecules that reportedly play a central role in regulating post-transcriptional gene expression during embryonic stem cell development, myogenesis, adipogenesis, fat metabolism and

glucose homeostasis.For assessment of the effect of loss of myoastatin signalling on gene expression in skeletal muscle, RNA from post developmental myostatin trasgenic and wild type littermate mice was analysed with Solexa deep sequencing. Sequencing data were analysed using miRDeep software V. 2.1.2. A Total of 461 mature known miRNAs were identified out of which 57 miRNAs were found to be differentially expressed. Expression pattern demonstrated that Mmu-miR-22 was most abundant miRNA, mmu-miR-133a and mmu-miR-378a were abundant and significantly differentially expressed miRNAs. Sixty nine novel miRNAs were also identified out of which three NMu-1, NMu-14 and NMu-36 accounted higher read count. For these 3 novel miRNAs and 5 known miRNAs, the expression profiling was done using Q-PCR analysis. Twenty most abundant and differentially expressed miRNAs were selected for target prediction and pathway analysis. Total 4,583 targets were identified out of which FST, SMAD3, TGFBR1, ACVR1a and MEF2c genes which plays vital role in MSTN signaling, were found to be targeted by miR-101, miR-425, miR-199a and miR-582 in TGFb signaling pathway which activates MSTN signalling. Hence these miRNAs could prove crucial candidate miRNAs in skeletal muscle development.
Conclusion: The present study proffers an initial miRNA transcriptome profile in skeletal muscle development of transgenic and control mice. Findings aided identification of miRNA and their targets which can possibly contribute to skeletal muscle development. Information generated in this study can be further utilized to investigate the role of identified miRNAs and their targets in regulation of skeletal muscle development.

P3094 microRNA-122 targets the genes related to liver metabolism in chicken. Xingguo Wang, Fang Shao and Jianfeng Yu (Department of Life Science and Technology, Changshu Institute of Technology), Honglin Jiang (Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University), Daoqing Gong (College of Animal Science and Technology, Yangzhou University) and Zhiliang Gu (Department of Life Science and Technology, Changshu Institute of Technology)

microRNAs (miRNAs) are small non-coding RNAs regulating various biological processes by targeting genes and affecting their expression. miR-122 is a very important miRNA in mammal livers, but the study about miR-122 in chicken liver is limited. In the current study, 364 of 11891 chicken genes were predicted as the targets of miR-122 by the computational algorithm "miRanda" with "TargetScan" principle. Among the 364 predicted targets, we selected five genes related to lipid metabolism for further study, of which 1 had conserved target sites across species (P4HA1), 1 had conserved seed region across species (PKM2), and 3 had chicken specific target sites (TGF-β3, FABP5 and ARCN1). We determined the expression patterns of miR-122 and 5 of the predicted target genes, P4HA1, PKM2, TGF-β3, FABP5 and ARCN1 in liver and hepatocytes, finding that miR-122 is highly expressed in chicken liver and it is up-regulated in liver during development and down-regulated in primary hepatocytes after cultivation, P4HA1, TGF-β3 and ARCN1 were down-regulated during the isolation of hepatocytes, and FABP5 was down-regulated and up-regulated during the isolation and cultivation of hepatocytes, respectively.. Experiments of co-transfection of miR-122 expression plasmids and pMIR with 3'-UTR of target genes into CHO were used to confirm that these five genes are real targets of miR-122 and the overexpression of miR-122 in DF-1 revealed that miR-122 regulates TGF-β3 in mRNA level. P4HA1 and PKM2 are also up-regulated in the hepatocytes while miR-122 was inhibited. These findings suggest that miR-122 targets some genes to regulate liver metabolism in chicken.

P3095 Expression study of porcine perilipin

family genes: identifying a positive factor in the regulation of pig intramuscular fat deposition. Roberta Davoli, Martina Zappaterra, Silvia Braglia and Paolo Zambonelli (1Department of Agricultural and-Food Sciences (DISTAL), University of Bologna)

Fat content, fatty acid composition and lean cut amount are the most important traits defining pig meat quality. The latter must satisfy both consumers' and pig industry's requirements: as consumers demand a healthier meat with a lower fat content, quality pig products require an adequate content of intramuscular fat. Hence, in pig selection schemes an essential goal is represented by the reduction of excessive carcass fat content without affecting intramuscular fat deposition. However, there is still poor knowledge of genes involved in and related to lipid metabolism in porcine muscle and fat tissues.

To this aim, we studied gene expression level of a novel gene family composed by genes Perilipin, Adipophilin, TIP47, S3-12 and MLDP/OXPAT (also known respectively as PLIN1, PLIN2, PLIN3, PLIN4 and PLIN5). Perilipin family genes encode for lipid droplet coat proteins, which play an essential role in mobilisation of stored lipids. Tissue samples were collected at slaughterhouse from 912 Italian Large White pigs, and among this population two groups of 20 pigs with divergent breeding values for intramuscular fat content were chosen. mRNA was extracted from pig muscle tissue and quantified by qRT-PCR; finally transcription levels of the analysed genes were compared between divergent groups for intramuscular fat content. Different expression levels were identified, suggesting divergent roles of Perilipin genes in intramuscular fat deposition. In particular, OXPAT presented significantly different expression levels (P values < 0.05) between samples with high and low intramuscular fat content and was identified as positive factor in the regulation of intramuscular fat deposition. This result suggests an important role of OXPAT gene in lipid metabolism and further studies are in progress to clarify the molecular functions of the porcine perilipin family genes.

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P3096 MicroRNA-x targets the transcription factor E2F3 promoting G1 arrest in C2C12. Meng Wang, Chuncheng Liu, Tianyu Lu and Qingyong Meng (State Key Laboratories for Agrobiotechnology, College of Biological Sciences, China Agricultural University)

MicroRNAs (miRNAs) are a class of small non-coding single-stranded RNA, a length of about 20-25nt. It could inhibit the expression of genes by binding to target mRNAs. Its roles are very extensive, involving in a variety of biological processes including cell proliferation, apoptosis and differentiation. Mir-x appears to play a crucial role in cancer pathogenesis where they exert their effect as tumor suppressors. But its roles in skeletal muscle development are unclear. Here, we found that the expression of miR-x was upregulated in differentiated myoblasts or myotubes. Overexpression of mir-x in C2C12 cell lead to induction of G1 cell cycle arrest. Meanwhile the expression of cyclin E1, CDK2, cyclin D1 and CDK6, paly an essential role in cell cycle, were downregulated. Furthermore, we demonstrate E2F3, was an important transcription factor for the induction of S phase, was identified as a target of miR-x in C2C12 by dual-luciferase reporter assay and Western blotting. Together, our study demonstrated that miR-x could regulate C2C12 cell cycle through E2F3.

P3097 Gene expression profile of equine Mesenchymal Stem Cells (MSCs) exposed to inflammatory environment. Laura Barrachina, Ana Rosa Remacha and Beatriz Ranera (Laboratorio de Genética Bioquímica (LAGENBIO). University of Zaragoza), Antonio Romero and Francisco José Vázquez (Laboratorio de Genética Bioquímica (LAGENBIO). University of zaragoza), Jorge Albareda (Área de Traumatología y Ortopedia. Universidad de Zaragoza), Marta Prades (Dept. Medicina i Cirurgia Animal. Universitat Autónoma de Barcelona. Spain) and Pilar Zaragoza, Inmaculada Martín-Burriel and Clementina Rodellar (Laboratorio de Genética Bioquímica (LAGENBIO). University of zaragoza)

MSCs are being investigated as a new treatment for equine osteoarthritis (OA) because of their immunomodulatory ability and capacity of changing their immunophenotype in response to inflammatory conditions. The aim of this work is to analyse the gene expression profile of bone marrow derived MSCs (BM-MSCs) exposed to two different inflammatory environments. BM-MSCs from three animals were exposed to two different inflammatory environments: a) 20% allogeneic inflammatory synovial fluid (SF) and b) a pro-inflammatory cytokine cocktail (CkC) consisting of TNFa (50 ng/ml) plus IFNg (50 ng/ml). After 72 hours, expression of 24 immunomodulatory-related genes was analysed by RT-qPCR. BM-MSC proliferation and differentiation abilities after inflammatory exposure were also investigated.The analysed genes were grouped into four blocks: 1) Pro and anti-inflammatory molecules: *TNFa, IL6, IL1b, CXCL1, IFNg, IL10, TSG6, TGFb1, TGFb1-R, CXCR*; 2) Immunomodulatory-related enzymes: *COX1, COX2, IDO-1, iNOS, Cyclin D2*; 3) Adhesion molecules: *ALCAM, VCAM, CD44, CD29*; 4) Antigen presenting-related molecules*: MHC I, MHCII, CD80, CD40, CD40L*.The results reflected that the effect of CkC culture conditions on BM-MSCs was remarkably stronger than the inflammatory synovial fluid.BM-MSC proliferation and differentiation potential remained unaltered under SF exposure; however both properties were diminished after CkC culture conditions.A higher gene expression of *TNFa, IL6, COX2, IDO, VCAM, MHCII* and*CD40* was observed under CkC conditions. *ALCAM* expression was also significantly higher under SF conditions. On the other hand, significantly lower expression of *Cyclin D2* and *CD40L* was detected in CkC pro-inflammatory environment. These results suggest that BM-MSCs change their immune and inflammatory-phenotype according to the surrounding environment. These findings provide us a better understanding about the potential gene expression behaviour of BM-MSCs inside the inflamed joint besides constituting a mandatory step before clinical application of MSCs as a therapeutic tool in equine degenerative joint disease.

Genetic Diversity and Polymorphisms

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P4001 Avian populations in Algeria (Aurès); Phenotypic Characterization of Local Breeds. Abderrahmane Mehdaoui (university of Batna) and Rachid Bouhadad (USTHB)

In Algeria, local chickens (*Gallus gallus domesticus*) are part of the traditional breeding; they occupy the first place without competition among avian species. They are characterized by extremely varying phenotypes, such as coloration of plumage, types of peak, crest, feathered legs, and other features which are hardly recorded. Many of these features are monogenic traits controlled by a single gene and / or, in some cases, by two or three genes. To characterize the local chicken population in Aures region, have spent more than thirty outputs, which were spread over four months in the majority of the villages that belonged to : Oued Taga; Bouzina; Lambèze; Timgad; TniatElAbed; Ine yagout; N'gaousse; Fes dis; Djerma; Ichemoul; Seriana; Kais; T'kout; Ouled fadel; Cecher; Elyabouce; Bouhmama; Merouana; Khirane; El hamma. A database was constructed based on various characters: coloration of the beak, the peak and the plumage; the presence of the crest, feathered lugs, the presence of ergot in females and their absence in males and bare neck. Recording of different phenotypes allowed to deduce both allele and genotypic frequencies of these populations which are considered as natural breeds. The presence of several allelic forms showed that these populations conceal a high genetic variability.

P4002 Genetic diversity of native Indian pigs by microsatellite markers. Nihar Sahoo (Indian Veterinary Research Institute), Nasimun Nesa (Manipur University), Soumen Naskar and Santanu Banik (NRC on Pig), Prabhat Pankaj (Central Research Institute for Dryland Agriculture) and Monalisa Sahoo (Indian Veterinary Research Institute)

We determined the genetic diversity and evolutionary relationships among geographically and phenotypically diverse two registered breeds (Ghoongroo and Niang Megha) and a local pig type (Tenyi Vo; yet to be registered) native to one of the two global biodiversity hotspots of India (i.e. covering eastern and northeastern part) using a panel of 22 ISAG recommended microsatellite loci. The Na ranged from 3 to 15 with an average per locus as 8.18 ± 0.62; 6.95 ± 0.54 and 7.18 ± 0.45 for Ghoongroo, Niang Megha and Tenyi Vo respectively whereas, the mean Ho found were 0.71 ±0.04; 0.61 ± 0.04 and 0.68 ± 0.05. There were abundant genetic variations displayed within breeds with mean He of 0.75 ± 0.02, 0.67 ±0.03 and 0.69 ± 0.03. The PIC values ranged between 0.55 (S0026) to 0.85 (S0068) in Ghoongroo and between 0.52 (S0026) to 0.86. (S0218) in Niang Megha and between 0.45 (S0026) to 0.87 (Sw936) in Tenyi Vo pigs. The moderate *F*ST value (0.115 ±0.01) indicated degree of genetic differentiation among the native Indian breeds. The Nei's unbiased genetic distance estimates were found to be 0.6589 between Ghoongroo and Niang Megha, 0.6681 between Ghoongroo and Tenyi Vo pigs, and 0.2909 between Niang Megha and Tenyi Vo pigs, indicating closer relationship between Niang Megha and Tenyi Vo pigs. Furthermore, a total of 90 private alleles exclusive to a particular breed (45 in Ghoongroo; 23 in Niang Megha and 22 in Tenyi Vo pigs) were described. The estimated divergence time was found to be 4454 and 1939 generations between Ghoongroo- Tenyi Vo and Niang Megha- Tenyi Vo pigs, respectively. Bottleneck analysis indicated no genetic bottleneck occurred during the most recent decline. These results may be useful to facilitate conservation decision making and it is proposed that Tenyi Vo pigs may be recognized as distinct pig breed like the other two.

P4003 Analysis of dense genome-wide single nucleotide polymorphisms unlocks the genetic

structure of nondescript Ethiopian village chickens. Takele Desta and David Wragg (University of Nottingham), Judy Bettridge and Stacey Lynch (University of Liverpool), Tadelle Dessie (International Livestock Research Institute), Paul Wigley (University of Liverpool), Pete Kaiser (University of Edinburgh), Rob Christley (University of Liverpool) and Joram Mwacharo and Olivier Hanotte (University of Nottingham)

Following their domestication and dispersion, village chickens have been kept by smallholder farmers as source of food and income and for socio-cultural uses. Extensive phenotypic diversity is a common feature of most village chickens due to the lack of stringent selection criteria in favour of specific phenotypes. Here, we investigated the genetic structure of village chickens sampled from two geographic locations in western and eastern Ethiopia that considerably differ in their agro-ecological and socio-cultural setup. After quality control 375213 SNPs and 747 birds (380 from Horro and 367 from Jarso) were available for analysis. High genetic diversity was observed within population (for example, expected heterozygosity was 0.332 in Horro and 0.316 in Jarso). Principal component and genetic admixture and relationship analyses clearly differentiate the two populations. Pairwise Fst analysis reveals weak genetic differentiation (0.042) between the two populations. This might however be partly attributable to the large sample size included in the analysis. A clearly defined pattern of isolation by distance explains this spatial genetic structure and reflects minimal gene flow between the two populations. Demographic history, natural selection for adaptation to niche environments and mild artificial selection for traits of socio-cultural significance might have contributed to the genetic divergence observed. In line with our sampling strategy, birds sampled from the same household were not genetically more related than birds sampled from different households. However, this might not reflect the extant level of inbreeding in the sampled flocks given that we deliberately excluded birds with known pedigree relationship upon sampling. Our results demonstrate that the use of a sufficiently large number of informative genetic markers, and a large number of samples, provide strong statistical power to elucidate the cryptic genetic structure observed in nondescript village chickens.

P4004 Sharing of maternal lineages between aurochs and domestic cattle in the Iberian Peninsula: introgression and/or local domestication? Catarina Ginja (Centre for Environmental Biology, Faculty of Sciences, Lisbon University), Simon Davis (Laboratório de Arqueociências, Direção-Geral do Património Cultural-DGCP), Ana Elisabete Pires and José Matos (Unidade Estratégica de Biotecnologia e Recursos Genéticos, Instituto Nacional de Investigação Agrária e Veterinária-INIAV), Carlos Fernandez (Facultad de Filosofía y Letras, Universidad de León), Catherine Hänni (Paleogenomics and Molecular Evolution group, Institut de Génomique Fonctionnelle de Lyon/École Normale Supérieure), Emma Svensson (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences) and Anders Götherström (Archaeological Research Laboratory, Stockholm University)

In cattle, mitochondrial DNA (mtDNA) provides strong evidence for a major domestication event in the *Fertile Crescent* during the Neolithic. While most ancient and domestic cattle matrilines belong to the macro-haplogroup T, which diverged ~16,000 years BP, Southwest-Asian taurine cattle hold the greatest diversity. In addition, the finding of distinct P and E matrilines in North and Central European aurochs appears to indicate that these are not direct ancestors of modern cattle, whereas T-lineages were found in Southwest-Asian

aurochs. The hypothesis of a single domestication event for taurine cattle in Southwest-Asia gained further support from recent Bayesian simulation analyses, which indicate a strong founder effect and limited subsequent admixture between wild and domestic stock. However, a different scenario is suggested for South European cattle. The finding of rare ancestral R-lineages in native Italian cattle suggests local contribution of wild cattle populations. We hypothesize that local aurochs also played a role in the origin of cattle in the Iberian Peninsula. We identified a total of 14 mtDNA haplotypes in 7 aurochs and 15 domestic cattle specimens, collected in Iberia and North Africa from the Chalcolithic to the Roman periods. A wide sharing of T3-lineages between aurochs and domestic cattle in the Chalcolithic of northern Spain was detected. Osteometric analysis ascertained wild status for three of the four T3-like specimens. This finding suggests that either introgression occurred and/or that Iberian aurochs carried T-lineages. Extensive introgression of domestic cows into the wild aurochs population is not expected, thus we cannot discard the possibility that Iberian aurochs carried T3-matrilines. If this was the case, the high frequency of T3-matrilines found in Iberian cattle could also derive from aurochs domesticated locally.

P4005 Gene number, copy number variation and expression analysis of *NK-lysin* in cattle. Junfeng Chen, Mi OK Lee, Leif Andersson and James Womack (Texas A&M University)

Antimicrobial peptides (AMPs) play significant roles in the host immune system, and are active against various microorganisms, including bacteria, fungi, protozoa, viruses and even tumorous cells. Human granulysin and its counterparts in several animal species have been identified as a new class of AMPs, which belong to the saposin like protein (SAPLIP) family, and they are secreted from the granules of cytotoxic T lymphocytes and natural killer cells. To date, the structure and function of NK-lysin peptides have been well studied while their genomic structure and variation have received much less attention.
This study focused on the genomic structure and organization of bovine NK-lysin genes. Reference sequence analysis suggested that there are at least four NK-lysin genes in the bovine genome, NK1, NK2, NK3 and NK4. Sequence analysis of NK1, NK2 and NK3 in 4 homozygous animals, which were chosen based on the genotyping result from a 770K HD SNP array, clearly showed that at least three NK-lysin genes exist in the cattle genome, and NK1, NK2 genes are located within independent copy number variation regions (CNVRs). To avoid the complexity of allelic variation, NK1, 2 and 3 sequences were studied in two BAC clones, and these data further confirmed the hypothesis that the bovine genome contains multiple NK-lysin genes. These two BAC clones were then sent to the PacBio for sequencing and complete genomic sequences for four genes were extracted. Expression profiles of the four genes in six respiratory-related tissues are currently being studied by taqman assay.

P4006 Whole-genome resequencing of Japanese native horses for SNP and INDEL discovery. Teruaki Tozaki (Genetic Analysis Department, Laboratory of Racing Chemistry), Taichiro Ishige (Genome Research Center, Tokyo University of Agriculture), Satoshi Sano (Ulvaniac Inc.), Hironaga Kakoi, Kei-ichi Hirota and Hitoshi Gawahara (Genetic Analysis Department, Laboratory of Racing Chemistry), Masaki Takasu (Department of Veterinary Medicine, Gifu University), Tomohiro Kono (Laboratory of Animal Developmental Biology, Department of Bioscience, Tokyo University of Agriculture) and Kei Hanzawa (Laboratory of Animal Physiology, Department of Animal Science, Tokyo University of Agriculture)

To date, horse genome sequencing/resequencing

has identified over 1.1 million single-nucleotide polymorphisms (SNPs), which have mainly been used for breeds in Europe and USA, including Thoroughbred and Quarter horses. In Japan, there are 8 breeds, namely, Hokkaido, Kiso, Misaki, Noma, Taishu, Tokara, Miyako, and Yonaguni horses. Japanese horses originated from Mongolian horses and have isolated from European breeds. Many novel SNPs and insertion/deletions (INDELs) are expected in Japanese horses because these have phenotypic characters that differ from those of European breeds. Here, we describe the identification of SNPs and INDELs by whole-genome resequencing of Japanese horses sequenced as paired-end 100-bp reads using HiSeq 2500 (Illumina). Compared with the EquCab 2.0 reference sequence, a Hokkaido horse had 5,821,844 SNPs, including 18,052 non-synonymous, 30,646 synonymous, and 5,939 untranslated region (UTR) SNPs in exons and 1,493,856 SNPs in introns. Additionally, there were 118,698 SNPs in the 2 kb upstream of genes, 97,287 in the 2 kb downstream of genes and 4,057,366 in other intergenic regions. The Hokkaido horse also had 335,954/384,820 INDELs compared with EquCab 2.0, of which 1,875/3,134 were in exons, 665/1,121 in UTRs, and 92,805/106,343 in introns. Furthermore, there were 9,377/14,144 INDELs in the 2 kb upstream of genes, 6,549/7,906 in the 2 kb downstream of genes, and 224,683/252,172 in other intergenic regions. Although another Hokkaido horse (5,812,513 SNPs, 332,887 insertions, 381,860 deletions) had a similar number of SNPs and INDELs, a Kiso horse (5,460,962 SNPs, 328,217 insertions, 365,123 deletions) and a Yonaguni horse (5,320,104 SNPs, 321,223 insertions, 351,697 deletions) had less SNPs and INDELs than the Hokkaido horses. It is considered that the bottleneck effect of decreases in the population sizes of Kiso and Yonagni horses would lead to an increase in homozygous SNPs. We also discuss the characteristics of Japanese horses in terms of their genomic structures.

P4007 Whole Transcriptome SNP Discovery and Analysis of Genetic Diversity in Five Duck (Anas platyrhynchos) Populations. Xiaoyong Du, Shuhong Zhao, Jianhua Cao and Shijun Li (Huazhong Agricultural University)

One of the goals of animal genomics research is to identify the genetic differences responsible for variation in phenotypic traits. The completion of the duck (*Anas platyrhynchos*) genome sequence and recent advances in NGS technology allow for in-depth characterization of the genetic variations present in duck. In this study, we performed transcriptome sequencing of distinct breeds for the purpose of identifying and annotating novel SNPs in duck. Alignment of RNA-Seq data of 15 individual ducks from four domesticated populations and one wild population was used for the discovery of 167,230 reliable SNPs, which subsequently be used for the analysis of genetic diversity among the different populations. The application of RNA-Seq data rather than WGS or WES lead to enrich variants in coding exons (9.6%), UTRs (2.9%), introns (19.2%) and downstream/upstream (37.2%) and thus increases the power to detect functionally important SNPs. The SNPs we discovered were highly abundant in these four categories. Only a small fraction (28.8%) fell into intergenic region. Heterozygosity of all individuals ranged from 38.2% to 58.7%, and the average frequency of heterozygous SNPs was 46.1%. These sites presented a transition-to-transversion (ts/tv) bias of 2.29, which is higher than the overall ts/tv ratio of 2.0–2.1 for the entire human sequence and thus is a good reflection of the genomic variation in transcribed regions. The SNP variant detection from RNA-seq data provides more detailed evolution ary information and insight into adaptation mechanisms.

P4008 Assessment of genetic bottleneck and

population structure of captive Red junglefowl (*Gallus gallus murghii*) populations in India. Mukesh Thakur (wildlife Institute of India, Dehradin), Merwyn Fernandes (Wildlife Institute of India, Dehradun), Jianlin Han (CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS)) and Sambandham Sathyakumar (wildlife Institute of India, Dehradin)

The Red Junglefowl (RJF) is the single most important species to mankind as it is believed to be the wild ancestor of all domestic chicken in the world. Establishment of genetic characterization and relationship among captive populations is crucial for zoos/conservation breeding centers to avoid inbreeding and increased extinction risks due to loss of genetic diversity and fixation of alleles. We investigated the genetic variation, population genetic structure, level of inbreeding and the presence of any historic bottleneck footprints in four captive RJF populations of India. Each of these populations was tested for heterozygosity excess or deficiency since any bottlenecked population would undergo transient heterozygosity excess. The results were supported by three statistical methods: (i) Wilcoxon sign-rank test; (ii) a mode shift test of allele distribution pattern; and (iii) the ratio of the number of alleles to the range of allele size, *M*-ratio test. The RJF populations of the four captive centers showed significant levels of inbreeding but none of the populations had undergone any severe bottleneck in the recent past. Bayesian cluster analysis revealed three distinct groups among the four captive RJF populations. Interestingly, birds of Kufri population were assigned together with Gopalpur as well as with Morni populations, indicating their shared genetic ancestry. Among the four populations, Morni population displayed the richest genetic attributes and was therefore presumed to be a key source of genetic variation. Nine birds of Morni population were relatively pure (q-value >0.98) and carried about 50% of the total private alleles of Morni population. Thus, being the foremost reservoir of allelic diversity, these nine birds may be selected for launching alien alleles to other RJF populations to rescue their loss of genetic diversity arising from inbreeding.

P4009 Trace back analysis of paternal lineages in local goat breeds of turkey by using Y-chromosomal DNA. Bengi Cinar Kul and Nüket Bilgen (Ankara Universitesi Veteriner Fakultesi Genetik Anabilim Dali Diskapi Ankara), Ozgecan Korkmaz Agaoglu (Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Zootekni Anabilim Dali Burdur), Bilal Akyuz (Erciyes Üniversitesi Veteriner Fakültesi Zootekni Anabilim Dali Kocasinan Kayseri) and Okan Ertugrul (Ankara Universitesi Veteriner Fakultesi Genetik Anabilim Dali Diskapi Ankara)

Genetic variations in chromosome Y of mammals are important to detect the genetic diversity of populations. Studies on chromosome Y have focused mainly on the domestication of a particular species. Researches on Y-chromosome haplotypes have shown that the origin and divergence of domestic goats all over the world can be traced. We have analysed two polymorphic Y chromosome genes, which are the amelogenin-*AMELY* and zinc finger-*ZFY* in seven Turkish native goat breeds (n=149). At the end of the study, the C1 haplotype was determined in 115 goats, and the C2 haplotype was determined in 33 goats. In addition, one goat carried a new haplotype. The genetic distances between breeds were examined using the Wright's FST and Nei's Dxy methods. According to these analyses, the Kilis breed was found to be statistically more varied than the other breeds. This result was significant for the evolutionary history of goat breeds. This study revealed that *Capra aegagrus* (bezoar) is the paternal line of the examined goat breeds and also relatively low level genetic diversity of Y chromosome

indicates that effective sizes of males are lower than females in domestication process in Anatolia. ***This study was supported by TUBITAK (TOVAG- 111O799)**

P4010 Everything matters in a promoter. New insights on the ovine *HSP90AA1* gene regulation.. Judit Salces-Ortiz and Carmen Gonzlez (nstituto Nacional de Investigacin y Tecnologa Agraria y Alimentaria (INIA)), Alfonso Bolado-Carrancio (Department of Molecular Biology), JC Rodguez-Rey (2Department of Molecular Biology) and M. Magdalena Serrano (nstituto Nacional de Investigacin y TecnologaAgraria y Alimentaria (INIA))

Polymorphisms at the *HSP90AA1* ovine gene, which encodes the Hsp90α chaperone, have been related with the *scrapie* incubation period, the adaptation to this specie to differential thermal conditions and the sperm DNA fragmentation rate. All these facts are consequence of the differences in the gene expression rate caused by some of the polymorphisms existing at its promoter. The region between -1500 and -250bp before the transcription start site (TSS) contains the main core of the regulation of the gene and is highly polymorphic (14 polymorphisms). Therefore it has been deeply studied in this work by three different approaches. a) - By studying the methylation pattern of this region and its relationship with the previously detected SNPs. It has been enclosed for the first time in the ovine HSP90AA1 gene the limits of its promoter which has previously described with a typical core promoter of TATA box, even so a different promoter pattern has also been observed. This characteristic can be the responsible of a more dispersed initiation of transcription and could be the clue of the differences of expression patterns. b) - By studying the differences of joining affinity of the transcription factors (TF) to a sequence where a SNP, INDEL or a methyl group is located. Using EMSA analysis, we studied the SNPs independently. This is possible even for those SNPs that cosegragate together. c) -By in vitro expression assays of the promoter region, using the luciferase reporter gene. It permitted us to quantify the exact differences of in vitro transcription, based on only one change in the sequence of the promoter that allows or blocks the joining of transcription activators or repressors.

P4011 Genetic structure and diversity of Australian Bullmastiffs. Sally-Anne Mortlock, Mehar Khatkar and Peter Williamson (The University of Sydney)

Increased incidence of lymphoma in the Australian Bullmastiff population provides a unique opportunity to investigate the genetic mechanisms behind lymphoma predisposition. The present study was undertaken to characterise the genetic profile of Australian Bullmastiffs prior to genome-wide association analysis. A total of 66 (including 6 affected with lymphoma) Australian Bullmastiff dogs from across eastern Australia were genotyped using the 170,000 SNP Illumina CanineHD Beadchip. Fine-scale population structure was investigated and visualised using NETVIEW, a population analysis pipeline. An unsupervised network clustering procedure, Super Paramagnetic Clustering (SPC), was used to create a fully connected population network in which individuals are clustered based on genetic distance. The clustering of individuals within the network was then visualised in Cytoscape. The high definition network visualisation showed detailed relationships among individuals within and between subpopulations. Three subpopulations were identified within the 66 dogs genotyped, with families from two different states clustering separately highlighting the close genetic distance within family lines. Genetic diversity and the degree of genetic homogeneity within the breed were investigated through the calculation of multilocus heterozygosity (MLH), runs of homozygosity (ROH) as proportion of the

genome, and inbreeding coefficient. The mean MLH was 0.346 while on an average ROH covered 28.77% (ranged from 21.08% to 44.03%) of the genome. The mean inbreeding coefficient based on a total of 16,378 Bullmastiff pedigrees from year 1980 to 2013 was estimated as 0.039 which was higher than the estimate of 0.023 using genotype data. Investigation into the extent of linkage disequilibrium, haplotype diversity and the effective population size (Ne) is ongoing. Understanding the genetic profile of this breed is essential to interpret genome-wide association analysis, allowing regions associated with lymphoma predisposition to be better defined.

P4012 Genetic status of red deer *(cervus elaphus)* in China based on mitochondrial DNA control region sequences. Jian-feng TU (Institute of Special Wild Economic Animal and Plant Science, Chinese Academy Agricultural Sciences)

Red deer *(cervus elaphus)* is the special animal, and its' antler is rare medicinal materials in China. To better protect and utilize genetic resources of red deer, we obtained 201 complete sequences of the mitochondrial genome control regions from 14 geographic populations at 6 subspecies in this study. 155 mutation loci and 57 kinds of haploid were detected. The genetic diversity between subspecies is rich, but within populations genetic diversity is poor relatively. There were shared and unique haploid between different subspecies, in which may be genetic exchange. Phylogenetic analysis showed that the relationship of different groups correlated to the geographical position.

P4013 CD4 polymorphism in Microminipigs. Tatsuya Matsubara (United Graduate School of Veterinary Sciences, Gifu University), Masaki Takasu, Naohito Nishii and Noriaki Imaeda (Faculty of Applied Sciences, Gifu University), Asako Ando and Yoshie Kametani (Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine), Jerzy Kulski (Centre for Forensic Science, The University of Western Australia) and Hitoshi Kitagawa (Faculty of Applied Sciences, Gifu University)

Microminipigs are extra-small sized novel miniature pigs developed for biomedical research in Japan. In the process of analysis of lymphocyte subpopulations, CD4 positive cells could not be detected in the peripheral blood mononuclear cell (PBMC) analysis in some groups of Microminipigs by flow cytometry using anti-pig CD4 antibodies (clone 74-12-4, MIL17, and PT90A). Two kinds of alleles in the CD4 gene, CD4.A and CD4.B, were identified in Microminipigs by nucleotide sequencing of RT-PCR products using CD4 specific primer pairs. In the CD4.A gene, one amino-acid substitution was found in exon 3 region compared with CD4 sequences in Sscrofa10.2 (NP001001908). In comparison, nine amino-acid sequences were different between CD4.B and CD4 sequences in the exon 3 region of Sscrofa10.2. Amino acid sequences of CD4.A and CD4.B in exon 3 region were identical to those of CD4.c in Japanese boar (AB250925) and CD4.2 in NIH miniature pigs (X65630), respectively. Homozygous and heterozygous CD4.A and CD4.B alleles were identified simply by using the RFLP technique and the restriction endonuclease, BseRI. Allele frequencies of CD4.A/A, CD4.A/B and CD4.B/B in a herd of Microminipigs (n=198) were 16.2%, 54.0% and 29.8%, respectively. Both of the CD4.A and CD4.B expression in PBMCs of pigs with CD4.A/B were confirmed by sequencing of the RT-PCR products. Only CD4.B/B gave rise to a loss of reactivity with the CD4 antibodies that we used in flow cytometry. Microminipigs with CD4.B have grown normally and not shown any abnormal clinical signs. No significant difference of immunoglobulin G and M concentrations in plasma were observed between the pigs with CD4.A/A and CD4.B/B. As exon 3 region of

CD4 molecules is essential for binding to MHC class II molecules, further analyses of their polymorphic relationships in Microminipigs might be important.

P4014 Increase of luteinizing hormone beta promoter activity in the Moroccan D'man breeds with high breeding prolificacy.. Redouane Aherrahrou (Institut für Integrative und Experimentelle Genomik (IIEG)/Moulay Ismaïl University, Faculty of Sciences, Department of Biology BP 11201 Zitoune Meknes , Morocco.), Zouhair Aherrahrou (Institut für Integrative und Experimentelle Genomik (IIEG)), Frank Kaiser, Diana Braunholz and Jeanette Erdmann (Institut für Humangenetik Lübeck) and Mouhieddine MOUMNI (Moulay Ismaïl University, Faculty of Sciences)

LH-ß plays a key role in reproduction. Common Moroccan breeds D'man, Sardi and Timahdite were found to differ in litter size. Recently we amplified and sequenced the promoter region of this gene from the three breeds and revealed many genetic variants within the promoter region that may affect the promoter activity using an *in-silico* approach. The functional role of these variants on the promoter activity remained unclear. In this research project we aimed to use *in-vitro* assay to functionally investigate the role of these identified variants on the promoter activity using luciferase assay. Our analysis demonstrated a high increase of the promoter activity from D´man with high prolific breed compared to low prolific breed Sardi and Timahdite. We generated different fragments with different lengths and tested the presence of informative promoter region with high promoter activity. Interestingly a small region of 541 bp was identified to retain a high promoter activity. This region contains the two palindromic sequences (P1 and P2) strongly relevant for the promoter activity after stimulation with *Forskoline*. Using sequence alignment within this region, 7 variants were identified to differ between D'man and Timahdite/Sardi. Two variants were found informative and localized within a transcription factors binding sites (TFBS) rich region including GATA-1/GATA-2, E4/th1, CP2 and c-Ets. Using site directed mutagenesis we could demonstrate that the -559 A/G polymorphisms affect dramatically the promoter activity and may alone explain the difference in the promoter activity observed between D'man and Timahdite/Sardi breeds.

P4015 Genetic diversity and population structure of indigenous sheep population of eastern Ethiopia. Helen Nigussie (Ambo University)

In eastern Ethiopia, indigenous sheep significantly contributed to the livelihood of pastoralists, agro pastoralists and smallholder farmers. The increased demand for mutton both in domestic and export market outstrip the current productivity of sheep. A comprehensive evaluation of eastern Ethiopian sheep population has not been previously performed; therefore, ten indigenous sheep populations from three breeds (Afar, Black head Somali, Hararghe highland) were genotyped at 21 microsatellite markers recommended by Food and Agriculture for the United State and International Society of Animal Genetics. A total of 300 individual sheep were tested to assess genetic variations within and between sheep populations (viz. Amibara, Awash, Gewane, Babile, Jijiga, Harshin, Shinile, Deder, Gorogutu and Meta) based on morphology and sheep owners' identification. The polymorphic Information Content (PIC) values ranged from 0.497 (for marker BM1824) to 0.90 (marker MCM140) with an average value of 0.77, showing that the microsatellite panel used was highly informative. The overall observed and expected heterozygosity values were 0.60 and 0.72, respectively. The FIS (0.20) and FIT (0.25) values indicated relatively high level of inbreeding within sheep populations while a low

FST estimate (0.07) shows that low genetic sub differentiation among sheep populations. Bayesian cluster analysis indicated that ten sheep populations were grouped into two major clusters (K=2) viz. Amibara, Awash, Gewane, Jijiga and Shinile were clustered into group one while Babile, Deder, Gorogutu, Harshin and Meta populations were clustered into the second group; at K=3 and beyond there was high degree of population genetic admixture and it was difficult to separate one cluster from the others. The low genetic differentiation and high genetic admixture between populations might be due to proximity between the study areas, marketing system and breeding practice of the sheep owners. The current finding will help to set up sustainable genetic improvement and conservation program.

P4016 Genetic differentiation and persistency of linkage phase among six South African cattle populations. Sithembile Makina and Faria Muchadeyi (Agricultural Research Council), Este Van Marle-Koster (University of Pretoria), Michael MacNeil (Montana State university) and Azwihangwisi Maiwashe (Agricultural Research Council)

Genomic selection relies on the assumption that phases of linkage disequilibrium between markers and quantitative trait loci are the same in the selection candidates and reference population. if maker phase are correlated across multiple breeds, it could be possible to pool several breeds into one common reference population. This study investigated genetic relationships and persistence of linkage disequilibruim phase amonst six South African cattle populations including Afrikaner (42), Nguni (54), Drakensberger (45) Bonsmara (46), Angus (31) and Holstein (29). Genetic differentiation between breeds was estimated using Wright F-statistics (*Fst*) and Principal Component Analysis (PCA). Model-based clustering was used to infer ancestry of individuals in the breeds. Consistencies of SNP phase between populations was inferred from the signed *r* values. Among the indigenous and local developed breeds the greatest genetic differentiation was observed between Afrikaner and Drakensberger (*FST = 0.159)* pair while the least genetic differentiation was observed between the Bonsmara and Drakensberger (*FST* = 0.043). The PCA indicated a clear genetic differentiation of the six contemporary populations. ADMIXTURE analyses revealed some level of admixture between indigenous and local developed breeds that could be explained by their respective population histories. The highest correlation was observed between Nguni and Bonsmara cattle (0.6) for marker pairs separated by 10 kb. Analyses of genetic variation revealed that there is still great degree of diversity amongst South African cattle populations. Therefore this suggested the necessity of breed-specific reference populations or the need to include adequate representation of each breed in the reference population if a multi-breed reference is to be used.

P4017 The characterization of MHC class II *DRB1* in native sheep from Xinjiang China . Mairepati palati (The university of Tokyo; RIKEN), Yoko Aida and Shin-nosuke Takeshima (RIKEN), Jueken Aniwashi Aniwashi (Xinjiang Agricultural University) and Mahmut Halik (Xinjiang university)

This present study was designed to determine the nucleotide sequences of exon 2 of the *Ovar-DRB1* locus and to investigate *Ovar-DRB1* gene frequencies and characterize genetically the *Ovar-DRB1* exon 2 in Xinjiang native sheep, namely Karakul Ram and Bashibai, and three hybrid generations derived from a cross between Bashibai and Altai Argali wild sheep. Results: this study identified 12 novel alleles and 30 previously reported alleles. A neighbor-joining tree of the amino acid sequences of these 42 alleles did not reveal species specific clustering of alleles, alleles shared across the study

populations. Furthermore, pairwise comparisons of alleles found similar variation with respect to the average nucleotide and amino acid frequencies in all populations, and also similar levels of synonymous and non-synonymous substitutions. The value of non-synonymous vs. synonymous in ABS is remarkably higher than that of all sites. Wu-Kabat analysis showed that between 29 and 32 amino acid positions were significantly polymorphic in the five populations. All populations showed high degree of genetic diversity and the genetic variability may be maintained by balancing selection. By contrast, analysis of allele frequencies in each population indicated that *DRB1*K18cC*(21.2%), *DRB1*2F10c8* (13.2%) and *DRB1*0803* (13.2%), *DRB1*2F16c2* (17.6%), *DRB1*1601* (14.3%), and *DRB1*0803* (20.0%) were the most frequently occurring alleles in Karakul Ram, Bashibai, F1, F2, and F3 generations, respectively. Thus there are significant differences in allelic frequency between Karakul Rams and Bashibai sheep, but not between the Bashibai breed and subsequent hybrid generations arising from the Bashibai × Altai Argali cross. A population tree based on the *Ovar-DRB1* allelic frequency in each population indicated that the Bashibai breed and three hybrid populations were similar, with Karakul Ram being genetically distinct. Our study may provides information for the scientific design of improved livestock breeding strategies in the future

P4018 Whole genome sequencing of three native cattle breeds from northernmost cattle farming regions. Melak Weldenegodguad (Department of Biology, University of Eastern Finland), Ruslan Popov (Yakutian Research Institute of Agriculture), Ying Xiong and Jiabao Xu (Beijing Genomics Institute) and Juha Kantanen (Biotechnology and Food Research, MTT Agrifood Research Finland)

The Eurasian taurine cattle (*Bos taurus*) have adapted to a range of diverse environments and agricultural conditions as a result of 8,000 - 10,000 year domestication process. Finland in Europe and Sakha (Yakutia) in Siberia in the Russian Federation in Asia belong to the northernmost regions where cattle farming has been traditionally practiced. In these northern regions, locally adapted native cattle, Finncattle and Yakutian cattle, still exist although the breeds are currently endangered. Genetics of these breeds have been previously analyzed using e.g. autosomal microsatellites. In the present study, we sequenced genomes of 15 individuals (five in each breed) of the native Eastern Finncattle, Western Finncattle and Yakutian cattle using Illumina technology, examined genetic diversity and unfolded loci under natural or artificial selection. We reached 13-fold coverage after mapping the sequencing reads on the bovine reference genome (UMD 3.1). We detected selective sweep signatures using the identified SNPs applying Linkage disequilibrium and Composite of Likelihood Ratio methods. More than 14 million SNPs were identified in the samples and a total of 10% and 20% of SNPs found in the Finncattle and Yakutian cattle, respectively, have not been previously detected. Yakutian cattle displayed a higher level of within-population variation in terms of number of polymorphic SNPs and observed heterozygosity than the two Finnish breeds. This is in contrast to the previous microsatellite study where the Finnish breeds were clearly more diverse than Yakutian cattle. In addition, Yakutian cattle showed genetic distinctiveness. We identified a number of genomic regions that have been under selection and may have affected by positive selection for the northern and arctic environments, including genes involved in diseases resistance, growth and reproduction. The present study indicates that the gene pools of the studied breeds are valuable genetic resources for the northern agriculture.

P4019 Genetic diversity and relationship

among 16 Asian and European cattle populations using 121 autosomal SNPs genotypes by the DigiTag2 assay. Riku Yonesaka and Shinji Sasazaki (Graduate School of Agricultural Science, Kobe University), Hiroshi Yasue (Tsukuba GeneTechnology Laboratories Inc.), Satoru Niwata (Kurabo Industries Ltd.), Fumio Mukai (Wagyu Registry Association) and Hideyuki Mannen (Graduate School of Agricultural Science, Kobe University)

Native cattle breeds are important genetic resources for future breeding improvement. In this study, we assessed genetic diversity, structure and relationship among Eurasian cattle populations. We genotyped 121 autosomal SNPs for 470 unrelated cattle from 16 populations (*B.taurus* populations: Black Angus, Hereford, Japanese Holstein, Japanese Black, Tosa Japanese Brown, Higo Japanese Brown, Japanese Shorthorn, Japanese Polled, Hanwoo, Mongol. *B.indicus* populations: Laos, Cambodia, Myanmar, Vietnam, Bangladesh, Bhutan), using DigiTag2 assay. The genetic indices suggested that the genetic diversity of *B.indicus* populations was lower than that of *B.taurus* populations. PCA and phylogenetic analyses showed that *B.taurus* and *B.indicus* populations were clearly distinguished. Ten *B.taurus* populations clustered loosely together, and then partial separation between European and Asian groups was observed. Japanese Shorthorn and Japanese Polled clustered with European populations. The STRUCTURE analysis also revealed distinct separation between *B.taurus* and *B.indicus* (K=2 with maximum ⊿ K value), and between European and Asian populations (K=3). In addition, Japanese Shorthorn and Japanese Polled were clustered into European populations, and Japanese Holstein revealed an admixture pattern with Asian and European cattle (K=3). It suggested that these breeds have substantial genetic influence by European breeds. At K=11, all *B.taurus* populations were illustrated as independent clusters, although Mongolian population showed an admixture pattern with different two ancestries (K=13). *B.indicus* populations revealed uniformity genetic structure (K=2-11, 16), suggesting low genetic diversity in *B.indicus*. However, Bhutan and Bangladesh populations formed a different cluster from other *B.indicus* populations (K=8, 12-15). Vietnam native cattle was subdivided into two groups, southern and northern areas (K=14). In conclusion, our study using 121 SNPs markers could sufficiently explain genetic diversity, relationship, structure and admixture of Asian cattle population well.

P4020 Association between an alternative promoter polymorphism and sperm deformity rate is due to modulation of the expression of KATNAL1 transcripts in Chinese Holstein bulls. Changfa Wang (Dairy Cattle Research Center, Shandong Academy of Agricultural Science)

Association between an alternative promoter polymorphism and sperm deformity rate is due to modulation of the expression of KATNAL1 transcripts in Chinese Holstein bullsXiaojian Zhang, Changfa Wang*, Yan Zhang, Zhihua Ju, Xiuge Wang, Jingming Huang, Qiuling Li, Fangxiong Shi, Jifeng ZhongDairy Cattle Research Center, Shandong Academy of Agricultural Science, Jinan 250131, PR China. *Wangcf1967@163.com We evaluated one novel splice variant, and characterized the promoter and a functional single nucleotide polymorphism (SNP) of the bovine Katanin p60 subunit A like1 (KATNAL1) gene in order to explore its expression pattern, possible regulatory mechanism, and relationship with semen traits in Chinese Holstein bulls. A novel splice variant, KATNAL1 transcript variant 2 (KATNAL1-TV2) of the retained 68 bp in intron 2, was identified by RT PCR and compared with KATNAL1 transcript variant 1 (KATNAL1 TV1, NM 001192918.1) in various tissues.
Bioinformatics analyses predicted that

KATNAL1 transcription was regulated by two promoters: P1 in KATNAL1-TV1 and P2 in KATNAL1-TV2, respectively. Results of qRT-PCR revealed that KATNAL1-TV1 had higher expression than KATNAL1TV2 in testes of adult bulls ($P < 0.05$). Promoter luciferase activity analysis suggested that the core sequences of P1 and P2 were mapped to the region of c.-575 ～ c.-180 and c.163-40 ～ c.333+59, respectively. One novel SNP (c.163-210T>C, ss 836312085) located in intron 1 was found using sequence alignment. The SNP in P2 resulted in the presence of the DeltaE binding site, improving its base promoter activity ($P < 0.05$); and we observed a greater sperm deformity rate in bulls with the genotype CC than with the genotype TT ($P < 0.05$), which indicated that different genotypes were associated with the bovine semen traits.
Bioinformatics analysis of KATNAL1 protein sequence predicted that the loss of the MIT domain in KATNAL1-TV2 transcript resulted in protein dysfunction.

P4021 Large scale geographic mitochondrial DNA analysis provide insights on the demographic and evolutionary history of the dromedary (*Camelus dromedarius*). Faisal Almathen (Ecology and Evolutionary Genetics Group, School of Life Sciences, The University of Nottingham, Nottingham UK), Pauline Charruau (Vetmeduni Vienna, Department of Biomedicine, Institute of Population Genetics, Vienna Austria), Joram Mwacharo (Small Ruminant Genetics and Genomics Group, International Centre for Agricultural Research in Dry Areas, Addis Ababa Ethiopia), Majed Alnaqeeb (Kuwait University, Faculty of Science, Department of Biological Sciences, Khaldiya Kuwait), Abdussamad Muhammad Abdussamad (Department of Animal Science, Faculty of Agriculture, Bayero University, Kano State, Nigeria), Raziq Abdul (Lasbela University of Agriculture, Water and Marine Sciences, Regional Cooperation for Development (RCD) Highway, Uthal, Pakistan), Marzook Al-Eknah (Department of Clinical Studies, College of Veterinary Medicine and Animal Resource, King Faisal University, Al-Hasa, Saudi Arabia), Bernard Faye (CIRAD-ES, UMR 112, Campus International de Baillarguet, Montpellier cedex, France), Olivier Hanotte (Ecology and Evolutionary Genetics Group, School of Life Sciences, The University of Nottingham. UK) and Pamela Burger (Vetmeduni Vienna, Department of Biomedicine, Institute of Population Genetics, Vienna Austria)

The dromedary (*Camelus dromedarius*) or "the ship of the desert" has been a key livestock species in the emergence, development and expansion of trading networks, societies, civilizations and contacts across inhospitable habitats for over three millennia. Although, archeological findings seem to suggest that the species was domesticated in the southern part of the Arabian Peninsula, its exact geographic centre of origin remains in dispute, as is the timing and the routes of dispersal across their present day home range. Here, we address these issues by sequencing an 867 bp fragment of mtDNA spanning the *D*-loop region (549 bp), cytochrome B (184 bp) and the threonine and proline tRNA genes (134 bp) in 759 animals from 21 countries spanning the modern day geographic range of the species. We reveal two mtDNA haplogroups both with clear signals of expansion. These might have been "domesticated" at the same time from a single ancestral population, two closely related populations, or one ancestral haplogroup might have been domesticated first and the second subsequently introgressed into the domestic genepool. Bayesian coalescent simulations reveal an earlier signal of demographic expansion in the southern Arabian Peninsula, the proposed geographic center of domestication. A weak phylogeographic structure is observed, a likely outcome of the dynamic long-range movements and dispersals of the species across history. Our

findings support the unique role played by ancient land and sea trading networks in shaping the modern day genetic diversity and structure of a livestock species, while providing unique insights on the pattern and process of trading in ancient times across the drylands of the Old World.

P4022 Mitochondrial DNA diversity in Nepalese indigenous goats (*Capra hircus*). Neena Gorkhali and Bhola Shrestha (Nepal Agriculture Research Council), Yuehui Ma (Chinese Academy of Agriculture Sciences) and Jianlin Han (Chinese Academy of Agriculture Sciences, ILRI)

Origin and Genetic Diversity of Nepalese Indigenous Goats (*Capra hircus*) *N. A. Gorkhali**†, B. S. Shrestha†, Y. H. Ma*and J. L. Han*‡ *Institute of Animal Science, Chinese Academy of Agricultural Science (CAAS), Beijing, China, †Animal Breeding Division, Nepal Agriculture Research Council, Kathmandu, Nepal, ‡International Livestock Research Institute (ILRI), Nairobi, Kenya

The domestic goat (*Capra hircus*) is an important livestock species in Nepal and four phenotypically defined breeds and many non-descript goats exist in the country. In this study, the mitochondrial DNA (mtDNA) d-loop hyper-variable region of 93 Nepalese goats belonging to Khari, Chyangra, Terai and Sinhal breeds from different parts of the country were sequenced to examine their phylogenetic relationship and within-breed genetic diversity. High mtDNA diversity was observed among Nepalese goat breeds and all mtDNA haplotypes were classified into the four previously defined mDNA haplogroups (A-D). Haplotypes of haplogroup A were observed in most of the Nepalese goats whereas only one breed (Chyangra) contained all the four haplogroups. These sequences were compared with published sequences of Asian domestic and wild goats to determine the relationship of Nepalese goats among goat genetic resources of the region. Keywords: Nepalese goat, Mitochondrial DNA, Genetic diversity

P4023 Domestication and geographic origin of Chinese goats: insights into the evolutionary history of Eurasian goats. Tao Zhong (Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University), Jiazhong Guo (College of Animal Science and Technology, Sichuan Agricultural University), Jianlin Han (CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS)), Yuehui Ma (Institute of Animal Science, Chinese Academy of Agricultural Sciences) and Hongping Zhang (College of Animal Science and Technology, Sichuan Agricultural University)

Goats were one of the first domesticated animals around 10,500 years ago, and were introduced into Europe mainly via the Mediterranean route. A scenario of multiple maternal origins and weak phylogeographic structures, which were mainly inferred from mtDNA variations, was revealed in Eurasian goats. However, the global phases of mtDNA and Y-chromosome gene flows remain unknown. In this study, variations from both mtDNA and the non-recombining Y-chromosome (NRY) gene were genotyped in 2,147 unrelated goats from 40 Chinese indigenous breeds. The gender of each sample was confirmed using the SE47/SE48 sex identification primers. Direct sequencing of PCR products was carried out to scan the polymorphisms in the D-loop region and the SRY gene. A total of 710 D-loop sequences were used to construct the phylogenetic trees with consensus Bayesian, NJ, and ML methods. The results showed that Eurasian goats were dispersed in the five haplogroups A, B1, B2, C and D, which were consisted with NETWORK phylogenies. Another haplotype G was only observed in Turkish goats. Based on the

polymorphisms in SRY gene (-1769g.G>A, -1080g.G>A and -95g.T>G in promoter region, 2603g.T>A, 2670g.G>A, 2863g.T>A and 2990g.A>G in 3'-UTR region), four NRY haplotypes were identified in 508 Chinese male goats. Haplotypes NRY1, NRY2 and NRY3 were consistent with the haplotypes Y1A, Y1B and Y2 in European goats. The rare NRY4 was first identified in Chinese goats. Furthermore, extensive mtDNA and Y-chromosome gene flows were revealed in both Asian and European goats. Based on the mtDNA and NRY haplotypes, the geographic origin of Chinese goats were assigned to two main areas. In the present study, we provided molecular inferences on the origin of Chinese goats and particularly on their paternal evolution complementary to those from mtDNA data. However, further views based on ancient specimen with known provenance are warranted.

P4024 The mechanism of molecular evolution and epigenetic regulatory that muscle development-related genes H19 have in the process of the pig domestication. cencen li (Huazhong Agricultural University)

The domestic pig originated from Sus scrofa about 10,000 years ago. During domestication, there have been drastic changes between domestic pigs and wild boars, involving morphological, physiological, behavioral aspects, under artificial selection and natural selection. An important non-coding gene, H19, located in an imprinting cluster which has been reported to have the role of regulating muscle development in humans and mice, whereas its function role in pigs is still unknown. In order to explore genetic and epigenetic contribution of H19 in pig domestication and breed differentiation process. Firstly we analyzed the molecular evolution of this gene using the published re-sequencing data and identified 14 polymorphic sites in exon 1 and 5, including 1 singleton variable site in the position 1993 and 13 parsimony informative sites in the position 403, 465, 1140, 1399, 1481, 1547, 2020, 2028, 2050, 2139, 2212, 2292, 2412 and 17 haplotypes. Then we implement PCR amplification on SNP covered area using DNA sample of the 17 local representative pig breeds and a wild boar which we have. Using a series of evolutionary analyses to detect selection signals, analyze population dynamics, we try to reveal the molecular evolution patterns of this gene in the process of pig domestication and identify potential trait-related SNPs. Our results shows that the genetic diversity of domestication pig was significantly high than wild boar and domestication pigs gain 12% genetic diversity during the domestication process and Tajima's D test shows H19 was regulated by negative selection during the domestication process. Secondly, we analyzed the methylation pattern of H19 in pigs using the published MeDIP data and found the region in the updream 4 Kb of H19 is the important differential methylation region (DMR). Next we are systematically comparing the methylation status among the pigs breeds which we have. Combine the molecular evolution analysis and methylation analysis on H19 to disclose evolutionary mechanism and significance of noncoding imprinting gene under artificial selection.

P4025 Paternal genetic structure of Asian goats using SRY gene. Aoi Waki and Shinji sasazaki (Graduate School of Agricultural Science, Kobe University), Eiji Kobayashi (NARO Institute of Livestock and Grassland Science) and Hideyuki Mannen (Graduate School of Agricultural Science, Kobe University)

In previous study, we showed that one of the mitochondrial DNA lineages, mt-lineage B, was detected only in eastern and southern Asia. Therefore, paternal genetic information would contribute to deducing the ancestral history of Asian domestic goats. The aim of this study was to detect the paternal genetic variations and to estimate the genetic structure using SRY gene for 182 Asian goats (Myanmar; 34, Vietnam; 7, Laos;

14, Cambodia; 36, Bhutan; 24, Philippines; 16, Mongol; 31, Japan; 11, Bangladesh; 9). Sequencing comparison of SRY 3' untranslated region (498 bp) among 182 Asian goats revealed four different haplotypes (Y1A, Y1B, Y2A and Y2B) derived from four variable sites (bp 2711, bp 2778, bp 2971 and bp 3098). We compared the haplotypic frequencies of Asian goats with those of European goats (data from previous studies; Canon *et al*. 2006, Pereira *et al*. 2009, 544 samples). In Asian goat, predominant haplotype is Y1A (0.62) and second is Y2A (0.30). Asian goats have no haplotype Y1B, which was observed with moderate frequency in European goats. Haplotype Y2B was detected only in Mongolian goats out of Asian goats. In previous study by mtDNA analysis (Lin *et al*. 2012), we found that the frequency of mt-lineage B was higher in mountain areas than in plain areas. Therefore, in this study, we investigated the geographical distribution of SRY haplotypes in Myanmar and Cambodia. The haplotype Y1A was predominant in plain areas (1.00), while Y2A was observed with moderate frequency in mountain areas (0.36). Since the recent infiltration of modern goat breed into Southeast Asia, especially into plane area, has been reported, our results suggested that the Y2A may be an old and original SRY haplotype in Asian native goats. The present results by caprine SRY gene sequence indicated the geographical aspect and genetic construction in domestic goats.

P4026 Genetics of Creeper trait and correlation analysis between shank length and body weight in Xingyi Aijiao chicken. Sihua Jin, Zhuocheng Hou, Wei Yan, Junying Li, Guiyun Xu and Ning Yang (College of Animal Science and Technology, China Agricultural University)

Xingyi Aijiao chicken, originated from Guizhou province, is a valuable genetic resource characterized by Creeper trait, good uniformity, and quality meat with good taste. However, the genetic determination of Creeper trait has not been determined in birds. In this study, Xingyi Aijiao chickens were used as experimental populations. The segregate families from two types of matings, including group Ⅰ (Creeper (♂) XCreeper (♀)) and group Ⅱ (Creeper (♂) X normal (♀)), were constructed to generate their progenies. In incubation experiment, the eggs were candled each day and the embryos were observed referring to diagram of chicken embryonic development. Shank length (SL) and body weight (BW) were recorded at every two weeks from 0 to 40 wks of age. The aim of this study was to investigate the inheritance of Creeper trait and estimate the relations between SL and BW in Xingyi Aijiao chicken. The results showed that segregation ratio of Creepers and normals closely approaches 2:1, and all Creepers were heterozygous in group Ⅰ. It was also observed that the dominant homozygous embryos(Cp/Cp) were lethal at the fourth day of incubation. As progenies of group Ⅱ, the ratio of Creepers and normals yielded 1:1. There was a 24.66% difference in early mortality for two groups, which was approximately 25%. Furthermore, Pearson's correlation coefficient between SL and BW was 0.8936 ($P<0.05$) at 8 wks of age. The results of this study were consistent with those reported by Landauer and Dunn (1930). In conclusion, the Creeper trait was controlled by Creeper (Cp) gene, which was an autosomal dominant homozygous lethal gene. The results of this study could contribute to conservation, selection and utilization of Xingyi Aijiao chickens. Further studies are needed to perform genomic mapping and elucidate biological functions of Cp gene in chickens.

P4027 Isolation of novel tetranucleotide microsatellite markers from a *Vicugna pacos* BAC library (CH246) and their characterization in Dromedaries and New World Camelids. Haifa Khoory (University of Western Australia)

The Arabian camel (*Camelus dromedarius*) is the

most important camelid species in the Arabian Peninsula for over thousands of years. Microsatellites are one of the molecular markers that have proven very useful for genetic improvement in many livestock species. They are widely used for parentage verification, individual and breed identification and resolving forensic disputes in racing camels. All genotyping studies done so far on dromedaries have used dinucleotide markers.Tetranucleotide repeat markers are easier to score as compared to dinucleotide repeats due to lack of stutter. The aim of this study was to create a panel of tetranucleotide markers for parentage testing system in dromedaries. We performed an *in silico* screening of *Vicugna pacos* BAC library (CH246) clones for tetranucleotide repeats. After an initial screening, 28 tetra nucleotide markers were selected and evaluated for their effectiveness and polymorphism in 336 dromedaries and 46 New World Camelids. Some of these markers were sequenced and their sequences were compared to the Alpaca BAC clones. In dromedaries, 22 out of 28 markers were amplified of which 10 markers were found to be polymorphic. In New World Camelids, 27 out of 28 were successfully amplified of which 26 were found to be polymorphic. The heterozygosity, PIC and Fis values and sequence of these markers will be presented.

P4028 Early Holocene chicken domestication in northern China. Hai Xiang (China Agricultural University), Jianqiang Gao (Hebei Provincial Institute of Cultural Relic), Baoquan Yu (Xushui County Office for Preservation of Ancient Monuments), Hui Zhou and Dawei Cai (Jilin University), Youwen Zhang, Xiaoyong Chen and Xi Wang (China Agricultural University), Michael Hofreiter (University of Potsdam) and Xingbo Zhao (China Agricultural University)

Chicken represent by far the most important poultry species, yet the number, locations and timings of their domestication(s) have remained controversial for more than a century. Here we obtained ancient mitochondrial DNA sequences (totally 485bp) from the earliest archaeological chicken bones from China, Nanzhuangtou site, Cishan site, Wangyin site and Jiuliandun Chu Tomb, dating back up to 10,000 before present (BP). Combined analyses of our ancient sequences with a large data set of published modern and ancient chicken mitochondrial sequences suggest that northern China represents one region of the earliest chicken domestication, dating as early as 10,000 year BP, closely related with regard to their mitochondrial haplotypes to the major modern domestic chicken haplogroups. Similar to the evidence from pig domestication, our results suggest that these early domesticated chicken contributed to the gene pool of modern chicken populations. Moreover, our results support the idea that multiple members of the genus *Gallus*, specifically, *G. gallus* and *G. sonneratii* contributed to the gene pool of modern domestic chicken. Our results not only suggest that the oldest archaeological chicken bones recovered so far indeed are from domestic chickens but also provide further support for the growing evidence of an early mixed agricultural complex in northern China.

P4029 Genetic diversity and classification of Tibetan yak populations based on the mtDNA *CO*Ⅲ gene. Qiaoqiao Song and Zhixin Chai (Southwest University for Nationalities), Jinwei Xin (Tibet Academy of Agricultural and Animal Husbandry Sciences), Shangjuan Zhao (Southwest University for Nationalities), Qiumei Ji and Chengfu Zhang (Tibet Academy of Agricultural and Animal Husbandry Sciences) and Jincheng Zhong (Southwest University for Nationalities)

As a 'unique' species on the Qinghai-Tibetan Plateau and in adjacent mountains and subalpine regions, the yak is a multi-purpose domestic animal utilized by local people. In order to

indicate the level of genetic diversity and the phylogenetic relationships of Tibetan yak populations, the mtDNA *CO*Ⅲ (cytochrome c oxidase subunit 3) genes of 378 yak individuals from 16 populations(I.e. Sibu, Riduo, Leiwuqi, Dingqing, Jiangda, Sangri, Cuona, Longzi, Pali, Sangsang, Kangbu, Zhongba, Jiali, Baqing and Nierong yak) were analyzed in this study. Results showed that the length of *CO*Ⅲ gene sequences is 781 bp. A total of 26 haplotypes were identified, with 69 polymorphic sites which included 11 parsimony-informative sites and 58 single nucleotide polymorphisms sites. No deletions/insertions were found in sequence comparison that indicated nucleotide mutation types were transitions and transversions. The transition/transversion ratio (Ts/Tv) was found to be 12.89. The majority type of base substitutions were T/C transitions, accounting for approximately 57% of all nucleotide mutation. The haplotype and nucleotide diversities were 0.562 and 0.00138 respectively, indicating a high level of genetic diversity in Tibetan yak populations. The haplotype and nucleotide diversities of Sibu, Gongbujiangda, Leiwuqi, Sangri, Longzi and Jiangda yaks were relatively higher, compared with additional populations. The genetic distance between the populations of Tibetan yaks was 0.001452. Phylogenetic relationship analysis indicated that Tibetan yak populations were divided into two groups which Dingqing yak was one group and the other yaks clustered in another group. Two subgroups were created between the 15 groups. Leiwuqi, Sangri Gongbujiangda, Baqing, Longzi, Nierong, Zhongba, Riduo, Sangsang and Jiali populations clustered into one subgroup. Sibu, Jiangda, Cuona, Kangbu and Pali populations clustered into another subgroup.

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P4030 Analysis on Mitochondrial DNA D-loop Region genetic diversity and phyletic evolution of 8 Tibetan yak groups. Qiaoqiao Song and Zhixin Chai (Southwest University for Nationalities), Jinwei Xin, Qiumei Ji and Chengfu Zhang (Tibet Academy of Agricultural and Animal Husbandry Sciences) and Jincheng Zhong (Southwest University for Nationalities)

The yak (*Bos grunniens*) is currently regarded as an important renewable genetic resources, as it thrives in extremely harsh environments with high altitudes, hypoxia, severely cold winters, cool moist summers and survives short growing seasons with limited grazing resources. 328 individuals from 8 Tibetan yak populations (I.e.Riduo, Leiwuqi, Dingqing, Cuona, Longzi, Zhongba, Nierong and Shenzha) were analyzed by means of the mitochondrial DNA control region (mtDNA D-loop) to explore the level of genetic diversity within populations and the genetic relationship between populations. Results showed that the length of the nucleotide sequences was from 887 bp to 895 bp. Nucleotide mutation types included deletions, insertions, transitions and transversions. A total of 91 haplotypes were identified with 135 polymorphic sites including 52 single nucleotide polymorphisms sites and 83 parsimony-informative sites. The 91haplotypes were divided into two clusters(I and II). Cluster I and II contained 16 haplotypes and 75 haplotypes, respectively. The 16 haplotypes(from Hap_76 to Hap_91) in cluster I covered 7 Tibetan yak populations: Dingqing, Zhongba, Nierong, Shenzha, Riduo, Longzi and Leiwuqi yaks, while the cluster II covered 8 Tibetan yak populations. The haploid type diversity and nucleotide diversity were 0.884 and 0.01027 respectively, indicating a high level of genetic diversity in Tibetan yak populations. The nucleotide diversities of Leiwuqi yaks was the highest(0.01438), while the nucleotide diversities of Cuona yaks was the lowest(0.00451). The genetic distance between Riduo and Cuona yaks was the least (0.006), while genetic distance

between Leiwuqi and Longzi yaks was the furthest (0.015). Meanwhile, the Tibetan yak populations could be divided into two major groupes. One clade included 45 individuals, containing parts of Riduo, Leiwuqi, Dingqing, Longzi, Zhongba, Nierong and Shenza yaks. Another clade included 283 individuals , containing 8 Tibetan yak populations.

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P4031 Copy number variation analysis in the chicken genome using next-generation sequencing. Guoqiang Yi, Lujiang Qu, Jianfeng Liu, Yiyuan Yan, Guiyun Xu and Ning Yang (China Agricultural University)

Copy number variation (CNV) is important and widespread in the genome, and is a major cause of phenotypic diversity and disease. Herein, we performed genome-wide CNV analysis in 12 chicken breeds based on whole genome sequencing. A total of 9,025 CNV regions (CNVRs) covering 100.1 Mb and representing 9.6% of the chicken genome are identified, ranging in size from 1.1 to 268.8 kb with an average of 11.1 kb. Sequencing-based predictions are confirmed at high validation rate by two independent approaches, including array comparative genomic hybridization (aCGH) and quantitative PCR (qPCR). The Pearson's correlation values between sequencing and aCGH results range from 0.395 to 0.740, and qPCR experiments reveal a positive validation rate of 91.71% and a false negative rate of 22.43%. In total, 2,188 predicted CNVRs (24.2%) span 2,182 RefSeq genes (36.8%) associated with specific biological functions. Besides two previously reported copy number variable genes *EDN3* and *PRLR*, we also found some promising genes with potential in causing phenotypic variants. Two genes, *FZD6* and *LIMS1*, related to diseases susceptibility and resistance were covered by CNVRs. Entire or partial duplication of some genes like *POPDC3* and *LBFABP* may have great economic importance in poultry breeding. Our results based on extensive genetic diversity provide the first individualized chicken CNV map and genome-wide gene copy number estimates and warrant future CNV association studies for important traits of chickens.

P4032 A genome-wide association study identifies novel alleles associated with tibetan chicken blue shank. Guangqi Li (Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University,), Dongfeng Li (Department of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Nanjing Agricultural University), Lujiang Qu (Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University,) and Zhuocheng Hou, Jiangxia Zheng, Guiyun Xu, Yang Ning and Sirui Chen (Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University)

Shank color of domestic chickens varies from black to blue, green, yellow or white, which is determined by the combination of melanin and xanthophylls in dermis and epidermis. Chicken blue shank is determined by sex-linked inhibition of dermal melanin (Id), which is located on the distal end of the long arm of Z chromosome, through controlling dermal melanin pigmentation. Previous results of GWAS in Silkie Chicken demonstrated that two SNPs located at 67.1 Mb and 72.3 Mb on the 74.6 Mb of chromosome Z in the chicken genome (WASHUC2) were significantly associated with black shank. But no causal sequence variations associated with chicken blue shank have been identified. In this study, we first used the 600K Affymetrix Axiom HD genotyping array which included~ 580,961 SNPs to perform a genome-wide association studies (GWAS) on Tibetan chickens to refine the

Id location. Genomic DNA of 19 Tibetan chickens with blue shank and 21Tibetan chickens with yellow shank were isolated from blood sample by using standard phenol-chloroform extraction. Association analysis was conducted by the PLINK software using the standard chi-square test, and then Bonferroni correction was utilized to adjust multiple testing. The genome-wide study showed that three SNPs located in less than 1Mb interval on the Z chromosome in the current assembly chicken genome (galGal4) were significantly associated with chicken blue shank. The interval we refined was partly converged with previous result in Silky fowl, suggesting that the Id gene is in or near our refined genome regions. Large scale gaps on two sides of the refined interval along with rare SNPs developed in this interval in the array indicating that the genomic context in this region is considerable complex. Further work is needed to identify causal genetic variants associated with chicken blue shank

P4033 Genomic scan of selection reveals candidates for genes associated with short stature in Chinese miniature horse. Adiljan Kader, Neena Gorkhali, Kunzhe Dong, Yao Na and Yuehui Ma (Chinese Academy of Agriculture Sciences)

Identification of genomic regions associated with phenotypic traits is one of the challenging areas of research in animal genetics. With the availability of high density single nucleotide polymorphism (SNP) markers in farm animals, selective signature analysis have shown to be useful for detecting candidate genes affecting phenotypic traits. In this study, we genotyped 32 Debao miniature horse (DB), 32 Yili (YL) and 32 Mongolia horses (MG) using Equine SNP74k Bead Chip. Genetic differentiation coefficient FST was applied to detect the selection signatures between DB and the other two horse breeds. Using PLINK software to set quality control standards, a total of 65,095 SNPs were selected for statistical analysis. Our study identified two selection signatures involved in height at withers on chromosome 14 and 19, which are close to the PROP1 and SOX2 gene, respectively. Both loci have already been shown to be related to dwarfism in humans. The findings of this study provide better understanding of the genetic basis underlying body size in Chinese miniature horse.

P4034 Molecular detection of genetic introgression of domestic chicken into green and red junglefowls. Chen Wang (Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS)), Hidayat Ashari (Research Center for Biology, Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia (LIPI)), Mukesh Thakur (Wildlife Institute of India), Le Thi Thuy (National Institute of Animal Husbandry), Jaime Cabarles Jr (College of Agriculture Resources and Environmental Sciences) and Jian-Lin Han (CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS))

It has been reported that avian leukosis virus (*ALV*) insertion in the intron 4 of *tyrosinase* (*TYR*) gene leads to a recessive white plumage phenotype in chicken. Using *ALV* as a molecular marker, 51 green junglefowls and 246 red junglefowls from five countries were assayed to identify whether they were genetically contaminated by the introgression of genes from domestic or feral chickens using a duplex PCR method. We also analyzed the recognition site and sequences of *ALV* insertions. The results showed that there was no *ALV* insertion in all green junglefowls and red junglefowls from Hainan province in China, Philippines and India. However, seven and one red junglefowls from Vietnam and Indonesia had *ALV* insertions in heterozygous genotypes, respectively. The recognition site of *ALV* in the intron 4 of *TYR*

gene was 5'-CAGTGT-3'. There was one SNP in the recognition site of *ALV*, and both 5'-CAGTGT-3' and 5'-CAGTAT-3' were present in red junglefowls while only 5'-CAGTAT-3' was found in green junglefowls. Sequence alignment showed that all the *ALV*s were the same among red junglefowls, and there were 74 SNPs and 11 SNPs in the *ALV* sequences obtained in our study when they were compared with reference sequences AY013303 and DQ118701, respectively. Our study suggested that some red junglefowl populations may have been genetically contaminated by the introgression of genes from domestic or feral chickens. The results also demonstrated that the *ALV* insertion in the genome of red junglefowls was the same retrovirus as in chickens and the recognition site of *ALV* insertion was specific.

P4035 Characterization of insertion and deletion variation in chicken genome using whole genome sequencing data. Yiyuan Yan, Guoqiang Yi, Lujiang Qu and Ning Yang (China Agricultural University)

Insertions and deletions (INDELs) are an important source of genetic and phenotypic variation in chicken, whereas received less attention than SNPs and large structural variations (SVs). To gain a better knowledge of INDEL variation in chicken genome, we applied whole genome resequencing on 12 diverse chicken breeds. A total of 1.2 million non-redundant short INDELs were obtained. Follow-up validation assay confirmed that most (88.68%) of our randomly selected INDELs represent true variations. The vast majority (92.12%) of our INDELs were novel, indicating that the INDEL discovery in chicken, at least short INDELs, is far from complete. The INDEL length ranges from 1 to 45 bp, with the majority (92.46%) less than 10 bp. The genomic INDEL density was estimated as 0.3 INDELs per kb, but the genomic distribution of INDEL was not uniform. Macro-chromosomes and intermediate chromosomes had significantly higher densities than micro-chromosomes. Nearly 567,000 (46.48%) INDELs were mapped to genic regions, and 1490 (0.12%) of which were located in exons, affecting 1188 (6.94%) unique Ensembl genes. Many genes were associated with economically important traits, in particular, two novel coding INDELs in *THRSP* gene worth further investigation about their associations with abdominal fat content and body weight. *MUC6* contained as many as five coding INDELs and could be used as a candidate gene for egg quality. Besides, some genes were homologues of human genes relating to common diseases. These INDELs are a valuable source of candidates for further elucidating the association between genotypes and phenotypes of interest. Our results provided the highest resolution map of INDELs in chicken so far and the valuable data will be beneficial for developing INDEL markers, designing IDNEL arrays, and molecular breeding in chicken.

P4036 Polymorphisms in *ATP2A3* gene are associated with eggshell quality traits in pedigree Rhode Island Red hens. Zhongyi Duan, Siri Chen, Jiangxia Zheng, Guiyun Xu and Ning Yang (China Agricultural University)

The improved quality of eggshell played an important role in reducing waste due to cracking during production. Eggshell breaking strength (ESS), eggshell thickness (EST) and eggshell weight (ESW) are important parameters for measuring eggshell quality. The process of eggshell mineralization not only needs a mass of calcium, but also a variety of other ions. Previous study showed that *ATP2A3* located in the endoplasmic reticulum played a role in calcium dynamic store and maintaining the low level of free Ca2+ in cytoplasm, and was overexpressed in chicken uterus compared with magnum and duodenum. The objective of this study was to investigate the effect of *ATP2A3* gene on eggshell quality. Eggshell quality traits were

detected in a pedigreed line of Rhode Island Red with 976 hens at the 55 weeks of age. Genotyping was performed for all the 976 hens. The association of single SNP and haplotypes with eggshell quality traits was performed with the least-squares method using a linear mixed model, as shown below: Yij=μ+Hi+Gj+eij where Yij represents the observed values of the traits, μ is the population mean, Hi is the fixed effect of house, Gj is the fixed effect of genotype or haplotype, and eij is the residuals. The result showed that the SNP rs15841856 and rs13574212 were significantly associated with EST, and the SNP rs14118603 was significantly associated with both of ESS and ESW. Moreover, these three SNPs were located in the same linkage disequilibrium block, and the corresponding haplotypes were significantly associated with EST. This suggests a crucial role of this transporter in uterine calcium transport and regulation during the process of eggshell mineralization. This study provides evidence that genetic variation in *ATP2A3* can influence eggshell quality. Keywords: eggshell quality, single nucleotide polymorphisms, *ATP2A3*

P4037 Genetic diversity of East and West African cattle: A genome-wide comparison. Abdulfatai Tijjani (National Biotechnology Development Agency), Hussain Bahbahani (University of Nottingham), Christopher Mukasa and Gerald Akpah (Ahmadu Bello University), Marcos da Silva (EmbrapaGado de Leite), Oyekanmi Nash (National Biotechnology Development Agency), Tad sonstegard (United States Department of Agriculture), Olivier Hanotte (University of Nottingham) and Heather Huson (Cornell University)

In this study, we evaluate the genetic diversity and population differentiation of indigenous African cattle breeds from Nigeria and Uganda using the BovineHD Genotyping BeadChip (illumina). The populations included were Ugandese zebu (n = 65) and sanga (n = 25) cattle and Nigerian taurine (n = 37), crossbreed (n = 37) and zebu cattle (n = 108). Also, included were one taurine population from Ethiopia (n = 18) and Guinea (n = 24), a zebu population from Kenya (n = 92), two breeds from Europe (Holstein, n = 59 and Jersey, n = 32) and the Nellore (n = 35), originally from India. These animals were genotyped with 786,999 SNPs. After quality control, including Hardy Weinberg equilibrium (HWE) and linkage disequilibrium (LD), 248,751 SNPs, spanning all bovine autosomal chromosomes were selected. Zebu cattle population from Nigeria and Uganda showed the highest genetic variation with mean observed and expected heterozygosities of 0.375± 0.010(*Ho*) and 0.385± 0.000(*He*) for Nigerian zebu, and 0.378±0.010(*Ho*) and 0.385± 0.000(*He*) for the Ugandese zebu. It was followed by the sanga cattle 0.378± 0.010 (*Ho*) and 0.386± 0.000 (*He*), and then the Nigerian taurine 0.347±0.010 (*Ho*) and 0.429±0.000(*He*). Genetic differentiation (*Fst*) was lower for Nigeria 0.00675 ± 0.00147 than for Uganda 0.01610 ± 0.00299 zebu population, between countries *Fst* for the two zebu populations equal 0.0167. Principal component analysis (PCA) supports closer relationships within and between zebu populations in Nigeria compared to Uganda. This study presents the first comparative analysis of the genome-wide diversity of East and West African cattle population paving the way to a global understanding of the genetic diversity of African cattle

P4038 The Development of Frizzled Follicle and Genetic Characteristics of Candidate Gene *KRT75* in Frizzled Feather Chicken. Lin Tao, Wang Du and Li Zhang (Guangdong Ocean Univerisity)

In order to analysis features of frizzled feather and roles of *KRT75* gene in feather follicle development ;We used this study compared the microstructure and the development of feather follicle between frizzled feather and plain feather

by Microscopic section technology . In addition to that , we cloned and analyzed *KRT75* gene expression during the formation of feather follicle ;The results showed that the frizzled feather rachis curved outward , barbules cannot hooked together to form a closed feather ; there were some differences in the medulla and barb ridge between frizzled feather and normal one from E12 to E15 ; *KRT75* was highly expressed in frizzled feather follicle from E9 to E17 , especially from E12 to E15(Fig.1) . No 69 bp deletion mututation was detected in CDS region of KiRin Chicken *KRT75* gene . But three SNPs (954bp : T>C ; 967bp : T>C ; 978bp : C>T)were found compared KiRin chicken with Princess chicken(Fig.3) . We Conclued that the frizzled feather of KiRin Chicken were not caused by 69 bp detection mutation of *KRT75* gene . The three SNPs(955bp : T>C ; 967bp : T>C ; 978bp : C>T)could be used as molecular markers in distinguishing frizzled and plain feathers .

P4039 Genetic Diversity and Phylogenetic Analysis of mtDNA *ND6* of Tibetan Yaks. Ting Hai and Cheng Zhong (Southwest University for Nationalities), Mei Ji (Tibet Academy of Agricultural and Animal Husbandry Sciences) and Bin Zeng (Southwest University for Nationalities)

To investigate the genetic diversity，clustering relationships and genetic differentiation of Tibetan yak (Bos grunniens) populations, we analyzed the complete sequence of the mitochondrial DNA ND6 gene of 150 individuals from 15 yak populations . Determining the polymorphic loci and the number of haplotypes, calculating the haplotype diversity (Hd) and nucleotide diversity (Pi). In addition, building the molecular cluster diagram and the haplotype phylogenetic tree. Results showed nucleotide bias in the 528 bp and no introns Tibetan yak mtDNA ND6 sequence, with nucleotide frequencies of 42.2%、7.6%、20.9% and 29.3% for T, C, A and G respectively. The majority of polymorphisms were 37 transitions and 2 transversions, showed a strong bias of the conversion. According to the variation between the sequences, there were totally 7 type single times in Tibet yak mtDNA ND6, Hap_1 is the mainstream Tibetan yak haplotypes, and the remaining six haplotypes groups as part of the unique. The average haploid type diversity (Hd) and average nucleotide diversity (Pi) were respective 0.2978 and 0.00191, indicating lower genetic diversity within Tibetan yak populations. According to the molecular cluster diagram constructed genetic distance showed 15 Tibetan yak groups can be divided into two categories. According to the seven kinds of haplotypes constructed phylogenetic trees, indicating that there are two maternal origin.

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P4040 Identification of SNPs in equine Chorionic Gonadotropin gene. Shuqin Liu (China Agricultural University)

Identification of SNPs in equine Chorionic Gonadotropin gene Shuqin Liu, Chunjiang Zhao1 Equine Center of China Agricultural University 1. Correspondence: College of Animal Science and Technology, China Agricultural University, Beijing, 100093, China. Equine Chorionic Gonadotropin (eCG) is a gonadotrophic hormone secreted by endometrial cups of pregnant mare, which is used to induce follicular development. In the present study, SNPs in 5'end of eCG gene were screened across 9 horse breeds, including 6 Chinese indigenous breeds and three imported breeds. 5 SNPs were identified in the 5' end of eCG α subunit gene, and 2 of them were unique in indigenous breeds. A missense mutation was also found in the first exon of the eCG α gene. Two SNPs were detected in 5' end of eCG β subunit gene, and one of them were only found in Chinese breeds. The analysis of allele frequencies indicated that the mutation at -310bp

of eCG α gene and ones at -850bp and -900bp of eCG β gene showed significant different allele frequencies among breeds, especially between the indigenous breeds and the imported breeds. Binding sites of transcription factor in the 5' end of eCG genes were also predicted with software in the present study. The results of the present study facilitate the further research on the bio-functions of the polymorphisms of eCG gene.

P4041 Genetic diversity, origin and germplasm characteristics of Chinese indigenous sheep. ChouSheng liu, Gang liu and Jian Lu

Background: Chinese indigenous sheep are precious resources, characterized by strong adaptation ability, high prolificacy and good endurance. With the rapid development of livestock husbandry and the excessive introduction of foreign breeds, however, genetic diversity and characteristics in Chinese domestic sheep faced a serious threat. Therefore, it was so important to conserve these genetic resource. In addition, the germplasm characteristics, origin and differentiation of sheep remains unclear, and need to be further investigated. Methodology/Principal Findings: In the study, genetic diversity and origin of 44 sheep breeds had been investigated by the mtDNA D-loop. In addition, the germplasm characteristics of Chinese typical domestic sheep also had been explored by using the ovine SNP50 Beadchip. Our results domenstrated that the range of haplotype diversity and nucleotide diversity in these populations was 0.727-1.000 and 0.00681-0.03089, respectively. It showed that there were farther distances between Chinese domestic breeds and abroad breeds. The neighbor-joining and Bayesian cluster revealed that Chinese sheep populations could be subdivided into four lineages: lineage A(Asian type), lineage B(European type), lineage C(China and Turkey), and lineage D(two individuals of Kazakh sheep). Based on MJ, there were at least three lineages in Chinese sheep. The results of signature of selection and Gene Ontology by ovine SNP50 Beadchip suggested that, some genes with the higher ranked SNPs, such as BMP2, BMP4, CDH23, RHOQ, might be related to the angle characters in Chinese sheep. Conclusions/Significance: The results were congruent with a demographic model, showing a large and sudden expansion as inferred from the mismatch distribution. It was suggested that the introgression from Agarli sheep might contribute to genetic background of Turfan Black sheep in Xinjiang, but require further evidences. This study provides a fundamental genetic profile for the conservation of these populations and better to understand the domestication process and origin of Chinese sheep.

P4042 Temporal changes of genomic diversity for farm animals under conservation programs. Wenting Li, Wenyan Yuan, Keliang Wu and Changxin Wu

It is well-known that the traditional method to estimate genetic diversity was based on coancestry calculating from genealogical data, but limited to incomplete pedigree. With the development of genomic techniques, genetic diversity could be estimated from genome-wide SNPs data. We generated a base population which had reached a mutation-drift equilibrium. Then, conserved population management was carried out with current conservation strategies. After settling different effective population sizes (Ne) and different SNP densities on each population, we monitored the temporal changes over generations with the measures of genetic diversity such as expected heterozygosity (He), observed heterozygosity (Ho) and allele richness (AR). At low SNP densities (d=50), He and Ho only remained 62.5%, 61.8% of the initial population for 50 generations when Ne was 50, 79.4%, 79.0% when Ne was 100 and 88.9%, 88.6% when Ne was 200. While Ne equaled to

50, genetic diversity at 50 th generation decreased 37.5%, 33.9%, 17.5% and 12.1% relative to the initial generation with their corresponding SNP density being 50, 100, 500, and 1000. Besides, with Ne keeping unchanged, the genetic diversity maintained using microsatellite DNA marker (SSR) was higher than SNP marker which density was 50 and 100, however, along with SNP density reaching 500 and 1000, SSR performance was much lower than SNP. Based on the conserved population data analysis, we came to the following conclusions: I. With marker density remaining constant, the lowing rate of He, Ho and AR decreased with increasing Ne; II. With Ne remaining constant, the performance of SNP marker improved with increasing marker density. And it doesn't mean the density higher, the better; III. Either Ne or marker density higher could maintain the genetic diversity at a high level; IV. SSR accuracy is between low density SNP and high density SNP accuracy with the same Ne. This work was partly supported by the program of 973 project and Changjiang Scholar and Innovation Research Team in University, China.

P4043 Wild boar (*Sus scrofa*) meat authentication using melanocortin receptor 1 (*MC1R*) gene. A.M. Samaraweera (Department of Animal Science, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka.), P. Silva (Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka), S. M. C. Himali and H. W. Cyril (Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.) and J.L. Han (International Livestock Research Institute (ILRI), Nairobi, Kenya.)

Wild boar meat is known as a delicacy among many societies. Though slaughtering of Sri Lankan Wild Boar (SLWB) is not prohibited, selling and transportation of SLWB meat are banned by the Fauna and Flora Protection Ordinance in Sri Lanka. Hence correct identification of SLWB meat is a prerequisite in legal enforcement of the country. Current practice of the use of phenotypic markers is not effective due to crossbreeding of native pigs with SLWB. Therefore this study was conducted to differentiate meat of SLWB from domestic pigs using Melanocortin Receptor 1 (*MC1R*) gene. The complete *MC1R* gene was amplified using forward (5'-GGGAAGCTTGACCCCCGAGAGCGACG CGCC-3') and reverse (5'-CGCCGTCTCTCCAGCCTCCCCCACTC-3 ') primers in 17 SLWB and three native pig samples. The amplified products were directly sequenced and analyzed using Chromas 2.0 and Mega 5.0. Four fixed single nucleotide polymorphisms (at codons 4, 17, 121 and 207) and three more polymorphic positions were identified from SLWB samples. All these mutations were synonymous substitutions compared to European wild boar (EU443645) which may not alter the function and expression of the wild type allele. So far, the mutation at codon 207 has been only reported from a Chinese native domestic pig (FJ665476). Sri Lankan native pigs carried two haplogroups based on the presence or absence of the two nucleotide insertions (+CC) at codon 23 which shifted the entire coding sequence. Moreover, six mutations were also reported in native pigs with three of them to be non-synonymous substitutions. Thus mutation at codon 207 in the wild type allele in SLWB can be used to distinguish SLWB meat from other pig meat. To detect the crossbreds, meat authentication should always be coupled with mtDNA D-loop variations, where the result of PCR-RFLP analysis is presented elsewhere. Funding sources, National Science Foundation (NSF) and International Atomic Energy Agency (IAEA).

P4044 Tissue Expression, Polymorphism and the Genetic Effects of LXRα on Duck Meat Quality. Zhang Yiyu and Li Wangui (Guizhou

University)

Liver X Receptor Alpha (LXRα) is a nuclear receptor that plays a crucial role in regulating the expression of genes involved in lipid metabolism. The aims of this study were to detect the mRNA expression and polymorphism of the *LXR*α gene and investigate their associations with duck meat quality. Using relative quantification PCR, *LXR*α mRNA was the highest in the liver. Moderate mRNA levels were detected in the lung, spleen, kidney, heart and hypothalamus. Low mRNA levels were observed in the breast muscle, glandular stomach, duodenum, colon, cerebrum and cerebellum. Using PCR-SSCP and sequencing, the amplicons were genotyped for 316 healthy female ducks at 70 days of age. Silent mutations 277C>G and 1396C>G were first identified in exon 2 and 5'-UTR of the *LXR*α gene, respectively. Association analysis revealed that the 277C>G genotype was significantly associated with shear force value. The 1396C>G genotype were significantly related to pH, water holding capacity, intramuscular fat, unsaturated fatty acid, polyunsaturated fatty acid and essential fatty acid. Interaction between the 277C>G and 1396C>G loci were significantly associated with pH, water holding capacity, shear force value, intramuscular fat, unsaturated fatty acid, polyunsaturated fatty acid and essential fatty acid. Expression profile of *LXR*α mRNA may reflect the relative importance of the *LXR*α gene in liver function and lipid homeostasis. Recent research has demonstrated that the *LXR*α gene has important effects on mammalian carcass and meat quality. Huang et al. reported that the T1530C mutation in *LXR*α exon 2 had a significant effect on back-fat thickness, carcass length and marbling score in Qinchuan cattle. Our research suggested that the 277C>G and 1396C>G mutations of the *LXR*α gene may be important genetic markers for duck meat quality, and may be used in marker-assisted selection (MAS) in duck breeding.

P4045 Genetic variation in the second exon of *DQA*1 gene in the breeds of Guizhou local goats. Bin Liu (College of Animal Science, Guizhou University, Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, Guiyang, China)

In this study, we analyzed the genetic variation of *DQA*1 gene exon 2 in three kinds of local goat breeds of Guizhou Province. Total 514 samples were collected and their targeted sequences were analyzed using the method of DNA pooling combined with direct DNA sequencing. We observed four SNPs in the sequences of the *DQA*1 gene exon 2 of the three kinds of goat breeds in our research. The four SNPs were A59G (Met→ Thr), G80A (Pro→ Leu), T183C (Asn→ Ser), and G212A (Samesense mutation), respectively. The results of bioinformatics analysis show that the G212A locus mutation leads to the disappearance of a transcription factor binding sites. We also find that the locus mutation of G80A does not cause changes in the structure of mRNA, but it reduces the minimum free energy and thus enhances the structural stability.

P4046 Polymorphism of ChREBP Gene and Its Association with Serum Biochemical Levels in Ducks. LI Wangui, Zhang Yiyu and Pan Lanbing (Guizhou university)

Carbohydrate response element binding protein (ChREBP) regulates lipogenesis and glucose utilization in the liver . Nowadays , there was no study on polymorphism of ChREBP gene in ducks . The aims of this study were to detect the polymorphism of ChREBP gene and its association with duck serum biochemical level , which may offer insight into marker-assisted selection (MAS) and help improve economic traits in duck breeding ,our experiments collected blood samples from 100 healthy ducks (♂50 , ♀50 , aged at 10 weeks) that belong to Cherry

Valley ducks , and based on the different exon of duck ChREBP gene , 14 pairs of primers were designed by using Primer 5.0 , polymorphism was detected by PCR-SSCP and sequencing , serum biochemical levels were measured by using the CX4 automatic biochemical analyzer , the results showed that 3 genotypes (AA , AB , BB) and g.246912G>A mutation were firstly observed in ChREBP gene exon 9 , the alignment of amino acid codon indicated that the g.246912G>A was synonymous mutation , the frequency of B allele was 0.625 , gene heterozygosity (h) and effective number of alleles (Ne) were 0.469 and 1.882 respectively , polymorphism information content was 0.359 , it showed that the locus belonged to moderate polymorphism , the test of Chi-quare demonstrated that genotype distribution deviated from Hardy-Weinberg and showed significant difference($\chi 2=7.778$，$P<0.05$) . The analysis on genotypes association with serum biochemical levels showed that the total cholesterol (TC) have a significant difference , AA was higher than BB , no significant differences were observed in other serum biochemical levels .

P4047 Genetic Characteristic Analysis of the Blackbone Sheep via Genome-wide SNP Chip. Yuanyuan Zhang and Xuemei Deng (China Agricultural University)

Blackbone sheep (Ovis aries) were found at 2001 in Lanping (County of Yunnan Province, China). These sheep have excessive melanin in inner organs, which has been proved to be inherited in cross-breeding studies. Genomic DNA of sixty Blackbone were extracted and genotyped using the Illumina Ovine 60K BeadChip. The SNP data of another eight Asian sheep were obtained from the International Sheep Genome Consortium. Only SNPs that mapped on the autosomal chromosomes were used for association study. Individual inbreeding coefficients (*F*) and Expected heterozygosity (*He*) of Blackbone sheep were 0.22 and 0.32, respectively. Application of principal components analysis (PCA) provide visual means of identifying population structure, and individuals within populations cluster together. The dated MPL tree was constructed on Reynolds distances among populations. Average linkage disequilibrium (LD) r2 between adjacent SNP of Blackbone sheep is 0.13, which is the lowest among these sheep populations, and the rate of decay in LD was surprisingly rapid. Effective population sizes (*Ne*) were estimated based on the LD at various distances, and the recent estimation of *Ne* of Blackbone sheep was 850 (50 generations ago). These suggest low selection pressure and highly heterogeneous in Blackbone sheep. In order to obtain the favored loci in Blackbone sheep, *FST* for all population and population pairs were calculated using Weir & Cockerham's method (1984), smoothed *FST* with a local variable bandwidth kernel estimator was also provided. Almost 400 genes covered by outlier SNPs were screened out. Interestingly, melanoma pathway was found within 18 functional groups, which will be benefit to understand genetic base of Blackbone sheep. Genetic analysis of the blackbone sheep provides insights into the genetic mechanisms associated with excessive melanin trait and assist in artificial breeding and population conservation.

P4048 Bioinformatics analysis on the SNP of Promoter region of *POLRMT* gene in local goat in Guizhou Province. Yan Sun (Guizhou University)

In order to improve the marbling performance of Guizhou Goat，the SNP site in the promter region of *POLRMT* gene in goat was screened and the effect of SNPs on function on elements of promoter was analyzed.Guizhou White Goat, Guizhou Black Goat and Qianbei Ma Goat were selected to construct DNA pools, SNP site was screened by bothway sequencing subsequently. Variety bioinformatics tools were used to predict the core region of the promoter, CpG island and

transfer factor. There are two SNPs site in *POLRMT* gene promoter region for respectively T-81A, T-289C. It demonstrated that the scope of the *POLRMT* gene core promoter was changed，some transcription factors disappeared based on the SNP found in this study，meanwhile one CpG island was predicted increase by MethPrimer. There were two SNPs existed in the promoter region of *POLEMT* gene that have important effects on promoter elements. The test results could lay the foundation for the further analysis of function of *POLRMT* promoter.

P4049 Polymorphism of STAT5A Gene and Its Relevance with the growth performance in Goat. Hai XIE (Guizhou university), XIE Hai-qiang, LIU Luo-yu, SUN Yan-yan, SONG Tao-wei, PAN Dao-xing Guizhou University, Guiyang, China

(Objective)The purpose of this study was to analyze the association of STAT5A gene polymorphisms with body weight and body size traits in goat. (Method) Guizhou White goats, Guizhou Black goats and Qianbei Ma goats from Guizhou native goat breeds were selected for testing subjects, the DNA pooling was constructed and the technology of direct sequencing of PCR products and PCR-SSCP were used to detect the single nucleotide polymorphism of STAT5A gene. (Result)The result showed that C69T and G71A were detected in exon 7 and 10 of STAT5A gene in these goat breeds, respectively. The SNP(G/A) was a missense mutation, which made Ala to Thr and was devided into three genotypes: GG, GA and AA. The study of relationships between genotype and body weight and body size traits revealed that body height of individuals of Guizhou Black goats with genotype GA and AA were significantly better than that of individuals with genotype GG($0.01<P<0.05$), but no significant difference between genotype and the others ($P>0.05$). (Conclusion)The consequence indicated that STAT5A gene was primarily deduced to be a potential major gene or linked to major gene effecting goat body height trait, and this SNP(G71A) might be a candidate molecular marker of MAS.

P4050 SNP Identification in part of intron 1 of goat *LPL* gene. Jana Rychtarova, Alena Svitakova and Zuzana Sztankoova (Institute of Animal Science)

Lipoprotein lipase (*LPL*) hydrolyses triglycerides in chylomicrons and in very low density lipoproteins, thereby provide necessary free fatty acids and promote lipoprotein conversion. The coding region of the goat *LPL* cDNA was 1,437 bp long and encoded a protein of 478 amino acid. In the present study, a located fragment was amplified of signal peptide and part of intron 1 of the *LPL* gene (NC_022300.1) using the PCR technique. The primers for amplification and extension were designed by using Primer3Plus software. Sequencing analysis followed to for 31 animals belonging to Czech national dairy goat breeds (White Shorthaired and Brown Shorthaired goat). The results revealed three novel SNPs in the intron 1 (G183T, C255T, and G299A). The frequencies of alleles G/T of G183T polymorphism were 0.919/0.081. The frequencies of the minor allele T and the rare genotype TT of the C255T polymorphism were 0.258 and 0.097 respectively. In G299A, the minor allele A was observed at a frequency 0.226, genotype AA was not typed.

P4051 The polymorphisms of candidate genes associated with QTL in Wild boars *(S. scrofa)* of European and Asian origin inhabited in Russia.. Veronika Kharzinova, Natalia Zinovieva and Olga Kostyunina (All-Russian State Research Institute of Animal Breeding of the Russian Academy of Agricultural Science), Igor Domsky (Russian Research Institute of Gamekeeping and Fur Farming of the Russian Academy of Agricultural Sciences), Elena Gladyr (All-Russian State Research Institute of Animal

Breeding of the Russian Academy of Agricultural Science), Ivan Seryodkin (Pacific Geographical Institute, Far East Branch of the Russian Academy of Sciences), Alexander Aconomov (Russian Research Institute of Gamekeeping and Fur Farming of the Russian Academy of Agricultural Sciences) and Gottfried Brem (Institute of animal breeding and genetics, VMU, Austria)

The development of molecular genetics knowledge opens the new opportunities in the characterization of animal populations, determining the degree of their genetic differentiation. The objective of our work was to evaluate the genetic differences between wild boars of European (n=80) and Asian (n=9) origins inhabited in Russia using ten DNA markers *RYR1, ESR, FSHB, NCOA1, BF, MUC4, IGF2, MC4R, POU1F1, ECRF18/FUT1*. The primary assignment of individuals to two groups of Europe and Asian origins was performed based on the territory of their habitat. To confirm the correct distributions of animals between groups the microsatellite analysis (12 loci) was carried out and the similarity coefficients (Q) using the method of Pritchard J.K. et al. (2000) for the number of clusters k=2 were calculated. The average Q values in individuals of European origin in the first cluster were Q1/2=0.984±0.005 whereas the average Q values in individuals of Asian origins in the second cluster were Q2/2=0.994±0.001 that confirmed their different origin. Five DNA markers (*RYR1, ESR, MUC4, IGF2, ECRF18/FUT1*) were monomorph in both of wild boars' groups analyzed. The non-significant differences in allele frequencies of *FSHB, BF, MC4R* and *POU1F1* genes between groups of European and Asian origins were observed: pA=0.462 and 0.250, pA=0.020 and 0.143, pA=0.013 and 0.000, pC=0.000 and 0.111, respectively. The studied groups were significantly differed in *NCOA1* allele frequencies: pA1=0.938 in group of European origin and pA1=0,000 in group of Asian origin. The presence of A2 allele of NCOA1 in the group of Europe origin could be due to the known introgression from the Chinese pigs (Giuffra E. et al., 2000) Taking in a point the presence of allele A2 of *NCOA1* in Russian domestic pigs population with the frequencies up to 0,278 (own data) the possible hybridization of the wild boars with domestic pigs couldn't be excluded.

P4052 Identification and characterization of a novel defective allele at the goat (*Capra hircus*) alpha s1-casein locus. Valentin Balteanu (University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca), Marcel Amills (Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Campus Universitat Autonoma de Barcelona) and Augustin Vlaic (University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca)

In this paper we describe the characterisation of a new defective allele at the goat alpha s1-casein (*CSN1S1*) locus, so called Och (ch=carpathian), identified by isoelectrofocusing of milk samples from Carpathian goat breed reared in Romania. The amplification of coding region of *CSN1S1* cDNA from goats harboring this new allele highlighted variability in size of the transcripts, suggesting alternative splicing events. Sequencing of three different size transcripts revealed a common feature *i.e.* the loss of the entire exon 17 (155 bp, including the first two nucleotides of the stop codon-TG), that encodes 51 amino acids between positions 149-199 of mature protein. Two types of the most abundant transcripts are characterized either by deletion of 7 nucleotides located in exon 12 (CAACGTG, between positions 27-33) or of the entire exon 12 (42 bp). These transcripts coexist in two isoforms characterized by the presence or deletion of the first triplet of exon 11 (a constitutive allele-independent event). In a less abundant transcript we found the deletion of the last 5 nucleotides (GTGAG) of the exon 9 ,

contemporary with deletion of exons 10, 11, 12 and 17. To map the causal mutations that might explain this polymorphic expression pattern, we sequenced the incriminated genomic regions. We found that the 7 nucleotides deletion from exon 12 is present at the DNA level, which explains either total or partial absence of this exon in the transcripts, and can be typed by *Sdu*I restriction enzyme. Furthermore, the exon 17 skipping is produced by a G to A substitution at the first position of intron 17, which inactivates the donor splice site, and can be typed by using *Hph*I restriction enzyme. Both features are unique to this newly identified allele, adding important information to the knowledge of CSN1S1 polymorphism in goat.

P4053 The mtDNA D-loop variations in Korean native ducks and phylogenetic relationships with other breeds. NU-RI CHOI and DONG-WON SEO (Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University), SEON-DEOK JIN (National Biodiversity Information Network, National Science Museum), SULTANA HASINA (Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University), KANG-NYEONG HEO (Poultry Science Division, National Institute of Animal Science, RDA) and JUN-HEON LEE (Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University)

Recently, the consumption of duck meat has been gradually increased in Korea. However, most of the duck breeds in Korea were imported from overseas. Based on the great demands for the native ducks, a new project for the commercial use of the Korean native ducks has been launched. As the results, two lines of native ducks, Korean native duck (KND) and white meat-type native duck (WMD), were restored in Korea. For the initial investigation of the relationships with other wild duck breeds, the sequences from *D-loop* control region in mitochondrial DNA (mtDNA) was used. The results from phylogenetic analysis indicated that KND and WMD were mainly classified as wild duck breeds. However, mallard ducks were not discriminated well with KND. The haplotype analysis indicated that these native duck lines have seven different haplotypes with nine SNPs. Three haplotypes (haplotype number 1, 3, 5) were appeared both in KND and WMD. On the other hand, other haplotypes were only appeared either in KND or WMD. With further verifications, the results presented here can be used for the conservation and commercialization of the Korean native ducks.

P4054 Tracing origin and genetic diversity of domestic Bactrian camel in China using microsatellites and mtDNA D-loop. Xiaohong He (Institute of Animal Science, Chinese Academy of Agricultural Sciences)

In this study, two kinds of molecular markers viz., autosomal microsatellites and mitochondrial DNA D-loop were used to analyze the genetic diversity and origin of nine Bactrian camel populations from China and one from Mongolia. Genetic variability and relationship between 452 individuals from the 10 populations under the study were estimated using 18 mircrosatellite markers. Genetic parameter analysis showed that there was high genetic diversity in the 10 populations. The average number of effective alleles was 4.18, and a total of 242 alleles were observed across the 10 populations with mean number of alleles per locus as 13.44. The mean observed heterozygosity (Ho) was 0.5528 and the average value of PIC was 0.5996. The analysis showed that genetic differentiation reached a very significant level ($P<0.001$), and 9.6% of the total variance was present among the populations and 90.4% was detected within the populations. Cluster analysis showed that the 10 populations were divided into two groups, which were

generally in agreement with geographic distribution. The mtDNA D-loop region of 205 individuals from the 10 populations were also sequenced and analyzed. In all 17 polymorphic sites and 21 haplotypes were discovered from 940 bp long sequence. Further analysis revealed that the Bactrain camels were more conserved than other domestic animals in terms of their limited mtDNA diversity. Phylogenetic analysis and network analyses showed that only one haplogroup was present in the ten camel populations. Over 80% individuals belonged to three predominate haplotypes. Together with the 86 sequences retrieved from the GenBank database, a total of 286 sequences were finally analyzed. The results showed same patterns of phylogenetic and network relationships existed in all the ten populations. The results indicate that the ten populations might originated from few maternal ancestors.

P4055 The interaction analysis of black feather gene locus and white feather gene locus in egg quails. you-zhi pang, xiao-hui zhang, li-sha wang and ying xu (Henan University of Science and Technology)

Introduction: The black quails and white quails were used to analyze the relationship of black feather gene locus and white feather gene locus, by feather color separation and sexual segregation in hybrid offspring.
Materials and methods: Two male quails and six female quails were randomly selected from black quails and white quails. The positive group consist of black feather quails (♂) and white feather quails (♀), while the reciprocal group consist of white feather quails(♂) and black feather quails (♀) in the ratio of 1:3, respectively. In the F1 generation of the positive test, 15 female quails and 5 male quails constituted the intercross group. In the F1 generation of reciprocal test, 6 female quails and 2 male quails constituted the backcross group. The sexual segregation and plumage colors in F1, F2 generation and B1 generation within backcross group were recorded and *Chi-square* test was used to examine all data.
Result and analysis: A total of 96 young quails were obtained from F1 generation in the positive test, in which the ratio of sexual segregation was 1:1; the whole number of 226 young quails were obtained from F2 generation and four kinds of feather colors appeared at the first day of hatching .The ratio of sexual segregation as male: female was 2:1 among the black feather, incompletely black feather and maroon feather by *Chi-square* test ($P<0.01$),while the white feather only appeared in female quails. The 176 male quails were incompletely black feather and 160 female quails were white feather in F1 generation of the reciprocal test group, which indicated that the auto-sexing appeared and the ratio of sexual segregation was 1:1 ($P>0.05$). All the 161 individuals of B1 generation in the backcross test was white feather and the sexual segregation was 1:1 ($P>0.05$). In conclusion, the reciprocal hybridization model was auto-sexing by feather colors and this was important in breeding and production of egg quails.

P4056 Performance of high density bovine SNP 80K Bead Chip in domestic and semi-domesticated bovine species. Md. Rasel Uzzaman and Zewdu Edea Bedada (Chungbuk National University), Mohammad Shamsul Alam Bhuiyan (Bangladesh Agricultural University) and Kwan Suk Kim (Chungbuk National University)

High density Bovine SNP 80K Bead Chip derived from *Bos indicus* breeds was employed in Bangladeshi zebu cattle (*Bos indicus*) along with the semi-domesticated gayal (*Bos frontalis*), African zebu (Ethiopia- Ogaden cattle) and Asian taurine (Korea- Hanwoo cattle) populations in order to evaluate its performance across different *Bos* populations. A large number of fixed (MAF= 0) alleles (n=14,287) were found in gayal whereas the lowest (n= 236) was detected in

Ethiopian zebu across four populations. Within the three cattle populations the fixed alleles (n= 15,503) were found highest in Hanwoo whereas the lowest (n= 236) was observed in Ogaden cattle. On the other hand, most of the monomorphic alleles in zebu populations (Bangladeshi zebu, 79.31%; Ethiopian zebu, 75.58%) were commonly found monomorphic in gayal. In contrast, only 38% of Hanwoo monomorphic alleles were commonly found monomorphic in gayal. Therefore, the results give a hint about the effect of this indicine originated chip to take into account while applying it in two sub species of cattle along with other *Bos* populations. Despite of the effect of the chip origin (Indicine Chip) we suspect that the higher number of the fixed alleles in gayal is because of their loss of heterozygosity which is due to the highly inbred gayal population. Therefore, a further research is warranted which might disclose novel findings in this unique species that could help differentiate gayal from domestic cattle and understanding the genetics a lot.

P4057 Phenotypic and molecular genetic analysis of the cat AB blood group system. Toshinori Omi, Chihiro Udagawa, Rina Makita, Junya Furuseki, Kazuhiko Ochiai, Daigo Azakami, Toshinori Sako, Makoto Bonkobara, Shuichi Tsuchida and Shigenori Ikemoto (Nippon Veterinary and Life Science University)

Knowledge of the cat AB blood group system is important in specifically cat transfusion studies, as antigen mismatching can cause acute hemolytic transfusion reactions. Such reactions occur when naturally occurring alloantibodies in the serum react with the antigens of the cat blood types A, B, and AB which are expressed on the surfaces of red blood cells. To clarify the distribution of cat AB blood group antigens in cat populations, the blood type of 348 purebred, mixed-breed, and unknown-breed cats was determined by the agglutination method. Blood type B occurred in approximately 5% of the cats surveyed and blood type AB was not detected in the study population. Of the 130 pure-bred cats in the 19 recognized cat breeds that were assayed, the blood type B antigen was detected in six breeds. RT-PCR analysis revealed that mRNA of the *CMAH* (cytidine monophosphate-N-acetylneuraminic acid hydroxylase) gene, which is associated with the AB blood group in cats, was ubiquitously expressed in various tissues. We also performed the genotyping of SNP (Single Nucleotide Polymorphism) and indel (insertion/deletion) polymorphisms of the *CMAH* gene in genomic DNAs associated with the different blood groups, and found a mismatch between phenotype and genotype in three cats with blood type B. The presence of such a mismatch suggests that a new allele may be associated with blood type B in cats.

P4058 The Synbreed Chicken Diversity Panel – a resource for fine scale diversity studies. Steffen Weigend and Ulrike Janßen-Tapken (Friedrich-Loeffler-Institut), Malena Erbe (Georg-August-Universität Göttingen), Ulrich Baulain and Annett Weigend (Friedrich-Loeffler-Institut), Johann Sölkner (University of Natural Resources and Life Science) and Henner Simianer (Georg-August-Universität Göttingen)

High density SNP genotyping arrays allow genome-wide assessment of genetic diversity within species. Domestic chickens show massive phenotypic and genetic variation. Similar phenotypes can be observed in various breeds separated by many generations, thus providing a unique resource for mapping genetic mechanisms underlying phenotypic variation. Under the umbrella of the SYNBREED project, an ongoing research project supported by the German Federal Ministry of Education and Research, molecular and phenotypic data of a wide range of chicken breeds have been collected. In this study,

a subset of 82 diverse chicken breeds of the "Synbreed Chicken Diversity Panel (SCDP)" was used. The set consisted of 68 chicken breeds mainly sampled from German fancy breeds of various origins. It was augmented by samples of two Red Junglefowl populations (Gallus gallus gallus and Gallus gallus spadiceus) as well as 12 commercial purebred chicken lines (brown layers, white layers, broilers) taken from the previous international cooperation project AVIANDIV. In this set, 1677 individuals were genotyped with the Affymetrix Axiom® Genome-Wide Chicken Genotyping Array encompassing ~ 580K SNPs. First analyses aimed at genome-wide evaluation of diversity and relationships between chicken breeds and, as a proof of concept, genome wide association studies (GWAS) to detect chromosomal regions harboring known mutations causing trait differentiation. The majority of individuals showed close association with their supposed breed origin. Commercial white layers, brown layers and fancy bantam breeds formed the corners framing all other breeds. Within the spectrum, breeds showed a wide variation of both the degree of polymorphism and the mean length an individual's genome is included in runs of homozygosity. Precise detection of regions associated to known mutations for traits as yellow skin color and rose-comb illustrates the usefulness of SCDP.

P4059 Genetic evidence for domestication of sheep in India. Satish Kumar (DBT-National Institute of Animal Biotechnology), Sachin Singh (CSIR- Centre for Cellular and Molecular Biology), Satish Kumar Jr (ICAR-Central Sheep and Wool Research Institute) and Atul Kolte (ICAR-National Institute of Animal Nutrition and Physiology)

To understand the domestication process of sheep, we have studied mitochondrial DNA sequences of 330 sheep representing 12 breeds from different agro-climatic regions of India and found three major lineages, namely; A, B and C. Consistent with previous studies from Asia our study showed lineage A is predominant in India with 84% of these animals having this lineage. Relative contribution of lineage B to various Indian breeds varied from 7- 48% and this differential contribution was responsible for breed differentiation. In addition our data also suggested that founding effects and or genetic drift in a few breeds might have led to breed differentiation in Indian breeds. Analysis of Indian sheep sequences along with published sequences from other regions of the world revealed several exclusive haplotypes of lineage A in Indian sheep accompanied by higher nucleotide diversity in these animals. Our results have provided strong genetic evidence for domestication of lineage A sheep east of Near East, possibly in the Indian sub-continent. Finally, our study suggests that lineage B and additional A haplotypes might have reached India from Near East.

P4060 The swine leukocyte antigen (SLA) nomenclature system, update 2014. Chak-Sum Ho (Histocompatibility Laboratory, Gift of Life Michigan), Sabine Essler (Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna), Asako Ando (Department of Molecular Life Science, Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine), Claire Rogel-Gaillard (INRA, UMR1313 Génétique Animale et Biologie Intégrative), Jun-Heon Lee (Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University), Lawrence Schook (Institute for Genomic Biology, University of Illinois), Douglas Smith (University of Michigan (retired)) and Joan Lunney (Animal Parasitic Diseases Laboratory, BARC, ARS, USDA)

The swine leukocyte antigen (SLA) system is among the most well characterized MHC systems

in non-human animal species. The International Society for Animal Genetics (ISAG) and International Union of Immunological Societies Veterinary Immunology Committee (IUIS VIC), SLA Nomenclature Committee was formed in 2002. The committee's primary objectives are: 1) to validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; 2) to assign appropriate nomenclatures for new alleles as they are validated; and 3) to serve as a curator of the IPD-MHC SLA sequence database (http://www.ebi.ac.uk/ipd/mhc/sla/), which is the repository for all recognized SLA genes, their allelic sequences and haplotypes. To date, there are 155 classical class I (SLA-1, SLA-2, SLA-3), 13 non-classical class I (SLA-6, SLA-7 and SLA-8) and 182 class II (DRA, DRB1, DQA, DQB1, DMA) alleles officially designated. There are 39 class I and 31 class II haplotypes at the high-resolution (allele) level designation. Recent evidence has suggested certain loci in the SLA system previously recognized as pseudogenes (e.g. SLA-9, SLA-11, DQB2 and DOB2) may be expressed at the transcript level for some haplotypes; the committee will determine if designation of the alleles of these loci is warranted as more evidence accumulates. A systematic nomenclature for the genes, alleles and haplotypes of the swine MHC is critical to the research in swine genetic diversity, immunology, health, vaccinology, and organ or cell transplantation. Continuous efforts on characterizing SLA alleles and haplotypes and studying of their diversity in various pig populations will further our understanding of the architecture and polymorphism of the SLA system and their role in disease, vaccine and allo- or xeno-graft responses.

P4061 Maternal Inheritance Background of Kazahk Horses from China and Kazahkstan Revealed by mtDNA. Gemingguli Muhatai (College of Animal Science,Tarim University), Aladaer Qi (Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences), Long Cheng (Faculty of Agriculture & Life Science, P.O. Box 84, Lincoln University) and Wumaierjiang Aizimu (College of Animal Science,Tarim University)

mtDNA D-loop region and *ATP6-Arg* gene were sequenced to determine the inheritance background of Kazahk horse from China and Kazahkstan. Results demonstrated that haplotype diversity and nucleotide diversity of *D-loop* region were both higher than those of *ATP6-Arg* gene.There was no significant difference in *Hd* and *Pi* between horses from the two countries. In comparison the *nps7974-9963* sequences data with 81 sequences from GenBank representing 18 major haplogroups of wrold horses, we found that Kazakh horses composed of 13 major haplogroups , accounting for 72% of all horse haplogroups, in which seven haplogroups' frequencies were higher than 5%, indicating an abundant maternal hereditary background of Kazakh horses. The inheritance character was almost the same except for differences in some haplogroups' frequencies. Haplogroups *C*, *N* and *R* were only found in Kazahkstan horsepopulations, but haplogroup frequency was lower than 5%; while haplogroup *J* exclusively was observed in Xinjiang horse populaitons, with frequence higher than 5%. The frequencies of haplogroups *L* and *O* in Kazahkstan horses were 14% and 16%, respectively; in contrast, those two values were both less than 5% for Xinjiang horses. Multiple sequences alignment was conducted with the *D-loop* data of Kazahk horse and additional 388 accessions retrieved from GenBank representing other horses, such as Fell, Highland and Shetland horse from Europe; Mesenskaya, Vayatskaya and Orlov horse from Russia;Mongolian horse and Yakutian horse. Furthermore, *NJ* phylogenetic tree was reconstructed with the alignment.The phlogeny revealed that Kazakh horse from Xinjiang and Kazakhstan were closely related, and they further

clustered with Mongolian horse. In conclusion, our study demonstrated a close hereditary relationship between Kazahkstan and Xinjiang Kazakh horses. Therefore the Kazakhstan horse could be used to improve the germplasm of Kazahk horse in China, considering the lagging horse breeding work in Xinjiang. We propose that the breeding experiences of Kazakhstan will be beneficial to the purification and renovation of Kazahk horses for Xinjiang.

P4062 Genetic Diversity of Four Domestic Chicken Breeds in Southern Part of Xinjiang Based on Microsatellite DNA Analyses. Wumaierjiang Aizimu (College of Animal Science, Tarim University,Alaer), Gemingguli Muhatai (College of Animal Science, Tarim University,Alaer,China), Aladaer Qi (XinJiang Institute of Ecology and Geography, Chinese Academy of Sciences,Urumqi), Long Cheng (Faculty of Agriculture & Life Science, P.O. Box 84, Lincoln University), Yong Wang (College of Animal Science, Tarim University,Alaer), Nuerguli Yusupu (Animal Husbandry and Veterinary Station of Hejing,Bazhou, China) and Yong Hong Liu (College of Animal Science, Tarim University,Alaer)

The genetic diversity of four domestic chicken breeds in southern part of Xinjiang was analyzed using 20 microsatellite markers. A total of 203 typical chicken samples were collected from four areas, namely Hejing, Baicheng, Aheqi and Tashikuergan. The results showed that all of the *STR* loci were highly polymorphic (polymorphic information content *PIC*=0.575-0.920) except locus *MCW0248* (*PIC*=0.487). All of the four chicken populations showed high level of polymorphism (observed heterozygosity*Ho*=0.455-0.833; expected heterozygosity *He*=0.542-0.927), with the lowest polymorphic level found in Taxkuergan chicken population. Inter-group analyses indicated that the genetic divergence between groups was overall not significant. The lowest genetic differences was observed between Baicheng and Aheqi chicken populations; The genetic distance between Tashikuergan chicken breed and Baicheng, Hejing, Aehqi chicken breed were larger than that of between Baicheng, Hejing and Aehqi chicken breed pairs. Nei's analyses revealed that the lowest genetic distance was between Baicheng and Aheqi chicken breeds; Genetic distance between Tashikuergan and the other chicken breeds were much larger. *UPGMA* tree inferred from Nei's genetic distance demonstrated that Tashikuergan chickens formed a single branch and sister to the branch comprised of the other three chicken breeds. Baicheng and Aheqi chicken breeds were clustered together, indicating a more close relationship between them. Tashikuergan is a county located in the Pamirs. It is hypothesized that due to the relative geographic isolation from the outside world, the genetic exchange of Taxkuergan with other chicken breeds was much rare, leading to a relative lower genetic diversity and farther genetic distance with other chicken breeds, and then gradually evolved into a more independent breed.

P4063 A whole−genome approach unlocks the genetic diversity and structure of Ethiopian cattle populations. Zewdu Edea Bedada (Chungbuk National University), Hailu Dadi Melka (Konkuk University), Kwan Suk Kim (Chungbuk National University) and Tadelle Dessie (International Livestock Research Institute (ILRI))

Comprehensive knowledge of genetic characterization is of great importance and prerequisite for sustainable utilization and management of domestic livestock breeds. In the past, genetic diversity studies in Ethiopian cattle populations used limited number and density of molecular markers. Recent advances in molecular genetics technologies have facilitated the development of high density molecular markers. High density single nucleotide

polymorphisms (SNPs) chips provide a higher resolution genome wide genetic diversity and structure analyses than by using microsatellite and mtDNA markers. In this study we sampled 294 animals representing *Bos indicus* and *Bos taurus* breeds. All animals were genotyped using a high density (80 K) SNP chip derived from the indicine breed to explore genome−wide genetic diversity and structure. The proportion of polymorphic SNPs, observed and expected heterozygosities were used to estimate diversity indices at the population or breed level. Patterns of population structure and introgression were inferred by applying STRUCTURE and principal component analyses. The overall mean minor allele frequency (MAF) ranged between 0.32 (Ethiopian cattle) and 0.27 (Hanwoo cattle). The expected heterozygosity varied from 0.26 in Hanwoo to 0.42 in Raya-Azebo (Ethiopian cattle). Sheko, the only remnant of East African short horned taurine cattle showed considerable levels of zebu introgression (58%). Genetic distances and STRUCTURE analyses revealed that the relationship among Ethiopian cattle populations reflects their history of origin and admixture rather than their phenotypic based discriminations. The high within population genetic variation observed in Ethiopia cattle populations could be untapped opportunities and prerequisite for sustained genetic improvement of local breeds in the changing environments and breeding goals.

P4064 Y chromosome genetic diversity of Polish native cattle breeds included in the National Rare Livestock Breeds Preservation Programme. Beata Prusak (Institute of Genetics and Animal Breeding, Polish Academy of Sciences), Wioletta Sawicka-Zugaj (University of Life Sciences) and Tomasz Grzybowski (Ludwik Rydygier Collegium Medicum, Institute of Forensic Medicine, The Nicolaus Copernicus University)

Y chromosome-specific markers provide information on migration and gene flow between populations. Fixed geographic correlation of the Y-chromosome haplotypes in cattle allows identification of likely ethnic origin of DNA samples. The aim of the study was to analyse the paternal gene pool of domestic cattle breeds selected for the NRLB Preservation Programme based on the haplotype diversity of the Y-chromosome microsatellite *loci*. We analysed Polish Red, Polish Whitebacked, Polish Black-and-White, Polish Red-and-White, Polish Holstein-Friesian and also Simmental bulls used for improvement crossings (395 bulls in total). We analysed five microsatellite *loci*: INRA-189, INRA-124, BM-861, BYM-1 and DYZ-1. The study identified seven alleles and three haplotypes. The most common haplotype had a total frequency of 89%, while the other haplotype was detected with frequency of only 0.7% exclusively in Polish Whitebacks. Overall, four breeds evidenced only one haplotype and in the remaining two, we detected two and three haplotypes. All Y-chromosomes were classified to be taurine (allele INRA124-132bp). Relationships among Y chromosome haplotypes including data for cattle breeds from other parts of the world were summarised in the MJ network. The reconstructed network indicates that the majority of the tested bulls belonged to the most common haplotype H11 described earlier by Kantanen et al. Within two old Polish breeds we recognized haplotype carried by all tested Simmentals but also recognized in other native cattle breeds from the neighbouring Slavic countries. The present study could be a prelude to the extended analysis of ancestral roots of Polish domestic cattle breeds and also expand the knowledge of the history of domesticated cattle.
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P4065 *LYZC* and *GAS41* polymorphisms in four chicken varieties. Huaxiang Yan (1. National Poultry Engineering Center, Shanghai Academy of Agricultural Sciences, Shanghai

201106, China; 2. Shanghai Poultry Breeding Co., Ltd., Shanghai 201419, China.) and Changsuo Yang and Junfeng Yao (1. National Poultry Engineering Center, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China)

Background: Lysozyme is an innate immune factor which is abundant in eggs and plays an important role in egg quality and embryo survivability. Chicken lysozyme gene(LYZC) is tissue-specific transcribed and expressed, being located within a short locus of 24 kb of chromatin. It contains all the elements required for position-independent and tissue-specific expression of the gene and displays enhanced general DNaseI sensitivity between two MARs. The locus also includes the constitutively transcribed Gas41 gene that codes for a component of the NuA4 complex. This research analyzed the *LYZC* and *GAS41* polymorphisms for marker assisted selection to improve chicken survivability, egg quality and hatchability.
Result: We studied four genetic sources of chicken varieties, Rohde Red Chicken, White Leghorn Chicken, Dongxiang Green Chicken, and Royal Chicken. Over five hens of each variety were extracted DNA to PCR and re-sequencing. *LYZC* is divided into 12 segments, from -6.3kb to 6.7kb.Referring to NCBI *LYZC* sequence, 161 polymorphism sites were found, 7 indels, 2 VNTRs and 152 SNPs. There were 5 synonymous mutations on 3 exons of *LYZC* and 3 synonymous mutations on 2 exons of *GAS41*. There were 20 bases mutation in *LYZC* and 6 in *GAS41* result in no CpG methylation. There were about one variation per 80 bases, however, the variation distribution was not evenly. Few of variations were found in the positions related toDNaseI sensitivity, CpG islands or 3' terminal regions. The polymorphism frequencies were significant different among four varieties, the sites of Royal Chicken were the most and that of White Leghorn Chicken were the fewest.
Conclusion: The chicken *LYZC* and *GAS41* show abundant polymorphisms. The distributions of polymorphism are not evenly along the gene sequence and significant different among chicken varieties.

P4066 Genetic differentiation among 6 populations of red deer (*Cervus elaphus L.*) in Poland based on microsatellite DNA polymorphism. Anna Radko (National Research Institute of Animal Production, Department of Animal Cytogenetics and Molecular Genetics), Agnieszka Szumiec and Dominika Rubis (National Research Institute of Animal Production) and Dariusz Zalewski (University of Warmia and Mazury in Olsztyn, Department of Fur-bearing Animal Breeding and Game Management)

Recently, there has been considerable interest in genetic differentiation in the *Cervidae* family. A common tool used to determine genetic variation in different species, breeds and populations is DNA analysis, which allows for direct determination of the differences and changes within a group of animals. The objective of the present study was to determine the genetic structure of 6 European red deer populations found in Poland and the genetic differentiation among Poland's red deer population.The study was performed with samples collected from a total of 793 red deer. Six groups (subpopulations) of red deer were defined according to region: Masurian (330 animals), Bieszczady (194 animals), Małopolska (80 animals), Sudety (76 animals), Lower Silesian (62 animals) and Lubusz (51 animals). The analysis involved 12 STR markers (BM1818, OarAE129, OarFCB5, OarFCB304, RM188, RT1, RT13, T26, T156, T193, T501, TGLA53), for whichone multiplex PCR reaction was established. The observed and expected heterozygosity values were high at over 0.529 (OarAE129) for HO and over 0.784 (OarAE129) for HE. FIS values calculated for all the red deer populations were higher for 4 *loci* (OarAE129, OarFCB5, RT13, T156), ranging from 0.1257 (OarFCB5) to 0.3584 (RT13). All

the markers showed a very high level of polymorphism. The lowest PIC value was 0.7062 (OarAE129) and the highest was 0.9105 (TGLA53). Based on the distribution and frequency of alleles at the 12 microsatellite loci, genetic distances between the red deer populations were determined by calculating the coefficients of genetic distance using two independent methods: Nei (DN) and Reynolds (DR). In both cases, the Sudety and Małopolska populations formed one cluster and the Lower Silesian and Lubusz formed another, while the Masurian and Bieszczady populations were relatively distant from the other groups (DN = 0.103 to DN = 0.376, and slightly lower DR values of 0.133 – 0.247).

P4067 Evaluation of the performance of parentage verification-dedicated SNPs in Polish Holstein cattle. Dominika Rubis (National Research Institute of Animal Production, Department of Animal Cytogenetics and Molecular Genetics), Artur Gurgul (National Research Institute of Animal Production, The Laboratory of Genomics) and Anna Radko (National Research Institute of Animal Production, Department of Animal Cytogenetics and Molecular Genetics)

Parentage verification is one of the important factors of regular animal breeding. Currently it is based on the analysis of 12 microsatellite (STR) markers with sufficient polymorphism to detect accidental pedigree errors. Together with the growing number of available data on SNP (single nucleotide polymorphism) genotypes generated during genomic selection programs, the standard use of this type of markers for parentage testing is being suggested. In this study we attempt to compare properties of the standard ISAG microsatellite panel with the data obtained from ISAG-designed panel of SNPs dedicated for parentage verification (ISAG core + additional) based on the data obtained from 859 animals of Polish Holstein cattle. STR genotypes were obtained by standard procedures, subsequently the SNP genotypes were retrieved from Bovine SNP50 assay (Illumina, San Diego, CA). Apart from two monomorphic markers from ISAG core panel (ARS-USMARC-Parent-DQ786764-no-rs; ARS-USMARC-Parent-EF034087-no-rs), the combined use of 197 SNPs gave substantially lower probability of identity (PI) than the microsatellites (8.16E-81 vs. 2.28E-12)and visibly lower combined non-exclusion probability (parent pair, 1-PEc) 7.41E-28 for SNPs and 3.0E-6 for microsatellites. Comparison of variability parameters between ISAG core and additional SNPs panel, showed that additional panel had better performance and delivered lower PI and 1-PEc values. It can be concluded that both SNP panels can be used for paternity testing in Polish Holstein cattle population. The ISAG additional panel has better properties and can be recommended for routine pedigree verification in Polish cattle.

P4068 Transcriptome analysis and gene expression changes leading extreme alkaline tolerance in Amur ide (*Leuciscus waleckii)* inhabiting soda lake. Jian Xu and Qiang Li (Centre For Applied Aquatic Genomics, Chinese Academy of Fishery Sciences)

Background: Amur ide (*Leuciscus waleckii*) is an economically and ecologically important cyprinid species in Northern Asia. The Dali Nor population living in the soda lake Dali Nor can adapt the extremely high alkalinity, providing us a valuable material to understand the adaptation mechanism against extreme environmental stress in teleost.
Results: The transcriptome of Amur ide was assembled into 53,632 cDNA contigs and 19,338 unique proteins were identified. A total of 10,395 microsatellites and 34,299 SNPs were identified and classified. A dN/dS analysis on unigenes was performed, which identified that 61 of the genes were under strong positive selection. Most of the genes are associated with stress adaptation and

immunity, suggesting that the extreme alkaline-saline environment resulted in fast evolution of certain genes. We also performed parallel comparisons of three tissues gill, liver and kidney of *L. waleckii* living in the soda lake Dali Nor and the fresh water lake Ganggeng Nor. We found 477, 2,761 and 3,376 differentially expressed genes (DEGs) in the gill, kidney, and liver, respectively. Further analysis revealed that well-known functional categories, which are associated with stress response and extreme environment adaptation, have been significantly enriched, including the functional categories of "response to stimulus", "transferase activity", "transporter activity" and "oxidoreductase activity", and signaling pathways of "mTOR signaling", "EIF2 signaling", "superpathway of cholesterol biosynthesis". We also identified significantly DEGs encoding important modulators on stress adaptation and tolerance, including carbonic anhydrases, heat shock proteins, superoxide dismutase, glutathione S-transferases, aminopeptidase N, and aminotransferases.
Conclusions: Overall, this study demonstrated that transcriptome changes in *L. waleckii* played a role in adaptation to complicated environmental stress in the highly alkalized Dali Nor lake. The results set a foundation for further analyses on alkaline-responsive candidate genes, which help us understand teleost adaptation under extreme environmental stress and benefit future breeding for alkaline-tolerant fish strains.

P4069 Genetic characterization through STR analysis of wild boar population in the italian Umbria region.. Massimo Biagetti, Fabio Vincenti and Carla Sebastiani (Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy), Francesca Vercillo (Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy), Umberto Sergiacomi (Umbria Region, Regional Wildlife Observatory, Italy) and Bernardino Ragni (Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy)

Wild boars, together with pigs, belong to the species *Sus scrofa*. In Umbria region they are strongly bound to man especially for the hunting world, but sometimes it can come into conflict with agriculture. Umbria region authority funded a scientific program for the genetic characterization of wild boars to evaluate whether an introgression of the domestic pig in the boar genetic pool has occurred. 251 wild boars were sampled in the whole regional territory during the hunting period. Genomic DNA was extracted and analyzed by 18 STR markers to obtain a genetic profile. Binning analysis was done by FlexiBin v2 software. Data analysis was performed: allelic frequencies, distance matrices and PCA analysis by Genealex 5.6 software; Hardy-Weinberg equilibrium, Fst, AMOVA by Arlequin 3.1 software; populations structure by Structure 2.3.4 software; phylogenetic tree analysis by Mega 5 software. All STR loci resulted polymorphic with an overall Hardy-Weinberg disequilibrium (heterozygotes deficit) as underlined also by the positive values of fixation indices (FIS). Population structure analysis revealed 3 main clusters of individuals and a further one constituted by only two animals (Group 4). Interestingly, when these clusters were put in comparison to a population of domestic pigs, the software included "group 4" boars in the domestic animals cluster suggesting a possible hybridization between wild boars and domestic pigs. PCA analysis revealed a good degree of separation between the three groups and a clear separation from the domestic swine. Phylogenetic analysis (Neighbor-Joining algorithm) confirmed the belonging of group 4 to the domestic pig branch. The three clusters are geographically quite well separated and divide Umbria region in three areas. Both "group 4" animals come from the same area. Further analysis will be performed to confirm the hypothesis of an hybridization between the two

genetic pools.

P4070 Genetic structure and relationships of Merino breeds assessed by genome-wide SNP analysis. Fabio Pilla (Department of Agricultural, Environmental and Food Sciences, Universit à of Molise), Emiliano Lasagna and Francesca Sarti (Department of Agricultural, Food and Environmental Sciences, University of Perugia), Mariasilvia D'Andrea (Department of Agricultural, Environmental and Food Sciences, Universit à of Molise), Vincenzo Landi (Department of Genetics, University of Cordoba) and Elena Ciani (Department of Biosciences, Biotechnologies and Biopharmaceutics)

Merino and Merino-derived sheep breeds are widely diffused in the world. Originated from Spain, they are raised mainly for wool production in Southern hemisphere while, in Europe, meat is the main production goal. Enhancing the knowledge of Merino breeds genetic structure and relationships can orient genetic variability preservation, management and exploitation practices. Several studies, each targeting a restricted number of Merino breeds, have investigated genetic variability by microsatellites. In our study an extensive analysis of Merino breeds genetic variability was performed by genome-wide SNP loci. OvineSNP50 BeadChip data were obtained from International Sheep Genomics Consortium and BiOvIta project on 37 breeds including 12 world Merino breeds, 10 relevant Spanish and Italian breeds, 10 primitive breeds and 5 among feral (mouflon) and wild populations. Within-breed genetic diversity parameters and MDS components were obtained using PLINK software. Levels of gene-flow and admixture were evaluated through the model based clustering algorithm implemented in ADMIXTURE. Relationships among breeds were also explored by Neighbor-network analysis using the Reynolds distance calculated by POWERMARKER. MDS data clearly showed the presence of four main genetic clusters, respectively attributable to the wild sheep, the mouflon, the primitive breeds and the modern ones including all the Merino breeds. The latter all clustered in the same Neighbor-network branch, although very close to Spanish not-Merino breeds, thus suggesting shared ancestry and/or geographical gene flow among Iberian breeds. Inside the Merino breed group, a complex and puzzling ADMIXTURE pattern was reconstructed for Spanish Merino, while being more homogeneous in the majority of the other Merino-derived breeds. Indeed, Spanish Merino displayed many genetic components, each of them predominant in a single Merino-derived breed, thus suggesting different Spanish Merino genetic stocks contributed to the development of the various Merino-derived breeds all over the world.

P4071 The IPD-MHC database; past, present and the future. Steven Marsh (University College London), James Robinson (Anthony Nolan Research Institute) and Keith Ballingall (Moredun Research Institute)

The Past: The major histocompatibility complex (MHC) contains many of the most polymorphic loci within the mammalian genome. The extensive allelic diversity at MHC loci provides a rich source of genetic markers for; a, analysis of genetic diversity within and between populations; b, studying the relationships between host genotype and disease phenotype; c, comparative studies of evolution and natural selection and d, to underpin vaccine development and infectious disease research. Advances in sequencing technology along with reductions in cost have resulted in a large increase in MHC alleles submitted to public databases. The consequence of this was an often confused array of sequences each using a different nomenclature system, high levels of redundancy and variable sequence quality. This necessitated the development of standardised nomenclature systems which may be used across species with associated nucleotide

and amino acid sequence databases. The IPD-MHC database (http://www.ebi.ac.uk/ipd/mhc/index.html) was set up to provide such a resource to the scientific community.The Present: The Comparative MHC Nomenclature Committee of ISAG in association with the Veterinary Immunology Committee (VIC) of IUIS has supported the development of the IPD-MHC databases by the HLA Informatics Group, Anthony Nolan Research Institute, for the last 10 years. These databases now provide standardised and official MHC nomenclature for many species including most livestock species. All the original work needed to implement the database was implemented at no cost to the wider scientific community. The Future: TheIPD-MHC database is now a growing resource which requires continued maintenance and further development to keep up with the demands for including new species specific sections and timely updates of those already included. Improvements to the software resources to simplify access, submission and expert curation of the species specific sections will require designated bioinformatics support. Options for continued support and future funding of the databases are currently being explored.

P4072 Genetics of the curly hair trait of the horse. E. Cothran (Texas A&M University), Laurent Schibler and Caroline Morgenthaler (INRA) and Rytis Juras and Anas Khanshour (Texas A&M University)

Horses with curly coats are becoming increasingly popular in the USA and many countries of Europe. This is partly because these horses are mostly hypoallergenic. In this study we set out to determine the genetic basis of curly hair in the horse. Seventy-one horses, half with curly coats and half with straight hair, were genotyped using the Illumina 50K SNP Beadchip. A total of 46,215 snps could be typed across all samples. The most significant association found was for a snp located on ECA11. This snp was located within a group of keratin genes with the closest gene being KRT25. We performed exon sequencing of Krt25 and Krt27. For Krt25 a G to A missense mutation was found which appears to be causal for the curly hair. We then genotyped approximately 100 horses registered a curly and over 250 horse from a diversity of breeds that do not have curly hair. No straight haired horse carried the A allele, however, a small proportion of the curly horses had the GG genotype. We checked with the registry and were told that the GG horses actually had what the registry call wavy coats. This result indicates that there must be another mutation associated with the wavy coat. We have sequenced a few of these wavy horses for KRT71, which is frequently associated with curly hair in other species but have not found any mutation that appears like it could be causal. In addition, we genotyped 5 curly horses from Mongolia and 5 from the tran-baikal region of Russia. All had the Krt25 genotype GG but they did not have the wavy coat phenotype. This suggest a third mutation associated with curly hair in horses.

P4073 Whole genome sequencing reveals genomic signatures of selection in native bighorn sheep populations. Marty Kardos and Gordon Luikart (University of Montana), Rowan Bunch (CSIRO), Sarah Dewey (Grand Teton National Park), Hank Edwards (Wyoming State Veterinary Laboratories), Sean McWilliam (CSIRO), John Stephenson (Grand Teton National Park), Fred Allendorf (University of Montana), John Hogg (Montana Conservation Science Institute) and James Kijas (CSIRO)

Understanding the processes that underpin adaptation is a central goal of evolutionary biology. Recent progress has been made through comparison of divergent breeds of animals, uncovering loci relating to dietary intake, reproduction, pigmentation and other changes associated with domestication. The identification of adaptive variation in free living wild species,

however, has proven more challenging. Bighorn sheep are adapted to living in extreme environments characterised by high altitude across the Rocky Mountains of North America. We generated whole genome sequence from three bighorn sheep populations sampled across Montana and Wyoming, and identified 3.23 M single nucleotide polymorphisms by comparison against the draft reference genome assembly of domestic sheep (OARv3.1). Patterns of SNP variability were examined for evidence of selection using two approaches, resulting in identification of 66 putative selective sweeps that overlapped genes. We will report evidence for selection at genes with demonstrated roles in traits that influence sexual fitness in bighorn, growth early in life and adaptation to high altitude. These results help to elucidate the genetic, physiological, and social mechanisms of adaptation in wild bighorn sheep.

P4074 The identification of the mutation for SLICK hair coat in Senepol cattle - an adaptive variant with effects on thermo-tolerance and milk production.. Tad Sonstegard and Derek Bickhart (ARS, USDA), Heather Huson (Cornell University), Antonio Landaeta (Universidad del Zulia), Yuri Utsunomiya (UNESP), Laercio Porto-Neto and William Barendse (CSIRO), Melvin Pagan-Morales (University of Puerto Rico-Mayaguez), Anthony Reverter-Gomez (CSIRO), Peter Hansen (University of Florida), Serdal Dikmen (Uludag University), Esbal Jimenez-Caban (University of Puerto Rico-Mayaguez), Daniel Null (ARS, USDA), Robert Godfrey (University Virgin Islands), Fernando Garcia (UNESP) and Curtiss Van Tassell (ARS, USDA)

The slick hair coat (SLICK) is a dominantly inherited trait typically associated with tropically adapted, Criollo-derived cattle. The trait is of interest relative to climate change, due to its association with improved thermo-tolerance and subsequent increased productivity. The goal was to identify the mutation underlying the SLICK locus, which was previously mapped to a 4 cM region on chromosome (Chr) 20. To refine map position, BovineHD genotypes were generated from a sampling (N=195 animals) of Senepol, Carora, Romosinuano, three additional slick-haired cross-bred lineages and a group of non-slick ancestral breeds. Genome-wide association analysis narrowed the SLICK locus to a 0.8Mb (37.7-38.5 Mbp UMD 3.1) consensus region, which contains SKP2 and SPEF2 as possible candidate genes. Three haplotype patterns were identified in slick individuals, all with zero frequency in non-slick individuals. In attempt to identify candidate causative mutations in this region, whole genome re-sequencing was completed for one Romosinuano and five Senepol animals. SNP discovery and annotation analyses revealed a putative causative polymorphism within *prolactin receptor* (*PRLR*), which would truncate an encoded domain involved in JAK/STAT5 signaling. Validation testing of this SNP and 37 others was done across a DNA panel (N=466) that included representation from five SLICK and seven non-SLICK breeds. The results strongly suggest the frameshift mutation in *PRLR* is the causative mutation underlying SLICK in Senepol and some Romosinuano cattle. However, the frameshift mutation was not present in SLICK Limonero cattle and GWAS analysis of these animals suggested a different mutation within *PRLR* was the underlying variant for a SLICK phenotype in this breed. In a subsequent study to test the effects of the frameshift mutation on milk production in SLICK Holsteins revealed a less drastic depression in milk yield during the summer. Results support the utility of introgression of the SLICK haplotype for reducing the impact of heat stress on dairy production

P4075 Linkage disequilibrium and population structure of South African indigenous goat

populations using genome wide SNP data. Edgar Dzomba (University of Kwazulu-Natal) and Farai Muchadeyi (Agricultural Research Council)

South Africa is endowed with a rich diversity of indigenous goat (*Capra hircus*) breeds that are often characterized by their phenotypes and geographical distribution. The extent of genetic diversity of these goats is not fully understood because of the absence of both pedigree and performance recording systems. The first objective of this study was to assess the patterns of linkage disequilibrium (LD) in the feral Tankwa (25); the two indigenous breed groups: commercial meat types (Boer (20), Savannah (19) and Kalahari Red (17)), and local ecotypes (Nguni (10), Tswana (20), Zulu (30), Venda (30) and Xhosa (19)) using the Illumina goat SNP50K beadchip. LD was calculated using the pairwise r2 analysis. LD ± SD averaged 0.33 ± 0.15 and was affected by breed, chromosome and SNP interval ($p<0.001$). High LD was observed in the feral Tankwa goat (0.49±0.24) and the commercial meat types Boer, Kalahari Red, and Savannah goats (0.40±0.17). The lowest LD was observed for the indigenous ecotypes (range 0.35 to 0.34). Analysis of the rate at which LD decays with physical distance showed that LD decreased rapidly with the distance between 0-500 kb and remained constant from 500 to 2000 kb. The second objective was to study the genetic diversity and population structure using the Principal Component Analysis and admixture on all 192 animals grouped into breeds or ecotypes. The combination of PC1 (4,18), PC2 (3,74) and PC3 (1,52) separated individuals into two non-overlapping clusters of the feral, and the indigenous goats (commercial meat types, local ecotypes). At K = 2, a considerable source of variation among goats was observed with the clustering of the populations into feral and the indigenous goats. Further variation was observed at K=8 among the indigenous population. These findings suggest diversity among SA goat breeds particularly the Tankwa goat is genetically distinct from all the other indigenous goat populations.

P4076 Genetic Variations in Growth and the Associated SNPs in the S.A. Goat populations. Edgar Dzomba (University of Kwa-Zulu Natal) and Farai Muchadeyi (Agricultural Research Council)

Growth is an economically important trait in goats and other livestock production. There is wide variation in growth performance within and between South African goat breeds. Several genes including the growth hormone affect growth. The growth hormone gene is 2.544kb in size, consists of 5 exons and 4 introns and is located on the goat chromosome 19q22. It produces the growth hormone from the anterior pituitary and it plays a role in the metabolism and growth in mammals. Variation in growth hormone has been observed to affect growth in a number of livestock species. In this study, targeted gene and next generation sequencing technology was used to generate full length of growth hormone and screen for SNPs in the South African Boer (n=2), Kalahari Red (n=1), Savanna (n=1) and veld/indigenous (n=3) goat populations. Seven additional sequences (Accession nos.: D00467.1, EU651859.1, DQ531712.1, GU355689.1, GU355688.1, GU355687.1, and GU355686.1) were extracted from the GenBank for comparison. SNP analysis resulted in a range of 14-40 SNPs per gene sequence with an average of 2 indels. Majority of the SNPs were observed at exon 2, 4, 5 and some at intron 2, 3 and 4. A polymorphism resulting in one amino acid change from Glycine to Serine was observed at exon 2. The maximum likelihood phylogenetic tree showed close relations within the SA goat breeds that were different from the Japanese and Indian and Sarda Italian goat breeds. Results are discussed in the context of within breed selection and crossbreeding for genetic improvement of South

African goats.

P4077 Selection of informative SNPs for breed assignment in South African indigenous and locally developed beef breeds. Avhashoni Zwane and Azwihangwisi Maiwashe (Agricultural Research Council), Sithembile Makina (Africultural Research Council), Ntanganedzeni Mapholi (Agricultural Research Council) and Este Van Marle-Koster (University of Pretoria)

Animal identification and verification are essential tools for ensuring safety of livestock animals and animal products, facilitating veterinary disease surveillance and control. Studies have shown that breeds are significantly differentiated at genetic level. Therefore verification of breed origin is relevant for food safety and brand authenticity. Currently high density single nucleotide polymorphism (SNP) chips are available with large number of informative SNPs which can be used for breed assignment. In this study, 224 animals from five cattle breeds (Nguni, Afrikaner, Drakensberger, Bonsmara and Angus) were genotyped using Bovine50k SNP assay. The data was used to select the most informative SNPs between the breeds. The genetic structure was examined using the principal component analysis (PCA), which clearly separated the breeds. Highly differentiated SNPs were detected throughout the genome using Wright's Fst. 185 SNPs with Fst value of more than 0.6 were identified across the breeds. The highest number of SNPs was found between Afrikaner and Angus breeds. There were seven highly differentiated SNPs identified between the indigenous breeds. Therefore, this study shows the need for developing SNP panels specific for indigenous and locally-developed South Africa breeds. This will provide a reliable method to discriminated among the breeds, detect breed adulteration and to distinguish hybrids among populations.

P4078 Analysing the autosomal diversity of Spanish goat and sheep breeds at a whole-genome scale. AMPARO MARTINEZ MARTINEZ (Animal Breeding Consulting S.L.; Universidad de Córdoba, Spain), Antonia Noce and Marcel Amills (Center for Research in Agricultural Genomics), Juan Manuel Serradilla (Universidad de Córdoba), Félix Goyache (Servicio Regional de Investigación y Desarrollo Agroalimentario), Juan Vicente Delgado (Universidad de Córdoba), Vincenzo Landi (Animal Breeding Consulting S.L.; Universidad de Córdoba, Spain), Armand Sanchez (Center for Research in Agricultural Genomics), Joaquim Casellas (Universitat Autònoma de Barcelona) and Consortium for the Characterisation of Spanish Ovine and Goat Breeds: Silvia Adán, Federación de Razas Autóctonas de Galicia (BOAGA), Spain; Valentin Balteanu, University of Cluj-Napoca, Romania; Luis Bermejo, Universidad de La Laguna, Spain; Juan Capote, Instituto Canario de Investigaciones Agrarias, Spain; Jordi Jordana, Universitat Autònoma de Barcelona, Spain; Arianna Manunza, Center for Research in Agricultural Genomics, Spain; M. El Ouni, Livestock & Wildlife Laboratory, Arid Land Institute Medenine, Médenine, Tunisia; Águeda Pons, Serveis Millora Agrària, Spain; Amadou Traoré, INERA, Burkina-Faso; Oriol Vidal, Universitat de Girona, Spain (Animal Breeding Consulting S.L.; Universidad de Córdoba, Spain)

In Spain, goats (2.9 million heads) and sheep (22 million heads) play a relevant role in the agroindustrial sector because of their extraordinary ability to feed on poor pastures and their adaptability to low rainfall and extreme temperatures throughout the year. So far, 22 goat and 43 sheep breeds have been officially recognized in Spain, of which 17 and 33 have an endangered status, respectively. By using the 50K Goat Beadchip, we have analysed the autosomal diversity of 7 Spanish caprine breeds (N=176) including Blanca de Rasquera, Bermeya,

Malagueña, Florida, Murciano-Granadina, Palmera and Mallorquina. We have also surveyed several goat populations from Europe (Carpathian and Saanen, N=30) and Africa (Tunisia, Djallonké and Sahel, N=50). A multidimensional scaling plot analysis revealed three well separated clusters: one containing European goat breeds (Spanish and non-Iberian populations), another one encompassing breeds of African origin and the third one represented by the Palmera breed from the Canary Islands. The remarkable differentiation of Palmera goats is consistent with a scenario of founder effects combined with prolonged geographic isolation. Notably, genetic variation and geography were closely associated, with North (Bermeya, Blanca de Rasquera and Mallorquina) and South Spanish (Florida, Murciano-Granadina and Malagueña) breeds forming distinct subclusters. The analysis of the data with Admixture revealed the same trends reported above. We are currently genotyping five ovine Spanish breeds (Ripollesa, Xisqueta, Canaria de Pelo, Gallega and Roja Mallorquina, N=132) with the 50K Ovine BeadChip in order to achieve a more comprehensive perspective about the genetic variation of small ruminants in Spain.

P4079 Simultaneous characterization of genomic regions associated with two feather traits and two eggshell colors in domestic Japanese Quail (*Coturnix japonica*). Bertrand Bed'Hom (INRA, AgroParisTech), Frédérique Pitel, Patrice Dehais and Sophie Leroux (INRA, ENVT, ENSAT), David Gourichon and Sandrine Rivière (INRA), Nicolas Bruneau (INRA, AgroParisTech), Christine Leterrier (INRA, CNRS, Université François Rabelais), Céline Chantry-Darmon and Marie-Noëlle Rossignol (LABOGENA) and Francis Minvielle (INRA, AgroParisTech)

As in many domestic animals, and particularly in chicken, the domestic Japanese Quail (*Coturnix japonica*) has accumulated many visible traits since domestication. In order to understand the genetic mechanisms governing such traits, the genomic regions associated with four autosomal recessive mendelian traits (two feather phenotypes and two eggshell colors) have been characterized. Feather phenotypes are Curly (CU) and Rusty (RU). CU chicks have calamus of adjacent growing wing feathers not independent but connected through the follicle walls which appear to be joined together. The plumage of RU chicks is rusty, with down underneath having the usual wild-type dark-slaty color. A similar color pattern is present in RU adults. Eggshell colors are Celadon (CE) and White (WE). CE color is glossy pale blue, WE color is pure white, whereas the wild-type eggshell is brownish, with spots. Two F2 crosses have been organized, each one segregating for one eggshell color and one feather phenotype. 425 animals, i.e. 12 F0, 16 F1 and 397 F2 (females only) have been produced and phenotyped. With DNA extracted from blood, all animals have been genotyped using a genome-wide Illumina iSelect 6K SNP panel. SNP information has been obtained from whole genome sequencing of individuals from several INRA experimental populations. Genomic SNP coordinates are based on the relatively close chicken (*Gallus gallus*) reference genome. After quality filtering, 2090 informative SNP have been used for association analysis using PLINK. RU, CU and WE phenotypes are associated with high significance (p-value < 1E-20) to quail genomic regions corresponding to parts of GGA1, GGA5 and GGA6 respectively. The CE phenotype is weakly associated (p-value < 1E-5) to a quail genomic region corresponding to a part of GGA16.

P4080 Genetic Diversity of Korean Native Chicken Using Microsatellite Marker. JooHee Seo (Genomic Imformatics Center, Dept. of Animal Life and Environment science, The General Graduate School, Hankyong National University) and Jong Jin Kim and Hong Sik Kong (1 Genomic Informatics Center, Hankyong

National University)

27 microsatellite markers have been used to analyze the genetic variability of 20 breeds of Korean Native Chicken(KNC). 100 KNC whole blood samples (5 of each) were collected from National Institute of Animal Science (Red KNC, Yellow KNC, Gray KNC, Black KNC, White KNC, Ogye, Leghorn F, Black Cornish, Rhode Island Red C, and Rhode Island Red D) and from Hanhyup (A,H,F,G,V,S,W-lines). The 5 to 15 of allele were observed in each marker. Expected heterozygosity and polymorphism information content(PIC) per breed were observed at 0.668 to 0.881 and 0.616 to 0.865 respectively. ADL0259 marker polymorphic shows the highest (0.865) whereas MCW0330 marker polymorphic shows lowest (0.616). Statistical association analysis revealed that 10 markers were significantly associated to Hardy Weinberg Equilibrium. UPGMA dendrogram shows genetic relationship among breeds. Rhode Island Red C and Rhod Island Red D have the closest genetic distance (0.028) and the longest distance is Leghorn K and V line(1.606)

P4081 The extreme genetic diversity of olfactory receptor genes in pigs indicates the importance of maintaining olfaction capacity to diverse olfactants for the species. Dinh Truong Nguyen, Hunduma Dinka, Minh Thong Le, HyeJeong Lee, Hyoim Jeon and Chankyu Park (Department of Animal Biotechnology, Konkuk University)

Olfactory receptor (OR) genes belong to the largest gene family in the mammalian genome and *S. scrofa* contains 1300 OR related sequences. We investigated the genetic diversity of porcine OR genes focusing on 22 representative pig OR genes which covers most of OR gene containing chromosomes and all the OR gene families classified in the pigs. We specifically amplified the 22 OR genes by designing PCR primers on the specific regions of the pig genome around the OR genes and analyzed the sequence diversity using direct sequencing and cloning. The analysis results were obtained from 56 pigs consisting of 7 different pig breeds. Our results showed that the level of polymorphism in pig OR genes was generally high, but their genetic diversity was difference among different OR genes. The number of SNPs per OR gene ranges three to as high as 45 SNPs in their coding sequences. Also there was variation among different breeds. Number of SNPs which results in changing amino acids was 55.2% which is significantly higher than most of genes in mammals. Although the number of individuals for each breeds were relatively small (n= 8), 14% of SNPs and 68% of OR gene haplotypes were unique to specific breeds. The Ka/Ks ratio indicates an absence of strong selective constraint and therefore results in greater diversification of the pig OR genes except for a few family members. Such high genetic diversity of OR genes is comparable to that of MHC genes, the most variable genes in the mammalian genome, suggesting the importance of maintaining olfaction capacity to diverse olfactants in pigs.

P4082 Assessment of biodiversity in Chilean cattle using the distribution of MHC class II *BoLA-DRB3* allele.. Shin-nosuke Takeshima, Taku Miyasaka, Yuki Matsumoto and Guangai Xue (RIKEN), Veronica de la Barra Diaz (LAVET), Andrés Rogberg-Muñoz and Guillermo Giovambattista (Universidad Nacional de La Plata), Manuel Ortiz and Jorge Oltra (CIA, Universidad Austral de Chile), Misao Kanemaki (Institute for Animal Science) and Misao Onuma and Yoko Aida (RIKEN)

Bovine leukocyte antigens (BoLA) are extensively used as markers for bovine disease and immunological traits. In this study, we determined *BoLA-DRB3* allele frequencies in 888 cattle from ten groups, including seven cattle breeds and three crossbreds: 99 Red Angus, 100 Black Angus, 81 Chilean Wagyu, 49 Hereford, 95

Hereford × Angus, 71 Hereford × Jersey, 20 Hereford × Overo Colorado, 113 Holstein, 136 Overo Colorado, and 124 Overo Negro cattle. Forty-six *BoLA-DRB3* alleles were identified in the ten Chilean cattle groups. Each group had 12 to 29 different *BoLA-DRB3* alleles. The highest number of alleles (29 alleles) was detected in Overo Negro, which is considered in Chile as an "Old type" European Holstein Friesian descendant. By contrast, 21 alleles were detected in Holstein, which is considered as a "Present type" Holstein Friesian cattle. Chilean cattle groups were compared by phylogenetic and principal component analysis (PCA) using four Japanese breeds as an out group. The phylogenetic tree showed that Red Angus and Black Angus were in the same clade, crossbreeds were positioned close to the original breeds, and Holstein from Chile was positioned in the same clade as Holstein in Japan. Therefore, this tree provided a good explanation of breed history. The tree also showed that Overo Negro was close to Holstein, consistent with the historical data that Overo Negro is the "Old type" Holstein Friesian cattle. PCA showed that the Chilean breed originated in Britain (Angus, Hereford, and Jersey) and was distinct from breeds originating in the European continent (Holstein, Overo Colorado, and Overo Negro) at the point view of first principal component mainly computed from the frequencies of 16 *BoLA-DRB3* alleles. This allelic information will be important for investigating the relationship between MHC and disease.

P4083 Genetic diversity and inbreeding status in an insular bird population, the Barbary partridge (*Alectoris barbara*) in Italy.. PAOLA MODESTO, Cristina Biolatti and Simone Peletto (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta), Anna Vidus Rosin and Alberto Meriggi (Dipartimento di Scienze della Terra e dell'Ambiente, Universit à degli Studi di Pavia) and PierLuigi Acutis (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta)

The Barbary partridge (*Alectoris barbara*) of Sardinia belongs to the oldest and the most phylogenetically divergent species of the genus *Alectoris*. Due to the increased interest in partridges as game species, in the past decades, breeding stocks were reared in Sardinia and some breeding farms for population restocking arose in the island. Inbreeding and genetic changes in captivity can lead to low fitness, so the release of captive animals can affect the breeding performances of wild populations. Aim of the study was to assess the level of genetic variability and the existence of population structure in Sardinian Barbary partridges; moreover the presence of different species of the genus *Alectoris* in free-ranging wildlife was investigated. DNA was isolated from tissue samples of 104 animals and analyzed using nine known polymorphic microsatellite loci; primers for each locus were labelled at 5' with fluorescent dyes and two multiplex PCR reactions were optimised. Amplification products were run on a 3130 Genetic AnalyzerTM (Life Technologies). Moreover, a 234 bp fragment of the hypervariable domain I of the mitochondrial DNA was sequenced. Number of alleles per locus, allelic frequencies, observed (HO) and expected (HE) heterozygosity and inbreeding coefficient were calculated. A total of 52 alleles were found. All markers showed HO values lower than HE values. The FIS value was 0.104 (p<0.01), lower than the value previously recorded (Scandura et al. 2007). No population structure was detected by STRUCTURE software. Sardinian partridges genetic variability observed in this study is comparable with data reported in literature, even though is lower than the one of other wild species of the genus *Alectoris*; mtDNA analyses showed no evidence of the introduction of DNA extraneous from *A. barbara*. Further analyses are ongoing to assess the genetic differentiation between reared and wild partridges.

P4084 Genetic diversity and population structure of Brown hares (*Lepus europaeus*) from three different protected areas of the Emilia-Romagna region, Italy.. Paola Modesto and Cristina Biolatti (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta), Alberto Meriggi and Enrico Tagliaferri (Dipartimento di Scienze della Terra e dell'Ambiente, Universit à degli Studi di Pavia), Enrico Merli (Provincia di Piacenza, Servizio Tutela faunistica), Luca Nelli (Dipartimento di Scienze della Terra e dell'Ambiente, Universit à degli Studi di Pavia) and Simone Peletto and PierLuigi Acutis (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta)

The Brown hare (*Lepus europaeus*) is widely distributed throughout Europe where it constitutes an important game species. A marked decline has been recorded since 1960s due to hunting and habitat loss. In Italy, during the past decades, restocking programs were carried out with the introduction of allochthonous individuals. Nevertheless, in some protected areas, where no allochthonous animals were released, allelic fixation and inbreeding could have occurred. The aim of the study was to assess genetic variability of brown hares from three Italian protected areas instituted for restocking, located in the Emilia-Romagna region.Blood samples from 176 animals captured in Val Trebbia (n=47), Val Nure (n=64) and Val Tidone (n=65) were tested using eight known polymorphic microsatellites loci: SAT5, SAT12, SAT13, SOL8, SOL33, LSA1, LSA2 and LSA6. Primers for each locus were labelled at 5' with fluorescent dyes (FAM; VIC; NED) and two multiplex PCR reactions were optimised. Fragment analysis was run on the 3130 Genetic AnalyzerTM (Life Technologies) with ROXTM 500 size standard (Life Technologies). Number of alleles per locus, allelic frequencies and observed (HO) and expected (HE) heterozygosity were calculated. Moreover, the genetic differentiation of the three populations was assessed.All microsatellites loci resulted polymorphic, with a total of 63 alleles found. The mean HO value was lower than the mean HE value in all three populations. The HO and Fis were comparable with data recorded in other brown hare populations in Italy (Modesto et al., 2011; Canu et al. 2013) and in Europe (Ben Slimen et al, 2008; Thulin et al 2013).In conclusion, the three populations showed a good genetic variability and exhibited no genetic differentiation that proved the presence of gene flow among them. On the basis of our results, the areas involved in this study appear to have been undergone to successful management programs.

P4085 Whole mtdna genome sequence analysis of different haplotypes from the Turkish native goat breeds. Bengi Cinar Kul and Nuket Bilgen (Ankara University), Ozgecan Korkmaz Agaoglu (Mehmet Akif Ersoy University), Ozge Ozmen (Ankara University), Ozlem Gucuyener Hacan (Afyon Kocatepe University), Bilal Akyuz (Erciyes University) and Okan Ertugrul (Ankara University)

Turkiye is located in an advantageous geographical location, which houses native animal genetic resources specific to Anatolia, Europe and the Middle East. As also evidenced through genetic and archaeological investigations; Anatolia is one of the well-known origins of domestication for cattle, sheep, goats and pigs like China. Proximity to the site of domestication explains why Turkish indigenous animal breeds have a high level of genetic diversity when compared to European and New World breeds. Thus, whole genome studies and bioinformatics analyses are required for the characterization and preservation of the local genetic animal resources of Turkey. In this context, preliminary molecular identification of native goat breeds in Turkey, previously was made by the project team based on sequence analysis of mitochondrial D-loop region. In this study; was aimed to examine of

whole mitochondrial DNA (mtDNA) genomes of the goats assigned to different haplotypes by using next generation genome sequencing (NGS) analysis with 454 Life Sciences (Roche GS FLX) and to measure the current variation and phylogenetic relationships to estimate the divergence time of haplogroups namely A (n=5), D (n=1) and G (n=6) by contributing the related literature. The mitochondrial genomes were amplified through long range PCR by two overlapping fragments with (8351 bp) and (9127 bp) to provide full mitogenomic coverage. The phylogenetic relations between individuals were investigated by NJ and ML tree constructions. The individuals were clustered according to haplogroups and divergence time estimation for separate 13 genes encoding proteins and total genomes. However one disadvantage was found through NGS for repetitive sequence (76bp) in D-loop. In conclusion; the phylogenetic relationships and also genetic, historical and geographical relation can be established through the mtDNA polymorphisms by using NGS in the some Anatolian goat breeds as well as many domestic animals.

P4086 SNP diversity of the *ELR1* gene in Criollo Argentino horses. Claudia Corbi-Botto, Sebastian Sadaba, Maria Zappa, Pilar Peral-Garcia and Silvina Diaz (IGEVET, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata)

Equine lentivirus receptor 1 (ELR1) has been identified as the receptor responsible for the entry of equine infectious anemia virus (EIAV) in horse macrophages. Several single nucleotide polymorphisms (SNPs) have been reported in members of this tumor necrosis factor receptor (TNFR) protein family. A missense variant G/A in the coding sequence on the fifth exon of *ELR1* causes a non synonymous substitution E/K in a cysteine-rich domain of the mature protein. The aim of this study was to assess the extent of ELR1 polymorphism by specifically detecting the sequence variant SNP c.617G>A by Pyrosequencing®. We have analyzed 100 horses from diverse genetic background, such as Criollo Argentino (CR), Criollo Chaqueño (CH), Arab (AR) and mixbreed (MB). Our preliminary results showed that expected heterozygosities were higher in CR and AR (He=0.50) than in CH (0.44). In addition, only Criollo Argentino population was not in Hardy-Weinberg equilibrium for SNP617 (p-val=0.000). SNP617G results the most frequent variant in Criollo populations but not in Arabs. However, no significant genic (p-value = 0.07516) nor genotypic (p-value = 0.02032) differentiation were detected amongst the horse populations. Since allelic polymorphism of cellular receptors appears to be responsible for interindividual differences in gene expression profiles, they could also have an effect on the specificity of the interactions between ELR1 and the protein of EIAV that mediate infection of target cells.

P4087 Extremely conserved ruminant keratin-associated protein 7-1 gene. Sarnai Arlvd (Key Laboratory of Farm Animal Genetic Resources and Utilization of Ministry of Agriculture, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS)), Eka Sari (Department of Animal Production, Faculty of Agriculture, Syiah Kuala University), Zhi-Jie Ma (Academy of Animal Science and Veterinary Medicine, Qinghai University), Hao Zhang (Tongren Polytechnic College), Tian-Wu An (Sichuan Academy of Grassland Science) and Han Jianlin (CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS))

Keratin-associated proteins (KRTAPs) play a critical role in cross-linking the keratin intermediate filaments to build a strong hair shaft. In this study the KRTAP7-1 gene, a member of the high glycine-tyrosine KRTAPs gene family, was directly sequenced for 108 domestic yak,

taurine and zebu cattle samples collected in China and Indonesia for examining their possible polymorphisms and associated functional implication. Only two single nucleotide polymorphisms (one non-synonymous at c.7C/G and another synonymous at c.21C/T) and three haplotypes (BOVIN-KRTAP7-1*A, B and C) were identified in the complete coding sequence of bovine KRTAP7-1 gene among all animals. There was no polymorphism across three Chinese indigenous yak breeds and one Indonesian zebu cattle population, all sharing the BOVIN-KRTAP7-1*A haplotype. The four taurine cattle populations also had the BOVIN-KRTAP7-1*A as the most common haplotype with a frequency at 0.80. The frequency of novel haplotype BOVIN-KRTAP7-1*B was only 0.07, present in one heterozygous animal each of the four taurine cattle populations while BOVIN-KRTAP7-1*C was only found in a Simmental and a local Chinese Yellow cattle populations with frequencies at 0.17 and 0.36, respectively. The monomorphic yak KRTAP7-1 gene in particular and highly conserved bovine, sheep and goat KRTAP7-1 gene in general were attributed to an extremely stringent selective constraint in evolution due to their unique intrinsic structural property (e.g. >21% high glycine content) and primary functional importance in supporting the mechanical strength and shape of hair.

Genetic Markers and Selection

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P5001 Genome-wide identification of signatures of positive selection in African admixed zebu cattle. Hussain Bahbahani (University of Nottingham), Heather Huson (Cornell University), Abdulfatai Tiijani (National Biotechnology Development Agency), Christopher Mukasa (Ahmadu Bello University), Martin Blythe (University of Nottingham), Mark Woolhouse (University of Edinburgh), Marcos Vinicius Barbosa da Silva (Embrapa Gado de Leite), Oyekanmi Nash (National Biotechnology Development Agency), Tad Sonstegard (United States Department of Agriculture) and Olivier Hanotte (University of Nottingham)

The introduction of humped zebu to the African continent has led to genetic introgression with the local African humpless taurine. A mosaic of zebu x taurine cattle populations adapted to the local environments (e.g. semi-dry desert, humid and sub-humid forested areas) has arisen as a result of this introgression. Examples are the small East African shorthorn zebu (EASZ) from Western Kenya, Ankole from Uganda and Azawak cattle from Nigeria. Here, we use genome-wide high density SNP genotypes and full genome sequence information to identify candidate signatures of positive selection across the genome in EASZ. To increase power, we combined information of SNP genotyping data from two Extended Haplotype Homozygosity (EHH)-based (*Rsb* and *iHs*) and one interpopulation derived allele frequency (ΔDAF) tests in a single composite analysis. The full genome of 10 pooled EASZ samples has been sequenced using SOLiD next generation sequencing platform. Depressions of heterozygosity (Hp) were assessed on 100 kb sliding windows across the full EASZ genome. Eleven genomic regions in the EASZ show signals of signature of positive selection for both SNP genotypes and sequences analyses. Eight and three regions were further confirmed through the analyses of SNP genotypes data from East and West African cattle breeds respectively. Comparisons of signals of selection across the different cattle breeds analyzed, allowed us to narrow down the regions to genomic interval up to 500 kb. These regions harbour several candidate genes providing new information on the selection pressures which may have shaped the genome of these admixed cattle populations.

P5002 Genomics and gut microbiome of the next big fish Asian seabass. Gen Hua Yue (Temasek Life Sciences Laboratory)

The Asian seabass *Lates calcarifer* is an important farmed foodfish species. The fish has the ability to tolerate all levels of salinity from fresh to seawater allowing them to be cultured in both environments. We expect that this fish will become the next big fish in the world. We have worked on genetic improvement of Asian seabass using traditional and molecular breeding approaches since 1998. We have developed a large number of genomics resources and tools, and applied them in analyzing genetic diversity, reconstructing pedigrees, mapping DNA markers, mapping QTL for important traits and identifying important genes to accelerate genetic improvement. After three-generation selections, we have improved growth over 45%, and established three lines each with a preferred trait. Hybrids are being generated for commercial production. Since gut microbes play an important role in growth and diseases resistance, we recently started to sequence and analyze the intestinal microbiome. In this paper, we will present some details of the genomic tools developed and the intestinal microbiome in Asian seabass.

P5003 Kappa-casein and growth hormone genes polymorphism associated with Simmental cattle main productive traits. Natalya Shkavro (Institute of Animal Science Ukrainian Academy of Agrarian Science)

Ukrainian Simmental cattle genetic structure

evaluated by the kappa-casein (*CSN3*) and growth hormone genes (*GH*) polymorphism investigation by PCR-RFLP. For the *CSN3* gene a high frequency of *A*-allele (q = 0,833), which is associated with increased general milk yield was obtained. Predominant number (83.3%) of animals were with *AA* genotype, about 18.0% - heterozygotes *AB* genotype, near 8% animals were with desired for high-quality cheeses production homozygous genotype *BB*. For the growth hormone gene the preference of *L* allele with a frequency of 0,872 was detected. The most research animals (74%) were with the homozygous genotype *LL*, the frequency of heterozygous genotype *LV* was 0,256. Animals with homozygous genotype *VV* were not detected. Animals with *AA* genotype of *CSN3* gene were characterized the best milk yield, milk fat and proteins indexes. Milk fat and protein content at the average level observed for animals with the heterozygous *AB* genotype, animals with *VV* genotype had all parameters lower than other. Milk yield parameters at the average level were detected for animals with homozygous *LL* genotype by *GH* gene (5582,82 kg/lactation), and their milk fat also was higher of the heterozygotes animals (3.94% against 3.87%), but the heterozygous *LV* animals were characterized of the better protein content indicators (3,15%). The highest indexes of milk yield (5721.32 kg/lactation) and milk fat content (3.96%) were characterized animals with complex *AALL* genotype, moreover, the highest number of studied Simmental cattle had this variant of genotype (54%). High level fat milk content was indicated for cows with *ABLL* genotype (3,94%) and milk protein content - *AALV* (3.17%). Thus, for the studied animals the *AALL* complex genotype by kappa-casein and growth hormone genes was identified as the best by the productive characteristics (yield - 5721 kg, fat - 3.96%, protein - 3.15%).

P5004 QTL analysis of meat quality related traits in a large F2 intercross between Landrace and Korean native pigs. In-Cheol Cho (National Institute of Animal Science), Sang-Hyun Han (Jeju National University), Sang-Rae Cho, Moon-Suck Ko and Won-Mo Cho (National Institute of Animal Science) and Hee-Bok Park (Chungnam National University)

This study conducted a genome-wide linkage analysis to identify quantitative trait loci (QTLs) influencing meat quality related traits in a large F2 intercross between Landrace and Korean native pigs. All experimental animals (830 F2 progeny) were subjected to the genotype analysis using 173 microsatellite markers located throughout the pig genome, and the GridQTL program based on the least squares regression model was used to perform the QTL analysis. On SSC12, QTLs affecting 10 meat quality traits were detected and all of these are reached the highly significant at genome-wide level. In particular, the QTL affecting crude fat percentage explained 22.5% of the phenotypic variance in this study. Interestingly, the QTLs on SSC12 affecting the meat quality traits showed an obvious trend for co-localization. The results from our study appear to verify several previously reported QTLs. Also, we identified novel QTLs affecting meat quality traits. The identified QTLs together with the associated positional candidate genes can give us an enhanced knowledge of the genetic structure underlying the variation of meat quality traits in pigs.

P5005 Association of the SNP in the *MYBPC1* gene with marbling in Japanese Black beef cattle. Bin Tong (Graduate School of Science and Technology, Niigata University, Niigata, Japan), Seiki Sasaki (Division of Animal Genetics, Maebashi Institute of Animal Science, Livestock Improvement Association of Japan), Tatsuo Fujita (Oita Prefectural Institute of Animal Industry) and Takahisa Yamada (Graduate School of Science and Technology, Niigata University, Niigata, Japan)

Marbling is an economically important trait in beef industry and genetic markers associated with marbling are required. We previously showed that the *MYBPC1* gene encoding the slow skeletal muscle isoform of the major myosin-binding proteins in vertebrate striated muscles possesses higher expression levels in high-marbled Japanese Black steer group than in low-marbled Holstein steer group in *musculus longissimus* muscle. In this study, we confirmed this marbling-associated *MYBPC1* expression pattern by real-time PCR using *musculus longissimus* muscle samples of 12 high-marbled Japanese Black steers and 8 low-marbled Japanese Black steers. Further, we detected 3 SNPs by the direct sequencing for high-marbled Japanese Black steers and low-marbled Holstein steers, and the detected g.70014208A>G SNP was genotyped by PCR-RFLP in 100 Japanese Black sires and 745 paternal half-sib Japanese Black progeny steers produced from 2 sires homozygous for A allele. We showed that the SNP is associated with marbling (P = 0.045 for sires and P < 0.0001 for progeny steers) in Japanese Black beef cattle, with the G allele resulting in high levels of marbling. Based on the results of association study, we hypothesized that the g.70014208A>G SNP might have an impact on *MYBPC1*expression and also marbling by affecting *MYBPC1* promoter activity. In conclusion, our results suggest that the *MYBPC1* g.70014208A>G SNP is a useful genetic marker for increasing the levels of marbling in Japanese Black beef cattle.

P5006 Haplotypic analysis of the Ovine fatty acid-binding protein (FABP4) gene. Wei Yan (Gansu Key Laboratory of Herbivorous Animal Biotechnology), Huitong Zhou (Gene-Marker Laboratory), Yuzhu Luo and Jiang Hu (Gansu Key Laboratory of Herbivorous Animal Biotechnology) and Jon Hickford (Gene-Marker Laboratory)

The adipocyte fatty acid binding protein (FABP4) plays an important role in the regulation of lipid metabolism in mammals. In this study, two regions of ovine *FABP4* spanning exon 2- intron 2 (region 1) and exon 3-intron 3 (region 2) were investigated in four hundred and twenty lambs derived from seven sires that typed as having heterozygous genotypes in these two regions. The two regions were typed using a Polymerase Chain Reaction Single-Stranded Conformational Polymorphism (PCR-SSCP) method to identify extended haplotypes that spanned the two regions. These regions have been shown to be variable, with three SNPs plus one indel and four SNPs respectively constituting five and four allele variants in the two regions. Fourteen different haplotypes identified in the progeny, but unexpectedly three or four paternally-derived haplotypes identified in the progeny of six of the seven sires. The paternity of all the lambs was confirmed by typing for variation in *PRNP*, *ADRB* and the *MHC-DQA2-DQA2-like* region. These results suggest that meiotic recombination occurs within ovine *FABP4*. A number of simple sequence motifs that have been reported to be associated with recombination hotpots in both prokaryotes and eukaryotes were found. These include: 1) a 14-mer sequence (GCTGGTGCTGGTGA) consisting of two partially overlapping *chi-like* sequences (GCTGGTGC and GCTGGTGA) in region 2; 2) a *CTE*-like sequence (ATGAAGTCA) in region 1; 3) a CCTCCCT motif approximately 2 kb upstream of region 1 and variants of this sequence in region 1 and region 2;4) a number of copies of a CCAAT in region 1, in region 2 and other regions of the gene. These motifs were targeted and these may facilitate recombination.

P5007 Effects of *LTBP2* Genotypes on the Carcass Traits in an F2 Population between Landrace and Jeju (Korea) Black Pigs. Sang-Hyun Han, Yoo-Kyung Kim and Hong-Shik Oh (Jeju National University) and In-Cheol Cho (Subtropical Animal Experiment

Station, National Institute of Animal Science, RDA)

This study was tested the association between genetic polymorphisms of *latent transforming growth factor* β*-binding protein 2* (*LTBP2*) gene and carcass traits in an intercross F2 population between Landrace and Jeju (Korea) Black pig. Genetic polymorphisms were screened by DNA sequencing and genotyping. A total of 8 nucleotide substitutions in the protein coding regions and 3 insertion/deletion polymorphisms in the intronic sequence were found in *LTBP2* gene. Among those, *LTBP* exon 32 c.4481A>C (p.1494H>P) showed the significant associations with meat muscle area (MMA), carcass weights (CW), and carcass length (CBL) ($p<0.05$), but not with backfat thicknesses at three different points (BFs), meat color (MC), marbling score (MARB), and eye muscle area (EMA) ($p>0.05$), respectively. The F2 animals harboring the *LTBP2* c.4481 *A/C* heterozygote showed relatively heavier body weights for carcass weights (79.601±0.634 kg) than those of *C/C* homozygotes (77.590±0.917 kg) ($p<0.05$). The F2 animals possessing *LTBP2* c.4481 *A/A* genotype were shorter levels of CBL than those of *A/C* and *C/C* genotypes ($p<0.05$), showing approximately 2.0 cm shorter levels than those of *A/C* and *C/C* animals. In addition, the F2 progeny carrying the *LTBP2* c.4481 *A* allele showed significantly larger MMA levels than those of *C/C* homozygotes ($p<0.05$). These findings indicate that *LTBP2* genotypes may be involved in the muscular development or body length growth, suggesting certain roles of the *LTBP2* gene in mediating osteogenic differentiation, ECM network, and cell adhesion. Thus, *LTBP2* genotypes can assist as molecular genetic markers for improving the Jeju Black pig and Landrace-related crossbreeding systems.

P5008 Discrimination of Korean native chicken lines using 600K SNP array.. DONG-WON SEO, HEE-BOK PARK, NU-RI CHOI, SHIL JIN and JUN-HEON LEE (Department of Animal Science and Biotechnology, Chungnam National University)

The establishment of chicken breeding stock project has been launched to develop native chicken industry in Korea. For this project, 68 birds from fourteen different lines were collected from National Institute of Animal Science (NIAS) and Hanhyup company. Multi-dimensional scaling (MDS) analysis was conducted using 600K SNPs chip data. As the result, most lines were clustered well and discriminated from each other. On the other hand, four lines from the Hanhyup population (H and F, S and W) and the four lines from the NIAS population (R and Y, C and D) were clustered together. The initial analysis indicated that number of specific SNPs in each line was ranged from 0 to 125. W line in Hanhyup population has the highest (125) and there is no specific SNP was observed between C and D lines in NIAS population. These results will provide important genetic information for breeding strategy of Korean native chicken and can be used for construction of traceability system in the market.

P5009 Effect of polymorphisms in the *estrogen receptor alpha* gene on marbling in Jersey and Limousin cattle. Rugang Tian, Andrew Egarr, Eric Yin, Wayne Pitchford and Cynthia Bottema (University of Adelaide)

Marbling is a valuable economic trait for beef production and a major factor that influences overall meat eating quality, including tenderness, taste and juiciness. Genes associated with marbling are being studied to better understand fat deposition in cattle and potentially discover DNA variants that may be used for selection of marbling. Estrogen is involved in the positive and negative regulation of various biological processes. Two nuclear receptors, estrogen receptors α and β, mediate the effect of estrogen and the estrogen receptor alpha (ESR1) has been

associated with adipogenesis in mice and humans. The *ESR1* gene is within a region that affects marbling on bovine chromosome 9, identified by quantitative trait loci (QTL) genetic linkage analysis and a genome wide association study (GWAS) in Jersey and Limousin crosses. The objective of this study was to identify DNA variants affecting marbling in *ESR1* gene. In total, sixteen DNA variants were found in the *ESR1* gene by sequencing the genomic DNA from the three Jersey- Limousin mapping sires. Of these, three DNA variants were evaluated in the Jersey-Limousin mapping herd for their effects on marbling and other fat related traits. The genotypes of the SNP 2 had no significant effect on marbling score, but did affect omental fat ($P<0.05$). The genotypes of the SNP 7 and SNP14 had significant effect on US marble score ($P<0.05$) and Aus-Meat marble score ($P<0.05$,), respectively. Only the additive effects of SNP14 were significant for Aus-Meat marble score, accounting for 9% of the variance. *ESR1* haplotype was not significant for any of the traits analysed. The 9% increase in marbling associated with the *ESR1* SNP 14 adds new evidence that *ESR1* may be an important gene for the improvement of marbling in beef cattle industry.

P5010 Association of polymorphisms in the 5' flanking regions of the *GH*, *PRL* and *Pit-1* genes with Muscovy duck egg production. zhenqiang Xu (Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, and Key Lab of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture, South China Agricultural University, Guangdong), jun He (Wens Nanfang Poultry Breeding Co. Ltd., Yunfu, Guangdong, P. R. China) and dexiang Zhang and xiquan Zhang (Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, and Key Lab of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture, South China Agricultural University, Guangdong)

Somatotropic axis-related genes contribute to reproduction of poultry. Five single -nucleotide polymorphisms (SNPs) in 5' flanking regions of growth hormone (*GH*), prolactin (*PRL*) and pituitary-specific transcription factor (*Pit-1*) genes were identified and were genotyped in a female population of Muscovy duck. The association analysis of these SNPs with Muscovy duck egg production traits was performed. Results showed that SNP G-515C of the *GH* was significantly associated with the eggs number at 59-w old (E59W) ($P = 0.0009$), and associated with the eggs number at 300-d old (E300D) ($P = 0.0022$). SNP C-441T of the *GH* was significantly associated with E59W ($P = 0.0014$). Significant associations of T-884C and T-335C of the *PRL* with A1D, E59W and E300D were detected in this population ($P < 0.0001$). The significance mentioned above was referred to Bonferroni correction. It was concluded that the above four SNPs would be useful as markers for increasing Muscovy duck E59W. Based on genetic parameter estimation, heritability of A1D, E300D, E59W, and molting time was 0.43 ± 0.04, 0.45 ± 0.04, 0.36 ± 0.04 and 0.04 ± 0.03, respectively. The genetic correlation between E59W and E300D was remarkably positive (correlation coefficient: 0.80), whereas it was negative between E59W and A1D (correlation coefficient: -0.80). Therefore, selection for improved A1D will increase E59W.

P5011 SNPs associated with adult onset myotonic muscular dystrophy in chickens using 600K SNP array. Huaijun Zhou, Perot Saelao, Jason Abernathy, Jackie Pisenti and Mary Delany (University of California, Davis)

Adult-onset myotonic muscular dystrophy (MD) in chickens is caused by a recessive mutation (*am*) that produces significant and progressive fast twitch muscle weakness in affected individuals. The chicken is a useful biomedical model in addressing this hereditary muscle disease. UCD-md line derived from a highly inbred

chicken line that carries the mutation shows symptoms of fast twitch muscle weakness in the pectoral muscles starting around five weeks of age. Birds are evaluated using a standard "flip" test. Birds that are not homozygous for *am* can turn over more than ten times without fatigue; homozygous *am/am* birds usually manage few if any "flips", even at five weeks of age. Most White Leghorn homozygous birds show extreme atrophy of the pectoral muscles, and are unable to extend their wings. The UCD-md line has been back-crossed repeated into UCD-003, a highly inbred line. DNA was isolated from 6 UCD-md (homozygous dystrophy)(3 males and 3 females) and 6 backcross of UCD-md by UCD003 (3 males and 3 females, heterozygous). A 600K chicken SNP array was used to genotype these 12 individuals and one UCD003 bird. SNPs showed heterozygotes across all six F1 cross and respective homozygous in all UCD-md individuals and UCD003 were identified. A total of 623 SNPs were found to be potentially associated with MD in the chicken. Of the 623 SNPs found, 620 were mapped to chicken chromosome 2. Interestingly, functional analysis revealed that 24 unique genes associated with these SNPs are involved with ATP synthesis, ion transport, neuronal signal transduction, and membrane protein transport. Many of the functional annotations suggested that these SNPs identified may be crucial to muscle function and active tension in chickens. Further functional analysis on these SNPs will provide new insights on the underlying molecular mechanism of adult onset myotonic muscular dystrophy in the chicken.

P5012 MiR-16, potentially mediated by a 54bp indel, functions on growth traits in chicken (Gallus gallus). Xinzheng Jia, Qinghua Nie and Xiquan Zhang (South China Agricultural University)

MiRNAs are known to involve in growth and metabolism process. This study aims to identify candidate miRNA for chicken growth. MiRNA array and small RNA sequencing revealed that miR-16 showed significantly lower expression in the skeletal muscle tissues from fast-growing chickens. We herein demonstrate that miR-16 with a 54bp indel mutation in the forward of precursor was found in many populations, including Chinese domestic and imported breeds through directly sequencing. Further association analysis in F2 resource population showed that this variant was significantly associated with chicken growth traits. Body weight during 28 days to 84 days for Ins/Ins birds was significantly ($P < 0.05$) greater than for del/del birds. Interestingly, over expressing pri-miR-16 vector including 54bp insertion segment in DF-1 cell induce lower miR-16 expression compared to the deletion type, and two abnormal splicing modes were merely found in insertion types, which may contribute to decrease the generation of mature miR-16. In vitro, over expression of miR-16 could inhibit the proliferation of DF-1 and skeletal muscle cells. Further study proved that miR-16 could identically down-regulate two severe growth related genes (*FOXO1A* and *ACVR2A*) by directly targeting their 3'UTR. These data suggest that miR-16, mediated by deletion variation in the primary RNA, has important function on bird's growth traits, and this mutation provide a novel promising biomarker for breeding in poultry production. However, much systemic investigates were needed to elucidate the regulated mechanism in the muscle growth process by miR-16 in the future.

P5013 Fine mapping of locus *ID* for green legs in Chinese indigenous chickens. jiguo xu and xiquan zhang (Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, and Key Lab of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, Guangdong, China)

Fine mapping of locus *ID* for green legs in a Chinese indigenous chicken breed.The phenotype of green leg is caused by black pigment deposition in the dermal tissues of chicken shank, influenced by *id* locus. Previous studies have confirmed that *id*locus is mapped to the distal end of the long arm of chromosome Z. Until now, the corresponding genes of *id* locus controlling this phenotype are still unknown. The present study indicated that *GRAMD3* (GRAM domain containing 3) might be the positional candidate gene for *id* locus. In a Chinese local chicken population (Gushi chicken), some individuals have white legs while some have green legs. The case-control association analysis showed that the region from 78.4 Mb to 79.5 Mb of GGAZ (Galgal4) was significantly associated with green leg phenotype. The expression of several suspicious genes (*FEMC1*、*ALDH7A1*、*GRAMD3* and *ZNF608*) in this region were examined. The results showed that expression of *GRAMD3* in dermis tissues were significantly higher in Gushi chickens presenting green shanks at 350-d old than those without presenting green shank phenotypes at 1-d old. However, no significant association was found between the variations in the coding regions of this gene and shank color, including 7 point mutations and 1 deletion and insertion (indel). Thus we hypothesize that variations in the flanking regions might have important roles in formation of green shank by regulating the *GRAMD3* gene expression at the transcriptional or translational level.

P5014 Candidate gene analysis for reproductive traits in indigenous pigs of India. Soumen Naskar and Yoya Vashi (National Research Centre on Pig (ICAR)), Ankit Magotra (Lala Lajpat Rai University of Veterinary and Animal Sciences), Santanu Banik (National Research Centre on Pig (ICAR)) and Nihar Sahoo (Indian Veterinary Research Institute)

Pig production in India is inextricably linked to nutritional security and livelihood of resource-poor and predominantly socially disadvantageous farmers, characterized by low input-low output production system. Majority of production is restricted in eastern and north eastern part of country using indigenous pigs with widely variable (re)production performances. In the present study, association of reproductive traits in indigenous pigs of eastern and north eastern India, namely Ghoongroo, Niang Megha and Tenyi Vo pigs, with estrogen receptor (*ESR*), prolactin receptor (*PRLR*), retinol binding protein 4 (*RBP4*), follicle stimulating hormone beta sub-unit (*FSHb*) and epidermal growth factor (*EGF*) genes are reported. Reproductive traits considered for the study were litter size and weight at birth and weaning, respectively (LSB, LWB, LSW and LWW). Significant differences ($P<0.05$) in the reproductive traits were observed between different genetic groups, with considerably superior performances in Ghoongroo pigs. Polymorphism with variable gene frequencies was observed between genetic groups except *PRLR* and *RBP4* genes. However, no significant association of reproductive traits with the genes under study was found. Considering large variability in (re)production performances in indigenous pigs of India, screening with larger panel of candidate markers is suggested.

P5015 Design and Demonstration of a High Throughput DNA Tracking System for Genetic Improvement and Brand Verification in the Canadian Beef Industry. Kajal Devani (Canadian Beef Breeds Council) and Graham Plastow (University of Alberta)

To meet consumer demand beef industries have differentiated their product with assurances of quality, safety, and other social licence attributes. Recently, the need for an audit system and label verification system for these branded beef products has become apparent. As with most major beef supply industries, Canada employs

radio frequency identification (RFID) tags to enforce a mandatory animal identification and animal movement tracking system for response to food safety crises. A practical DNA tracking system using high throughput DNA technology was designed to strengthen the current Canadian animal traceability system, and enhance it by tracking carcasses and branded beef beyond the slaughter house. DNA linkage of branded beef to animals raised according to specific program requirements enables an audit for producer brands both protecting their brands and allowing for enhanced management of their product. In addition, this allows for verification of differentiation labelling strengthening consumer assurances and establishing consumer trust. A demonstration of the system allowed for the calculation of minimum sampling thresholds significantly decreasing the cost of system adoption. In addition to addressing the historical cost related barrier to adoption, the demonstration also enabled the assessment of several DNA sampling and genotyping technologies for practicality at the farm, feedlot, slaughter house, and laboratory level. This system brings value to the entire beef production chain as DNA typing on feeder calves can be leveraged to link the calves and their performance at the feedlot and packing plant back to their contributing genetics for the purposes of genetic improvement.

P5016 *LPL* gene polymorphisms are associated with fatty acid composition in Japanese Black cattle. Satoshi Koga (Graduate School of Agricultural Science, Kobe University), Shinji Sasazaki (Graduate School of Agricultural Science, Kobe University,), Kenji Oyama (Food Resources Education & Research Center, Kobe University) and Hideyuki Mannen (Graduate School of Agricultural Science, Kobe University)

Fatty acid composition of adipose tissue in cattle has become an important trait in beef industry. In bovine adipose tissue, higher concentrations of monounsaturated fatty acid (MUFA) in the adipocytes and lower fat-melting point are considered to contribute to favorable beef flavor and may decrease the circulating concentration of LDL cholesterol. Lipoprotein lipase (*LPL*), involved in the metabolism and transport of lipids, regulate energy balance, fat deposition and growth traits. It can be considered as a functional candidate gene that regulates fatty acid composition. In this study, we searched polymorphisms in full length CDS of *LPL* and investigated the associations with fatty acid composition (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, MUFA, SFA). Five SNPs (c.154A>T, c.798A>G, c.813C>T, c.1242T>C, c.1419C>T) were identified by sequence comparison among eight Japanese Black cattle. One of these SNP (c.154A>T) was predicted to cause amino acid substitutions (T52S) and the other four synonymous SNP were presumed to be in moderate linkage disequilibrium. Therefore we selected two SNP (c.154A>T and c.1419C>T) for further analysis. We investigated associations between these genotypes and fatty acid composition in the Japanese Black population (N = 449). Tukey-Kramer's honestly significant difference test revealed that T/T genotype in c.1419C>T indicated higher percentage of C18:1 and MUFA than the other genotypes in Japanese Black cattle ($p<0.05$). These results suggested that *LPL* genotypes would contribute to production of high-grade meat as selection markers in beef cattle.

P5017 Population-scale whole-genome sequencing reveals signatures of local adaptation in domestic pigs. Huashui Ai (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, Nanchang, China), Xiaodong Fang (BGI-Shenzhen, China), bin yang, Hao Chen, Feng Zhang, Leilei Cui, Ying Su, Jing Li, Hui Yang and Xianhua Xie (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture

of China, Jiangxi Agricultural University, Nanchang, China), Rasmus Nielsen (Department of Integrative Biology, University of California, Berkeley, CA) and Jun Ren and Lusheng Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, Nanchang, China)

Domestic pigs have evolved genetic adaptations to their local environmental conditions, such as cold and hot climates. Here we sequenced the genomes of 69 pigs (25X coverage) from 15 geographically divergent locations in China. We detected 41 million SNPs and 5.6 million structural variations, substantially expanding the catalogue of porcine genetic variation. Through haplotype sharing analysis, we showed a genomic landscape of reciprocal introgression between Chinese and Western pigs. Moreover, we reconstructed the evolutionary history of Chinese pigs in a broad context of worldwide pig breeds. We identified a genome-wide set of loci that likely have a role in regional adaptations to high- and low-latitude environments within China. These loci correspond to a list of genes related to thermostatic regulation like hair cell differentiation, energy metabolism and blood circulation. Intriguingly, we found an exceptionally large (14 Mb) and low-recombination region on the X chromosome that appears to have two distinct sweeps in the high- and low-latitude populations, possibly underlying their adaptation to cold and hot environments respectively. Surprisingly, the adaptive sweep in the high-latitude regions has acted on DNA introgressed from an African warthog-like species, providing the first example of adaptive evolution trigged by inter-generic introgression in mammals. We identified candidate genes within the sweep region, including *AR*, *EDA* and *AWAT2*, involved in the production of hair and sebum. Our findings provide novel insights into the evolutionary history of pigs and the role of introgression in adaptation more generally.

P5018 Association between *Acetyl-CoA Carboxylase*-αSNPs and fatty acid profile in Holstein bulls. Jolanta Oprzadek, Ewa Polawska, Anna Brzozowska, Edyta Juszczuk-Kubiak and Marek Lukaszewicz (Institute of Genetics and Animal Breeding, Polish Academy of Science)

The objectives of this study were to identify single nucleotide polymorphisms in the promoter region of the *ACACA* gene and to evaluate the extent to which they were associated with fatty acids profile. For association between *ACACA* genotypes and fatty acid profile, genomic DNA was extracted from 110, 15 months old Polish Holstein bulls. Samples of *m. longissimus dorsi* were collected at the location of the anterior end of the fifth lumbar vertebra at the time of dissection. Samples were minced, vacuum packed in polyethylene bags and stored at -70 C until required for fatty acid analysis. The PROC GLM procedure of SAS was used to test the association between SNP marker genotypes of the *ACACA* gene and carcass and fatty acid composition. Significant associations between the genotypes of SNP1/2/4/5/6/8 and C18:1n9c, total monounsaturated fatty acid content and n6:n3 ratio were detected. The content of several SFA and MUFA were significantly associated with the genotype of g. 2350T > C. Animals with the g. 2350TC genotype had higher contents of pentadecanoic acid and palmitic acid, lower content of total monounsaturated fatty acids. The g.2203TC genotype was associated with a higher content of polyunsaturated fatty acids in the *longissimus*. Higher contents of C18:2 and total SFA were detected with the g.2350 TT genotype compared with those of TC and CC genotypes.

P5019 Investigation of the role of miR-29 in the skeletal muscle development of pig. Weiya Zhang (Key Laboratory of Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and

Technology, Huazhong Agricultural University)

MicroRNAs are a class of small non-coding RNAs, which were involved into many physiological and biochemical processes through post-transcriptional regulation of the expression of genes. MiR-29 family includes three members, which are miR-29a, miR-29b and miR-29c. Our previous study confirmed that miR-29a/b/c were up-regulated during the development of the skeletal muscle in pigs and mice. Also, we confirmed that miR-29a/b/c could inhibit the proliferation and promote the differentiation of C2C12 cells through targeting the p85α and AKT3 genes. In this study, we further investigated the function of miR-29 in the skeletal muscle development of pigs. We detected the expression of miR-29c in longissimus dorsi muscle of 50-day fetuses(E50d), 95-day fetuses (E95d) and adult pigs. The result illustrated that the expression of miR-29c was highly up-regulated in E95d and sdults when compared with E50d, and the increase times were approximately 1.8 and 169.9, respectively. The Luciferase analysis results showed that miR-29 could inhibit the *YY1* gene through binding its 3'UTR in pig. Consistent with these results, differential expression analysis showed that the expression of *YY1* gene was highly up-regulated in E50d than E95d and adult pigs. It has been reported that *YY1*gene played very important roles in skeletal muscle in mice. Based on these results, we conclude that miR-29 plays very important roles in the skeletal muscle development in pig and the *YY1*gene targeted by miR-29 can be one of the potential pathway to regulate the skeletal muscle development of pig.

P5020 Genome-wide patterns of adaptation to climate-mediated selective pressures in sheep. Feng-Hua Lv (Institute of Zoology, Chinese Academy of Sciences, China), Saif Agha (Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland), Juha Kantanen (MTT Agrifood Research Finland), Licia Colli (Università Cattolica del Sacro Cuore, Piacenza, Italy), Sylvie Stucki (Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland), James Kijas (CSIRO Livestock Industries, St Lucia, Brisbane, Queensland, Australia), Stéphane Joost (Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland), Meng-Hua Li (Institute of Zoology, Chinese Academy of Sciences (CAS), Chaoyang District, Beijing, China) and Paolo Marsan (Università Cattolica del Sacro Cuore, Piacenza, Italy)

Sheep (*Ovis aries*) have been well adapted to thrive in a diverse range of climates during the domestication and breed development process. These climate-mediated selective pressures have shown to influence phenotypic variation within and among breeds and, meanwhile, left genetic "footprints" in their genome. Unlike numerous studies that searched for evidence of selection using only population genetic data, here we have scanned the sheep genome for selection signals by integrating genetic and climatic data. From the International Sheep HapMap Project, genome-wide data of *ca.* 50K SNPs in a diverse collection of 32 old and autochthonous breeds, which have been under different regional climates for a long term, were selected for the analyses. We first performed a variety of selection tests to detect variants under natural selective pressures. We demonstrated strong evidence for selective signals at a total of 230 SNPs associated with local adaptation to different climates. A great majority (82%, 189/230) of the candidate SNPs showed significant ($P < 0.05$) correlations between allele frequencies and climatic variables in a large subset of native populations from a world-wide range of geographic origins and climates. Our results imply that adaptations to local climates have shaped the spatial distribution of particular variants and, thus, such loci are likely involved in sheep adaptation to environmental challenges. Further molecular and functional studies of candidate genes close to significant markers will

help to elucidate the genetic architecture of climate-mediated adaptive traits in sheep and other farm animals.

P5021 Cloning and association analysis of porcine interferon-γ inducible protein-10 gene. Jing Huang, Zhiwei Zhu, Xiaoyu Chen, Fuxian Yu and Jianzhi Pan (Zhejiang Academy of Agricultral Science)

The understanding of disease-resistance genes is important to improve genetic resistance to infectious diseases through gene selection in pig breeding. Interferon-γ inducible protein-10 (IP-10), also named chemokine (C-X-C motif) ligand 10 (CXCL10), is a member of the chemokine superfamily. IP-10 has been known to play a critical role in immune response, such as mediating the development of inflammatory disease and contributing to viral clearance. In this study, we characterized porcine IP-10 gene (poIP-10) and the result showed that the cloned full-length cDNA of poIP-10 is 1145 bp, containing an ORF of 315bp encoding a protein of 104 amino acid residues with a calculated molecular mass of 11.6 kDa and an isoelectric point (pI) of 10.05. Expression profile analyses showed that poIP-10 expressed extremely high in spleen tissue, and lower in heart, liver and kidney. The level of the poIP-10 mRNA was up-regulated treated with polyI:C (a kind of synthetic double-stranded RNA, which can induce immune response similar as viral infection). One single nucleotide polymorphism (SNP) site was identified in the 3' un-translated region (3'-UTR) of poIP-10 and this SNP was significantly associated with percentage of lymphocyte (LY%) of 20-day-old pigs in blood, percentage of monocytes (MO%), hematocrit (HCT), mean of red cell volume (MCV) and mean of corpuscular hemoglobin (MCH) of 80-day-old pigs in blood ($p<0.05$), and also had extremely significant associations with hemoglobin concentration (HGB) of 80-day-old pigs ($p<0.01$) in blood. The results indicated that this SNP might be used as a genetic marker for blood parameters involved in disease resistance traits.

P5022 Signature of Selection in Chinese and Western Pig Breeds. Yanan Wang (Huazhong Agricultural University), Eui-Soo Kim (Iowa State University), Kui Li (Chinese Academy of Agricultural Sciences), Bin Fan (Huazhong Agricultural University), Max Rothschild (Iowa State University), Zhonglin Tang (Chinese Academy of Agricultural Sciences) and Bang Liu (Huazhong Agricultural University)

Under multiple independent domestications, local pig types were developed in both Europe and Asia from the wild boar, resulting in large changes of phenotypes and underlying genotypes. However, the signatures of selection of genes associated with economic traits and other differences between Chinese indigenous and Western pigs still remain to be elucidated. Here we scanned for signatures of diversifying selection in Chinese and Western breeds utilizing the Porcine SNP60 Beadchip. 275 animals were tested in this study including 6 Chinese indigenous breeds and 3 Western breeds. By calculating a standardized score of pairwise Fst between a breed and the remaining breeds (di) and iHS (integrated Haplotype Score) of each breed, we found some unique and shared selection regions among these breeds, with several interesting genes involved in the immune response, growth and body size. These breeds were then classified into different groups. Two Chinese minipig breeds were chosen to compare with the three Western commercial breeds and this comparison suggested some strong signatures of selection involving genes associated with growth. We also classified our four Chinese white-spotted breeds and compared them with two white Western breeds (Large White and Landrace), and found selection regions around the *KIT* gene and some other regions with genes involved in immune response

and tissue growth. These findings could help to understand artificial selection and adaptive evolution, and illuminate the genetic differences among Chinese and between Chinese and Western breeds.

P5023 Characterization and expression profile analysis of porcine PDGFRA gene. Fei Teng, Jing Huang, Zhiwei Zhu, Xiaoyu Chen, Fuxian Yu and Jianzhi Pan (Zhejiang Academy of Agricultral Science)

Intramuscular fat content is a key index of pork quality. The main gene associated with intramuscular fat need to be discovered. Recent findings obtained from animals such as mice and human, demonstrated that platelet-derived growth factor receptor α+ (PDGFRα+) mesenchymal progenitors from skeletal muscles can efficiently differentiate into adipocytes in vitro and in vivo upon transplantation into injured muscles. We carried out a series of experiments and got the following results: 1) The cloned full-length cDNA of porcine PDGFRA gene is 4050bp containing an ORF of 3270bp encoding a protein of 1089 amino acid residues with a calculated molecular mass of 122.1 kDa. This gene spans approximately 148 Mb on the genome, and contains 23 exons and 22 introns; 2) qPCR was performed to analyze expression level of porcine PDGFRA gene in different tissues of Jinhua-Duroc crossing pig, including heart, liver, spleen, lung, kidney, small intestine and skeletal muscle. The results showed that porcine PDGFRA expressed higher in spleen and lung, while much less in heart and liver; 3) Six kinds of muscle, including soleus muscle, supraspinatus muscle, biceps femoris muscle, psoas minor muscle, gastrocnemius muscle and loin muscle, were selected to compare expression profile of PDGFRA gene between slow muscles and fast muscles. The qPCR results showed that the expression of slow muscles (e.g. soleus muscle) was much higher than fast muscles (e.g. loin muscle). We supposed that slow muscles are more enriched with adipogenic progenitors and have higher propensity to form adipose, comparing to the fast muscles.

P5024 Host of long-term bidirectional selection for antibody titers alter profile of gut microbiota in chicken. Shuyun Liu and Wenjing Zhao (Shanghai JiaoTong University)

The gut microbiota is a complex ecosystem that has a symbiotic relationship with its host. The high level of diversity, community structure, and composition of gut microbiota are strongly associated with host species and are very stable and consistent within host species. This study will use lines of chickens divergently long-term selected from a common founder population for either high or low antibody response 5 days post-injection of a non- pathogenic antigen, sheep red blood cells as a resource, to study host effects on their gut microbiome profile. Fecal samples from 125 adult chickens, which consisted of high antibody strictly selected 40 generations (HAS40) 30 females, 13 males, high antibody relaxing selected 17 generations (HAR17) 17 females, and 10 males, low antibody strictly selected 40 generations (LAS40) 20 females, 8 males, low antibody relaxing selected 17 generations (LAR17) 19 females, and 8 males, were collected. The taxon abundance of each sample was generated into family levels primarily using the RDP database, aided by Greengene, and SSU databases. V4 of 16S rRNA were amplified and used to classify taxonomy by MGRAST. We observed that 13 genus, such as Fusobacterium, Proteus, Aeromonas, Pseudomonas, show significant different between high and low antibody response, 2 genus were significant influenced by gender. More interesting, selection pressure different also significant influence 28 genus. Our results demonstrated that long term selection not only alters the frequency of genes which related to antibody response, but also changes the profile of the microbiota influenced by those genes.

P5025 Selective signatures reveal candidate genes for altitude adaptation and body size in Chinese native pig breeds. Kunzhe Dong (Institute of Animal Science, Chinese Academy of Agricultural Sciences)

The Chinese native pig is a crucial component of the word's pig genetic resources because of its indigenous adaptation and specific traits. For example, Tibetan pig (TBP) and Dahe pig (DHP) dwelling on Qinghai-Tibetan plateau are renowned for their extremely well adaptation to altitude environments. While TBP and Wuzhishan pig (WZSP) are characterized by their small adult size. Here, high density multilocus SNP genotype for 91 unrelated individuals including TBP (33), DHP (26) and WZSP (32) were generated using the PorcineSNP60 chip assay, integrating SNP chips data of 425 samples from 13 European breeds and Meishan pig provided by Wilkinson et al.. The test statistics lnRH and *Si* that identify genomic regions with significantly reduced levels of variable in one population relative to the others, were used to find genes under selection in TBP, DHP and WZSP respectively, with a major focus on detecting genes underlying altitude adaptation and short stature. Different subsets of samples were considered as reference populations for different purposes in each analysis of the three pig breeds. Finally, we identified three promising genes (*ECE1*, *MAPK10* and *CTNNB1*) for TBP and four (*PRKCQ*, *BRAF*, and *FRS2*, and *CTNNB1*) for DHP that may equip them for life at high altitude environments. Among these genes, *ECE1* has been implicated in a recent study reported in TBP and others were newly identified by this study. In addition, the identification of *CTNNB1* that shared by TBP and DHP may suggest common adaptive mechanisms in the two pig populations. Moreover, we identified several known dwarfism genes with signals of selection in the two miniature pig breeds. They are *DDR2* and *FGFR2* in TBP, and *LCORL* in WZSP. These findings provide important information on the genetic basis for local adaptation and body size in pig, as well as other species.

P5026 Genomic Selection of Main Economical Traits in Pigs Using Low-density SNP Chip. Laihui Lv, Dewu Liu, Zicong Li and Zhenfang Wu (South China Agricultural University)

Genomic selection (GS) is a recently developed approach which can provide higher accuracy for prediction of animal's breeding value, as compared to traditional selection method based on BLUP. However, current GS in pigs mainly relies on costly high density SNP panel, such as Illumina 60k gene chip, for genotyping. To develop low cost but accurate GS technology for swine breeding, we collect SNPs that have significant correlation with important economical traits in pigs. These SNPs include SNPs reported by most of previously published studies, SNPs identified by one of our Illumina 60k gene chip-based genome-wide association studies (GWAS) in Duroc, and SNPs found by de novo sequencing of Landrace's and Large White's genomes. All collected SNPs are pooled and used to make a customized low density SNP chip. This low density gene chip then will be used for GWAS to identify additional SNPs that are significantly correlated with important economical traits in different pig breeds or lines. In addition, the low density SNP chip will be used for GS in different pig breeds or lines. The prediction accuracy associated with low density chip-based GS will be compared with that associated with traditional breeding method.

P5027 An improved Genotyping-by-Sequencing (GBS) approach for pigs. Cheng Tan, Jiangli Ren, Bo Jia, Yuzhe Wang, Yiqiang Zhao, Ning Li and Xiaoxiang Hu (China Agricultural University)

Rapid advances in next-generation sequencing

technology have revolutionized the way populations are genotyped. Genotyping-by-sequencing (GBS) technologies have proven capacity for delivering large numbers of marker genotypes with potentially lower cost than SNP chips. Here, we established a more flexible GBS procedure for pigs in order to discover and genotype genome-wide genetic marker across the genome. By using the modified GBS procedure, 24 genomic DNA samples from Duroc pigs were digested with ApekI enzyme, then ligated to adapters containing unique barcode and sequenced on the illumina sequencing platform. The results showed that all 24 barcoded DNAs were represented evenly, and that on average 0.5 million reads with a barcode and cut-site remnant were produced per animal. From these, 4,373,234 unique sequence tags containing 145,790 SNPs were identified through TASSEL4.0 analysis package. However, the average call rate per individual was lower than 40%, mainly because of the low sequencing output. The problem can be solved by increasing the sequencing data to 5 million reads per animal. Even then, we can also keep the cost under $60 per animal. This study suggests that GBS technique is flexible, sufficiently high-throughput, and capable of providing acceptable marker density for genomic selection or genome-wide association studies at roughly one second of the cost of currently available genotyping technologies.

P5028 Identification of Novel SNPs in Keratin Gene Family and their Association with Wool Traits of Chinese Merino Sheep (Xinjiang type). yuezhen tian (xinjiang agricalture university)

Keratin gene family, which contains keratin intermediate filament (KRT-IF) and keratin associated proteins (KAPs), acts as a regulator of follicle initiation and development and has been implicated in the regulation of wool growth and in controlling the structure and function of wool.Polymerase Chain Reaction Single-Stranded Conformational Polymorphism (PCR-SSCP) analysis and DNA sequencing were used to identify variants of 5 KAP genes and 6 KRT genes. in the population of 418 Chinese Merino Sheep (Xinjiang type). Also a protein sequence and structural analysis were performed to predict the possible impact of amino acid substitutions on physicochemical properties and structure of the keratin protein.A total of 32 nucleotide substitutions were identified, all of these substitutions have not been reported previously.Of these32 substitutions, five substitution in exon potentially result in amino acid substitutions from 6 KRT genes. According to the significance analysis, the different genotypes of KRT36, KRT38 and KRT85 have significant difference with mean fiber diameter ($P<0.05$) and the different genotypes of KAP8.2, KAP16.4-1, KAP16.4-2 have significant difference with fleece yield ($P<0.05$). The genetic variation revealed in this study suggests these genes are more variable than hitherto reported and provides a foundation for future research into how these variations affect wool traits, and some novel molecular markers for molecular breeding of sheep.As the results of polymer effect analysis,26 combined genotypes were observed and FF-MM-PP is the best combined genotype. Six combined genotypes concluding EE-MM-PP 、 EE-NN-PQ 、 EE-NN-QQ 、 FF-NN-QQ、EE-MN-PQ、EE-MN-QQ show up the excellent performance for wool traits,which provide reference for the further development of multi-genes pyramiding breeding in Chinese Merino sheep (Xinjiang type) .

P5029 Association tests on pleiotropic-QTL regions of chicken chromosome 1 using the 60k Illumina SNP BeadChip. Millor Rosario (CCN/UFSCar), Denia Attilio and Ricardo Brassaloti (ESALQ/USP), Monica Ledur (Embrapa Su ńos e Aves) and Luiz Coutinho (ESALQ/USP)

Usually in animal breeding, performance and carcass traits present different levels of genetic correlation that is caused by pleiotropy or linkage. Pleiotropy is considered a permanent cause, because a same QTL controls several traits simultaneously. Therefore, we aimed to saturate a region of chromosome 1 (57-71 Mbp), where two pleiotropic QTL were previously associated with 4 and 9 performance and carcass traits, respectively, and detect associations between these traits and SNPs. Based on 641 SNPs from the 60k SNP chip that are located on this region, we selected the 144 most informative SNPs according to the heterozygosity level of five F1 couples which generated the 453 F2 assessed for 24 traits. Single-marker analyses were implemented in the SAS using linear (additive) and quadratic (dominance) regression models. Out of 3,456 expected association tests, 609 (17.6%) were considered significant (at least $p<0.05$), being 424 (69.6%) with additive effect and 185 with dominance effect (30.4%). BW41d presented the highest number of associations (123), while back weight was not associated to any SNP. Proportionally, the highest number of SNPs was associated close to the pleiotropic QTL 2 with 17 traits. On the other hand, the highest significance levels ($p<9.59x10-8$) for the additive effect were evidenced for 5 SNPs located close to the pleiotropic QTL 1 and associated only with BW41. Novel associations were detected for weight gain and feed conversion from 35-41d, intestine length, feet, head, liver, heart, gizzard and lungs weights, hematocrit, cholesterol, triglycerides, and tryglycerides+cholesterol levels, and percentages of crude protein, ether extract and ashes. These associations indicate genomic regions that may be explored in details for putative positional and functional candidate genes detection.

P5030 Identification of the SNPs and miRNAs associated with meat quality traits in Yorkshire pigs based on GWAS and deep sequencing. Qian Dong, Wei Wei, Congcong Li, Yuanxin Miao and Xinyun Li (Key lab of pig genetics and breeding, Huazhong Agricultural University), Shuhong Zhao (Key lab of animal genetics, breeding and reproduction of ministry education, Huazhong Agricultural university) and Jianhua Cao, Sheila Apopo and Guojian Ma (Key lab of pig genetics and breeding, Huazhong Agricultural University)

Meat quality is an important economical trait. To identify genomic regions responsible for meat quality traits in pigs, genome-wide association study (GWAS) was conducted for five traits including intramuscular fat (IMF) content, pH at 45 minutes and 24 hours, drip loss within 24 hours and water-holding capacity in 233 Yorkshire barrows using the Illumina PorcineSNP60K Beadchip. Moreover, miRNA deep sequencing was performed using the longissimus muscle (LM) samples from high IMF group (2.94±0.04%, n=59) and low IMF group (1.62±0.02%, n=59). Our GWAS results showed that 334 SNPs were significantly associated with meat quality traits. Interestingly, 323 SNPs were within the QTL regions previously reported for meat quality and 5 SNPs were located in the introns of 4 potential candidate genes related to meat quality, such as ASGA0048299 and H3GA0052676 fell in *PIP4K2A and RAB31*, respectively. Finally, 25 haplotype blocks were detected among all the significant SNPs. In addition, 635 mature miRNAs were identified in LM by using deep sequencing, of which 301 were known in pigs while 334 were novel. Further analysis of novel miRNAs indicated that 298 miRNAs were conserved among other species, while 36 miRNAs were porcine specific miRNAs. There were 17 differentially expressed miRNAs (DEMs) between high and low IMF groups whose target genes were involved in 18 pathways responsible for IMF deposition. Furthermore, 15 DEMs, including miR-144 and miR-365-3p, were implied to target the Wnt pathway members that were critical for adipogenesis. Our studies

offered new information on the identification of SNPs and miRNAs affect meat quality traits in pigs and will benefit to the improvement of the related traits.

P5031 Effect of IGF2 gene variants on productive traits in pigs of different breeds in Russia. Nare Akopyan, Olga Kostyunina and Natalya Zinovieva (All-Russian Institute of Animal Breeding)

IGF2 (insulin like growth factor 2) is of interest as a genetic marker because of its chromosomal location, paternal expression effects on myogenesis and participation in a wide variety of metabolic processes, and differentiation. The objective of this study was to investigate the polymorphism of *IGF2* in pigs of Russian origin comparing to pigs of Canadian and European origins in Russia. 3215 animals of the Large White, Yorkshire, Landrace, Duroc and Russian local pig breeds were genotyped on A3072G polymorphism of *IGF2* using the pyrosequencing method. High frequencies of the desired A allele (from 0.767 to 1.000) were detected in commercial breeds and crosses of European and Canadian origins whereas the Russian pigs were characterized by significantly lower values of allele A frequencies (from 0.000 to 0.528) which are probably the result of preferential selection of animals on reproductive performances and lack of BLUP-evaluation in animal breeding in Russia.The effect of *IGF2* variants on productive traits was evaluated in 5910 offspring of the large white breed produced from boars with different *IGF2* genotypes. It was observed that individuals descended from boars with the AA genotype were characterized by 2-7 mm lower back fat thickness, by increased growth capacity (the age of 100 kilo was less on 6-19 days) and on 0,14 -0,25 kg/kg decreased feed conversion. These animals had on 12.5 sm2 larger eye muscle area. Thus, the application of boars carrying AA genotype of *IGF2* allows to produce per 1,000 head of commercial pigs additionally 8.7 tonnes of live weight and 6.1 tonne carcasse. Our results indicate that *IGF2* genetic marker is of interest in breeding farms in Russia and *IGF2* genotypes can be introduced as an additional criterion for the selection of replacement boars.

P5032 QTL analysis of shank color traits in Korean native chicken. SHIL JIN, HEE-BOK PARK, DONG-WON SEO, NU-RI CHOI and MUHAMMAD CAHYADI (Department of Animal Science and Biotechnology, Chungnam National University), CHAE-KYOUNG YOO, JAE-BONG LEE and HYUN-TAE LIM (Department of Animal Sciences, Gyeongsang National University), KANG-NYEONG HEO (Poultry Science Division, National Institute of Animal Science, RDA), CHEORUN JO (Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University) and JUN-HEON LEE (Department of Animal Science and Biotechnology, Chungnam National University)

A conservation project for the Korean native chicken (KNC) was initiated in 1994 and has been performed primarily by the Korean government. As a result, five lines of KNC have been established, categorized mainly by plumage colors. When the lines were developed, charcoal gray and dark green shank colors were selected to differentiate them from broilers, which have yellow shank colors. After more than 20 generations of selection with these criteria, the shank colors in KNC still display large color variations. From an economic viewpoint, shank color is a very important trait because different consumer preferences are prevalent in different areas of Korea. In this study, 596 F1 birds from five KNC lines were used to objectively measure the shank color traits [i.e., lightness (L*), redness (a*), and yellowness (b*)] by using a spectrophotometer. Genotype data on 161 DNA markers representing 28 linkage groups have been produced for this F1 intercross. The total

map length was 2,813 cM. A multipoint variance components linkage approach was used to identify QTL for the shank traits. In GGA24, we identified a QTL affecting b* value (LOD = 21.11, nominal *P*-value = 6.23 x 10-23). In the same chromosome, we also detected a QTL affecting a* value (LOD = 7.77, nominal *P*-value = 2.21 x 10-9). These QTL will be useful for positional candidate gene study. The results in this study can provide useful information for desirable shank colors for the selection of the KNC after the QTL effects are verified in other KNC populations.

P5033 Polymorphism of *LPL* gene and its effect on milk production traits in Czech dairy goat. Alena Svitakova, Jana Rychtarova, Zuzana Sztankoova, Jitka Schmidova and Lubos Vostry (Intitute of Animal Science)

The lipoprotein lipase (*LPL)* gene plays a central role in plasma triglyceride metabolism. The missense mutation G50C which involved a Ser → Thr amino acid replacement at position 17 of the signal peptide of *LPL* gene was determined in 262 Czech dairy goats. The PCR-RFLP method was used to identify this polymorphism by using restriction endonucleases *PleI*. The most frequent genotype was GG (0.775) in examined Czech dairy goat population. The mixed model with repeatability was used to estimate the impact of polymorphism *LPL* gene on milk production traits (milk yield, fat percentage and protein percentage). The fixed effects were genotype, herd-year-season of birth and parity number. The random effect was the animal (repeated measurements per goat). A significant effect of *LPL* gene on fat percentage and protein percentage was found. The animals carrying the GG genotype produced milk with the highest fat and protein content. The *LPL* gene appears to be a strong candidate gene for determining milk production traits.

P5034 Genetics of complex phenotypes in chicken. Mostafa Nassar (Department of Animal Production, Faculty of Agriculture, Cairo University) and Gudrun Brockmann (Department of Crop and Animal Sciences, Faculty of Agriculture and Horticulture, Humboldt-Universität zu Berlin)

Most of the traits considered in genetic improvement programs of growth in chicken are of complex nature, e.g. body weight gain, muscle mass and fat deposit. Those traits are genetically determined by many genes. A genome-wide scan was performed to detect quantitative traits loci (QTL) that affect 24 growth performance and body composition traits in reciprocal F2 crosses (n = 579) between the inbred lines New Hampshire (NHI) and White Leghorn (WL77). The lines NHI and WL77 had been selected for high body weight at the age of 20 weeks and for low egg weight during laying period, respectively. Afterwards, the lines were inbred. NHI chickens show a two-fold higher body weight at selection age compared to WL77. Linkage analyses provided evidence for highly significant QTL controlling growth performance and body composition on GGA2, 4 and 27. The peak QTL positions for different traits were located on GGA2 between 33.1 and 112.4 Mb, on GGA4 between 75.2 and 79.3 Mb, and on GGA27 between 3.6 and 3.8 Mb. The distal region of GGA4 (42.1 - 88.4 Mb) showed the highest effects on all analyzed phenotypes. This region accounting for 4.6 to 40.2 % of the phenotypic F2 variances of the corresponding affected traits. Additional genome-wide significant and highly significant QTL for different analyzed traits were mapped on GGA1, 5, 7, 10, 11, 12, 15, 26 and 27. For intramuscular fat content, a suggestive QTL was located on GGA14. The majority of identified loci showed additive effects. The directions of the QTL effects were consistent in both reciprocal crosses, but the magnitude was higher in the high cross direction NHI x WL77. The analysed crosses provide a valuable resource for further fine mapping of growth genes and

subsequent gene discovery on GGA4.

P5035 Alternative mRNA splicing, expression and association analysis of porcine interferon regulatory factor 9 gene. Wenwen Wang (China Agricultural University)

[Background] Interferon regulatory factor 9 (IRF9) gene is a member of the IRF-family and has been shown to play functionally diverse roles in the regulation of the immune system. To determine whether the IRF9 gene has an effect on serum cytokine level in pig, a candidate gene analysis was performed herein through genotype-phenotype associations. [Method] Immune traits including IFN-γ and IL-10 concentrations in each serum sample were measured using a commercial ELISA kit (Biosource, Carlsbad, California) based on the standard instructions from manufacturer. All exons and partial adjacent introns were cloned and MALDI-TOF MS (Squenom MassARRAY®; Bioyong Technologies Inc.) assay was applied for genotyping of the identified SNP in 300 pigs. The association analysis between the genotypes of the SNP and immune traits were examined by fitting the following mixed models using in SAS software (Version 9.2). Expression levels of mRNA in seven different tissues of three 35-old-day Large White pigs were investigated by real-time quantitative PCR using LightCycler® 480 II instrument (Roche Diagnostics GmbH, Germany). [Result] Two alternative mRNA splicing expression pattern of porcine IRF9 gene were identified. Tissue expression results revealed that the IRF9 mRNA was expressed widely in all analyzed tissues. An SNP in the peptide-binding region of the IRF9 gene was identified and it was significantly associated with the level of IL10 (At day 20), IFN-γ (At day 35) and ratio of IFN-γ/IL10 (At day 35) in serum. [Conclusion] There was a certain influence of A→G base substitution in exon 4 of the IRF9 gene on cytokine levels. Our results indicted that IRF9 variants could be a pontenial molecular marker in porcine breeding program for disease resistance and provided basis for further systematic function validation of the IRF9 gene. Keywords: pig, IRF9, association analysis, alternative mRNA splicing, mRNA expression

P5036 Detecting selective sweeps using Equine 70k SNP array in two native Iranian horse breeds. Mohammad Bagher Zandi B.M., Ardeshir Nejati Javaremi and Abbas Pakdel (university of Tehran)

Historical and archeological evidences trace Iranian horse breeds back to severalthousand years ago. Horse breeds are useful in investigating the origin of domestication and consequent migration pathways. Detecting the genomic regions is one of the most important areas of research in animal genetics since locations of selection signatures are often correlated with QTLs affecting economically important traits. Here we attempt to identify regions of Iranian horse genome that have been subjected to selective sweep. Theta and FST analytical methods were used to detect selection signals by using Equine70K SNPs genotyping result. We performed the theta (θ) and FST analytical methods on 26 Turkmen horses and 22 Caspian horses. The present study indicated that there are at least seven genomic regions that underwent a selection sweep in the body size in horse. Population differentiation using FST and Theta statistics in Iranian breeds revealed seven genomic regions with the most genetic differentiation between these breeds. Almost all of these regions overlapped with QTLs that had previously been identified as affecting body size traits in horse and human. A genome wide association analysis (GWAS) was used to verify the selective seep results based on GWAS, three of these seven SNPs have significant effect on body size.

P5037 Identification of a QTL affecting

carcass weight on GGA19 in Korean native chicken. MUHAMMAD CAHYADI, HEE-BOK PARK, DONG-WON SEO and NURI CHOI (Department of Animal Science and Biotechnology, Chungnam National University), CHAE-KYOUNG YOO, JAE-BONG LEE and HYUN-TAE LIM (Department of Animal Sciences, Gyeongsang National University), KANG NYEONG HEO (Poultry Science Division, National Institute of Animal Science, RDA), CHEORUN JO (Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University) and JUN-HEON LEE (Department of Animal Science and Biotechnology, Chungnam National University)

Mapping of genomic regions that contain genes involving economically important traits have been performed using DNA markers. The aim of this study was to identify quantitative trait locus (QTL) for body weight traits in Korean native chicken (KNC) population. A total of 679 chickens which are constructed by two generations of KNC were used. Microsatellite markers that covered 28 chicken chromosomes were genotyped to map QTL region and variance component linkage analysis was performed. Body weights of chicken were measured in every two weeks until slaughtered at 22 weeks of age. The QTL mapping results indicated that a QTL for carcass weight was identified at 20 cM (LOD score = 3.60, nominal P-value = 4.68 x 10-5, MCW0266-MCW0287) on chromosome 19 (GGA19). This preliminary result can be utilized to trace mutations of candidate genes which are involved in chicken growth within the QTL region. Furthermore, both QTL and positional candidate genes identified in this work can be useful to optimize the breeding plans for genetic improvement of KNC.

P5038 Single nucleotide polymorphisms, haplotypes and combined genotypes of Histatherin gene and their associations with mastitis in Chinese Holstein cattle. zhihua ju (Center of Dairy Cattle Research, Shandong Academy of Agricultural Science)

Histatherin(HSTN) is a ruminant-specific gene that plays a role in host defence in the oral cavity and milk in cattle. To investigate whether the HSTN gene is associated with mastitis in dairy cattle, a DNA sequencing approach was used to identify single nucleotide polymorphisms (SNPs) in the gene. Three new SNPs were identified. A total of 500 individuals from Holstein cattle populations were genotyped for their SNPs using Created Restriction Site PCR (CRS-PCR) and PCR-RFLP methods. Correlation analysis showed that the g.635 A>T marker was significantly correlated to somatic cell score (SCS) and 305d milk yield ($P<0.05$), and the g.12624 T>G locus had significant effects on SCS and fat content, suggesting possible roles of these SNPs in the host response against mastitis. Eight haplotypes and twenty-seven haplotype pairs were found. Combined genotype H2H2 with the lowest SCS was favorable for the mastitis resistance. They may be used as a possible candidate for marker-assisted selection in dairy cattle breeding program.

P5039 Wildlife breeding in South Africa: A rare opportunity. Henriette van der Zwan (Inqaba biotec)

Applying biotechnology tools in South African wildlife breeding holds a rare opportunity to select superior breeding animals from species where very little selection has been applied. In the past 30 years changes in legislation have resulted in farmers now owning the wildlife on their farm. Farmers are realising the potential of breeding these animals commercially. On state regulated reserves (e.g. Kruger National Park) conservation is the main objective, while on the estimated 9,000 privately owned wildlife ranches in South Africa the focus is profit from animal breeding through recreational and trophy hunting,

tourism and live auctions. In 2013 over ZAR1billion worth of live wildlife animals were sold at auctions. Rare and exotic species like buffalo (*Synceruscaffer*) and sable (*Hippotragus niger)* are sold at exceptionally high prices. Breeders are moving from extensive towards semi-intensive breeding practices and are incorporating tools such as DNA parentage verification tests to select superior animals. Very few studies have been conducted to estimate breeding values for traits like horn length, female fertility and growth. As breeders start to record more data the information could serve as the starting point to discover SNP markers linked to the traits of interest that can be used for marker assisted selection studies. If faster growing animals are selected based on whole genome selection, more venison can be produced at lower input costs than cattle which, in turn, can contribute to addressing Africa's protein need. Scientific breeding of wildlife species provides a rare opportunity as for most wildlife species no research on breeding has been done. The aim of this paper is to provide an overview of wildlife breeding in South Africa and to highlight the opportunities if existing biotechnology tools are applied in this field.

P5040 Comparative transcriptomics research of fine wool sheep skin. Yunxia QI (Inner Mongolia Research Center for Prataculture, Chinese Academy Sciences), Yongbin LIU (Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences), Wenguang ZHANG (College of Animal Science, Inner Mongolia Agriculture University), Shaoyin FU and Xiaolong HE (Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences), Jianghong WU (Inner Mongolia Research Center for Prataculture, Chinese Academy Sciences) and Weiheng RONG (Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences)

Wool fineness is the key factor that determines wool prices, and skin hair follicle is the basis of wool growth. To identify genes that may play important roles in wool fineness regulation, Illumina/ Hiseq2000 sequencing technology was employed to catalog global gene expression profiles in skin of yearling female sheep. Two groups of sheep were examined, that is, sheep with fine wool (wool fiber diameter＜22μm) and sheep with coarse wool (wool fiber diameter＞27μm) in the same farm. The results showed that, RNA-seq analysis generated 286 millions clean reads with 19914 genes aligned to sheep genome, which provided abundant data for the analysis of fiber diameter related genes. A total of 467 genes were differentially expressed in fine wool versus coarse wool sheep skin. These differentially expressed genes were mainly enriched in metabolism of lipids and lipoproteins, immune system and interferon alpha/beta signaling pathway. Further strict screening was performed and picked out 182 differentially expressed genes, of which 121 genes were in the hair follicle expressed gene pool built in our own laboratory. These 182 differentially expressed genes were revealed to contain close related gene clusters and mainly enriched in hormone stimulus biological process, with 12 genes up-regulated and 170 genes down-regulated. It was also discovered that there were 34916 common cSNPs in both fine wool and coarse wool sheep, and that the expression levels of low-heterozygous genes were higher in coarse wool sheep than that of fine wool sheep. This indicated that these cSNPs may influence gene expression levels and then influence wool fiber diameter. The study provides valuable resources for characterizing the gene functions associated with wool fiber diameter as well as for breeding elite fine wool sheep species.

P5041 The g.841G>C SNP of *FASN* gene is associated with fatty acid composition in beef cattle.. Kiri Hayakawa, Atsushi Ishii and Keita Yamaji (Graduate School of Agricultural Science, Kobe University), Yoshinobu Uemoto, Nanae Sasago and Tsuyoshi Abe (National Livestock

Breeding Center), Eiji Kobayashi (National Institute of Livestock and Grassland Science), Naohiko Kobayashi, Tamako Matsuhashi and Shin Maruyama (Gifu Prefectural Livestock Research Institute), Ichiro Tabuchi and Takuya Nogi (Tottori Prefectural Agriculture and Forest Research Institute Livestock Research Center), Kenji Oyama (Food Resources Education & Research Center, Kobe University) and Hirokazu Matsumoto, Shinji Sasazaki and Hideyuki Mannen (Graduate School of Agricultural Science, Kobe University)

Fatty acid composition is an important factor in determining beef quality in commercial market. In our previous study, we performed a genome wide association study (GWAS) for fatty acid composition in Japanese Black cattle population (JB1, n=461) using Illumina BovineSNP50 BeadChip and identified a candidate region between 49 and 52 Mbp on BTA19, where fatty acid synthase (*FASN*) gene was located. The objective of the current study is to evaluate the association between fatty acid composition and*FASN* gene polymorphisms as responsible mutations. For this purpose, we selected seven previously reported SNPs in *FASN* gene, including one within promoter region (g.841G>C) and six non-synonymous SNPs (g.8805C>T, g.13126C>T, g.15532A>C, g.16024A>G, g.16039C>T, g.17924A>G), and genotyped them in the same population. Genotyping results revealed that g.8805 C>T and g.17924 A>G were monomorphic loci and we therefore excluded them from further analysis. Genome-wide association analysis using the other five SNPs revealed that only g.841G>C showed significant associations with the percentages of C14:0 (P = 1.59E-12), C14:1 (P = 2.70E-9), C16:1 (P = 1.18E-8) and C18:1 (P = 2.72E-7) at 5% genome-wide significance level (P = 1.23E-6). In order to further evaluate the effect of g.841G>C, we genotyped the SNP using additional two populations, including a Japanese black (JB2, n=450) and a Holstein cattle population (HO1, n=195), and investigated the association with fatty acid composition by analysis of variance. As a result, g.841G>C showed significant effects on C14:0, C14:1, C16:0, C16:1, C18:1 at significance level of PC would be a responsible mutation for fatty acid composition and contribute to production of high-grade beef as a selection marker in beef cattle.

P5042 Effects of a single nucleotide polymorphism in *EDG1* and *TTN* genes with meat production and carcass traits in Japanese Black beef cattle. Seiki Sasaki, Itoh Tomohito, Atsushi Ogino and Hirohisa Kimura (Maebashi Institute of Animal Science, Livestock Improvement Association of Japan), Takahisa Yamada (Graduate School of Science and Technology, Niigata University) and Mitsuo Morita (Maebashi Institute of Animal Science, Livestock Improvement Association of Japan)

Marbling defined by the amount and the distribution of intramuscular fat is an economically important trait of beef cattle in Japan. Previous studies have reported that two single nucleotide polymorphisms (SNPs) in endothelial differentiation sphingolipid G-protein-coupled receptor 1(EDG1:c.-312A>G) and titin (TTN:g.231054C>T) have effects on marbling in Japanese Black beef cattle population. Because the observations were obtained by the studies on limited areas and strains, it is important to confirm the effects of the SNPs on the other traits as well as marbling for the improvement of whole Japanese Black cattle.In this study, we collected environmentally controlled 1,351 DNA samples from the progeny test population that contain various strains of Livestock Improvement Association of Japan, Inc. Then we analyzed the effects of the SNPs on the meat production and carcass traits that include beef marbling score (BMS), start weight (SWT) , start height(SH), end weight (EWT), end height (EH), daily gain (DG), carcass weight (CWT), rib eye area (REA), and rib thickness (RT). To

exclude environmental effects, we used estimated breeding values obtained by the BLUP method. Statistically significant differences ($p<0.05$) of the both SNPs were detected in BMS, EWT, DG, CWT and RT but not in SWT, SH, EH and REA. The G allele of EDG1:c.-312A>G and T allele of TTN:g.231054C>T resulted in favorable score for the traits. *EDG1* is known to be involved in angiogenesis and *TTN* encodes a protein that is responsible for passive elasticity of muscle. The facts of these functions suggest that *EDG1* and *TTN* exert a function in physiological or anatomical milieux surrounding adipocyte linage cells rather than intramuscular adipocyte linage cells. In conclusion, our results suggest that the two SNPs might be able to use for breeding program for Japanese Black beef cattle.

P5043 Genome wide association study for coat type in Sapsaree (Korean native dog). Bong-Hwan Choi (National Institute of Animal Science, RDA, South Korea)

This study was carried out to identify number of loci and distribution of their effects significantly associated with coat type (straight to curly), resulting in understanding genetic architecture of coat type in Sapsaree (Korean native dog). There was 16 significant SNP ($P<0.001$) that was associated with coat type, located across the canine genome in single point regression analysis. Of the all significant SNP ($P<0.001$), the largest number of significant SNP was located on CFA27, which contained 38 % (6 SNP) of the significant coat type markers. The other were located on CFA2 (1), CFA4 (1), CFA10 (1), CFA16 (1), CFA17 (1), CFA22 (2), CFA37 (1) and CFA38 (2). In particular, results of Blast search showed genes of KRT8, LIMA1, SCN8A and CCDC91 which are involved with coat type on CFA27. Our results revealed that coat type in dog will be affected by many loci with small effects and these genetic markers can be utilized to prediction markers for coat type in dogs.

P5044 Analysis of functional and positional candidate genes for early embryonal lethality in holstein friesian cattle. M.Sc. Christin Wehrhahn, M.Sc. Philipp Wittwer, M.Sc. Stefan Beckmann and Bertram Brenig (Institute of Veterinary Medicine, Georg-August-University)

Early embryonic lethality in cattle reduces fertility and influences several male and female reproduction related traits indirectly. On chromosome 19 (BTA19) the *STAT5A* gene has been described as a marker for early embryonic death. Furthermore, a region from 42.7 Mb to 43.9 Mb on BTA19 flanking *STAT5A* was reported to be associated with early embryonic lethality previously. Selected candidate genes located 1.8 Mb upstream and 2.39 Mb downstream of *STAT5A* have been associated with embryonic development. To identify further trait related genes, we have analyzed coding regions, respective splice sites as well as regulatory regions of seven so far not in depth characterized genes, i.e. *CDC6*, *TUBG1*, *TUBG2*, *RAMP2*, *BECN1*, *HEXIM1*, and *HEXIM2*, adjacent to *STAT5A*. Synonymous SNPs were found in *CDC6* (g.6018C>T), *HEXIM1* (g.588T>C), and *BECN1* (g.6120T>C). *RAMP2* harbored three intronic SNPs, two of them with a lack of one homozygous genotype (g.411C>T, g.530G>A, g.1395A>G). Within*TUBG1* we found 19 intronic and 8 exonic synonymous SNPs. The intronic SNP at position g.155C>A showed an allele frequency of 0.95 (N=1,083) of the C-allele with a distribution of genotype frequencies deviating from Hardy-Weinberg-Equilibrium. A preliminary association study showed that a SNP at position g.155C>A in *TUBG1* wassignificantly associated with fertility breeding values. In depth association analysis in 1,083 HF cattle revealed a reduced number of animals with the AA genotype and a significant (a=0.05) difference for days open (AA=101.98, CA=107.45, CC=106.23). FACS cell cycle analyses of cultured leukocytes showed a significant (a=0.01)

higher proportion of cells in G2 of the AA compared to the CC genotype indicating an arrest of the cell cycle in this phase. These results suggest that the SNP at position g.155A>C in the *TUBG1* gene could be a marker for early embryonic lethality in cattle.

P5045 Progress of genetic improvement and the whole genome selection research program in large yellow croaker. Zhiyong Wang, Xiande Liu, Mingyi Cai, Shijun Xiao, Yangjie Xie, Qiurong Wang, Limin Lin, Fang Han and Dongling Zhang (Jimei University) and Jiongtang Li (Chinese Academy of Fishery Science)

Large yellow croaker (*Larimichthys crocea*) is one of the most important maricultured fish species in China. The annual production of the marketable fish is roughly 100 thousand tons with a market value of more than 6 billion. A novel variety named JD01, exhibiting a faster growth rate and higher survival rate, have been bred by Jimei University and Centre of Popularization of Fisheries Technology in Ningde through a combined strategy of selective breeding and gynogenesis technique and promoted in the aquaculture industry of China. Because female individuals generally grow faster than male ones, techniques for producing genetically all female population are established via the mating of sex reversed gynogens (neomales, XX♂) and normal females (XX♀). As a result, the aquiculture of the all-female population increases a product as high up to more than 20%, comparing to that of traditional counterparts.

To further push forward the breeding achievements of large yellow croaker, a new genetic improvement program based on whole-genome selection has been set up since 2012. A draft genome has been obtained through whole genome sequencing with a highly homozygous individual from a second generation gynogen population by our research team. We obtained the preliminary assembly of 700 Mb, with a contig N50 > 80kb and a scaffold N50 > 1Mb. By whole genome re-sequencing of 57 male and 57 female individuals, we identified over 3M reliable SNP/InDels. To construct a high density genetic map and perform Genome-Wide Associate Studies (GWAS), we have designed a Cap-Seq Kit to detect 50K high-frequency SNPs in large yellow croaker (50% in coding regions and 50% in non-coding regions). Further, more than 20 phenotype data from 30 lines have been collected to support the following GWAS studies. Our recent work provides a solid basis for the whole-genome selection program, and will finally improve mariculture efficiency by high-throughput molecular assisted breeding in large yellow croaker.

P5046 Effect of single nucleotide polymorphisms of *BTN1A1* gene on the milk production traits of Czech dairy goats. Jitka Kyselova, Zuzana Sztankoova, Alena Svitakova, Sona Melcova and Michaela Krejcova (Institute of Animal Science)

The butyrophilin (BTN1A1) is a glycoprotein of the immunoglobulin superfamily that is secreted in association with the milk-fat-globule membrane from mammary epithelial cells. The *BTN1A1* gene is considered as a candidate gene for the milk performance traits. The goal of this study was to characterize genetic variants of the *BTN1A1* and test their associations with milk performance traits in Czech dairy goats. DNA sequencing and PCR-RFLP methods were used to detect genetic variation in the 4th exon of*BTN1A1* gene in 262 Czech dairy goats. *BTN1A1* gene (526 aa) showed two novel single-nucleotide polymorphisms (SNPs): g.603T>C which is synonymous and monomorphic and g.599G>A, resulting in a missense mutation GAG (Glu)>AAG (Lys) at position of 184 aa (Acc. No. NCBI NM_001285618.1). Three genotypes were

distinguished with frequencies as follows: 0.019, 0.248, and 0.733 (for AA, AG and GG genotypes, respectively). Association testing revealed that the *HinfI* polymorphism was significantly associated with the milk yield ($P < 0.01$) and the milk protein content ($P < 0.05$). This study provides new SNPs extending the *BTN1A1* gene characterization as well as an evidence of the relationship between polymorphism and milk production traits in Czech dairy goat breed which contribute to implementing marker-assisted selection (MAS) in breeding and genetics in dairy goats.

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P5047 Genome wide analysis of the effect of 20 years of selection in the Italian Large White pig breed. Luca Fontanesi and Giuseppina Schiavo (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna), Giuliano Galimberti and Daniela Giovanna Calò (Department of Statistical Sciences "Paolo Fortunati", University of Bologna), Emilio Scotti and Antonia Bianca Samorè (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna), Maurizio Gallo (Associazione Nazionale Allevatori Suini (ANAS)), Vincenzo Russo (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna) and Luca Buttazzoni (Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Produzione delle Carni e il Miglioramento Genetico)

In this study we investigated if a selection program based on boar genetic evaluation obtained with a classical BLUP animal model can change, in a quite short period of time, allele frequencies in a pig population. All Italian Large White boars born from 1992 to 2012 with estimated breeding value reliability >0.85 (n. = 200) were selected among all boars of this breed. Boars were genotyped with markers in major genes (*IGF2* intron3-g.3072G>A, *MC4R* p.D298N, *VTNR* PRE1 insertion, *PRKAG3* p.I199V and *FTO*g.276T>G) and with the Illumina PorcineSNP60 BeadChip. Genotyping data were analysed grouping boars in 8 classes according to their year of birth. To evaluate the influence of time on allele frequencies of the genotyped markers, a multinomial logit regression model was computed. For four out or five gene markers (*IGF2*, *MC4R*, *VTNR* and *FTO*) frequencies of the alleles associated with favorable effects on traits under selection (higher lean meat content and ham weight, higher average daily gain, lower back fat thickness and favorable feed:gain ratio) changed significantly in the considered period of time, according to the directional selection of the selection program of this pig breed. In addition, a significant change over time in the allele frequencies was detected on other 464 single nucleotide polymorphisms ($P<0.05$, Bonferroni corrected). Most of them were very close or within annotated genes in the Sscrofa10.2 genome version providing potential information on the functional effects of these allele shifts. These results indicated that selection carried out during the last two decades modified substantially the genome of the Italian Large White pig population. Moreover, several candidate chromosome regions affecting production traits were identified.

P5048 SNP screening, molecular characterization and evolutionary aspect of chicken *Piwi* genes. Hong zhi Wang (College of Animal Science & Technology, Yangzhou University)

Piwi（P-element induced wimpy testis）gene get involved in the process of germ line stem cell self-renewal, meiosis, RNA silencing, and transcriptional regulation. *Piwi* genes are relatively conservative in different species, but the function of *Piwi* gene within poultry species

remains unclear. In this study, *Piwi* gene were sequenced by using target sequence capture assay in *Quail* and 28 kinds of chicken breeds, then SNPs and evolutionary aspects for these different chicken breeds were analyzed. We found that SNP sites existed mainly in introns of a few chicken breeds, afterwards, we selected SNPs on intron 4 for further verification by Sanger sequencing. The results of Sanger sequencing was consistent with the results of target capture sequencing assays. Furthermore, the results of evolutionary analysis showed the following characteristics: the mutations of *Chahua* chicken and *Leghorn* (SPF) were relatively richer compared with other breeds. The phylogenetic tree was divided into four main categories. The classification of data suggested that the *Piwi* gene was evolutionarily conserved and mutations in intron might be associated with the phenotype of gametogenesis. The screened SNPs can be used as the candidate markers for *Piwi* gene. Collectively, these observed results provide basic information for further study of *Piwi* gene function in poultry.

P5049 Effects of natural resistance-associated macrophage protein 1 and toll-like receptor 2 gene polymorphisms to the post-weaning piglet survivability. Hye-sun Cho, Won Kim, Hyoim Jeon, HyeJeong Lee, Dinh Truong Nguyen and Chankyu Park (Department of Animal Biotechnology, Konkuk University)

To predict resistance or susceptibility to infectious diseases according to animal's genotypes of disease related genes could be important in animal breeding and husbandry. We evaluated the potential influence of the genetic polymorphisms of four immune related genes, porcine beta defensin 4 (pBD4), interferon-induced GTP-binding protein Mx1, natural resistance-associated macrophage protein 1 (Nramp1) and Toll-like receptor 2 (TLR2) on post-weaning piglet survivability in farms. We selected a single SNP satisfying the criteria of nonsynonymous substitutions and the minor allele frequency >0.1 from each gene, and perform PCR-RFLP. Initially 371 randomly collected Yorkshire x Landrace F1 piglets consisting of post-weaning survival (n=185) and non-survival groups (n=186) were genotyped for selected SNPs. Nramp1 and TLR2 showed differences in genotype frequencies between the two group ($P<0.000005$ and 0.001, respectively). For further confirmation of the results, 390 additional animals from a different farm were further genotyped for the makers. The results were consistent to the initial analysis, suggesting that the genetic polymorphisms of Nramp1 and TLR2 are likely to affect the post-weaning piglet survivability. Theodd ratiosbetween the beneficial and non-beneficial genotypes to piglet survivability were 4.88 and 1.72 for Nramp1 and TLR2, respectively. The allele and genotype frequencies of SNPs from five different breeds, Berkshire, Yorkshire, Duroc, Landrace and KNP with 20 animals for each breed showed the fixation of an allele for Mx1 and TLR2 in some breeds. Our results are consistent to previous studies reporting the possible association of Nramp1 and TLR related genes with defense against pathogen infection.

P5050 Preimplantation analysis of bovine embryos using the GeneSeek® Genomic Profiler™. Deanne Waine, Kim Lyons and Emily Piper (The University of Queensland)

The ability to apply selection pressure prior to embryo implantation has the potential to drastically reduce the generation interval and increase genetic gain. It also presents an opportunity to skew sex ratios in the resulting generation, screen out carriers of deleterious alleles, and potentially rank and sell embryos with genomic breeding values attached. The GeneSeek® Genomic Profiler™ (GGP) for beef cattle is a genotyping platform based on the Illumina Infinium® BeadChip technology and is a tool used widely in genomic selection programs

across the USA and Australia. A particular attraction of this platform is the ability to diagnose a range of inherited diseases and traits, including recessive lethal conditions and selective traits such as horn/poll status and tenderness profiles. The present study was undertaken to determine whether a GGP genotype could be generated from embryo biopsies. Embryos were flushed from the donor cows 7 days after insemination and 20-30 cells excised from the embryo using standard dissection biopsy techniques. The biopsies were removed directly into 10 ul of DNA extraction buffer in a 96-well plate and incubated on a thermocycler at 95℃ for 20 min. The first step in the Infinium genotyping process is a whole-genome amplification step which was undertaken in the 96-well plate into which the biopsy was removed. Following the whole genome amplification, the contents were transferred to a deep 96-well plate and the genotyping completed as per the manufacturer's instructions. Genotyping call rates ranged from 50% to 97% with all but three samples achieving a call rate of over 80%. For the samples above an 80% call rate we were able to estimate gender and genotype several recessive conditions and selective traits.

P5051 Diagnostic marker panels for polled in Australian beef cattle. Sean Corley (The University of Queensland), John Henshall (CSIRO Animal Food and Health Science), Bruce Tier (University of New England) and Emily Piper (The University of Queensland)

The northern beef herd in Australia is overwhelmingly genetically horned. Dehorning is routinely practised but raises several welfare concerns (including calf losses of up to 2%) particularly when it is undertaken in older animals. Breeding for polled is a desirable alternative. In 2013, two marker panels to diagnose poll status in cattle were released: one from a German group (Medugorac et al.,) and one from our group. The markers published by the German group included a 202 base pair deletion which was associated with polled in beef breeds of Celtic origin, and another pair of deletions that flank a five SNP haplotype and which are associated with polled in dairy breeds of European origin. The test released by our group for the Australian industry is a 10 locus microsatellite-based haplotype test. We tested the German markers across our Australian populations of horned and polled cattle with genotype information available from the Australian haplotype test and some progeny test data. Across the Bos taurus breeds tested (Hereford, Limousin, Shorthorn, Simmental, Charolais and Blonde d'Aquitaine) the German marker test was in agreement with the Australian test in the vast majority of cases, and the genotype reported for both tests was consistent with the phenotype recorded for the animals. These Australian beef populations carried both the Celtic and Dairy mutations, the frequency of which varied from breed to breed. There were many homozygous polled animals in these populations that were in fact heterozygous for both the Celtic and Dairy mutations. The results of the German test were consistent with the Australian marker test results in the majority of Bos indicus animals tested (Brahman, Santa-Gertrudis, Brangus and Droughtmaster), although there were some horned Brahman animals which carried the Celtic polled mutation, and some polled Brahmans which carried neither mutation.

P5052 Genome-wide scan for selection signatures in Chinese Merino and Kasak sheep. San-Gang He, Shu-Dong Liu, Lei Chen, Wen-Rong Li and Ming-Jun Liu (Key Laboratory of Genetics, Breeding and Reproduction of Grass Feeding Livestock of Ministry of Agriculture, Xinjiang Academy of Animal Science)

Merino sheep has distinguished wool characters after experiencing the strong artificial selection

for wool traits. In this study, genetic differentiation coefficient (FST) was used to identify genome selection signatures between Chinese Merino non-horned strain and coarse-wooled Kasak sheep. Total of 262 Chinese Merino and 160 Kasak sheep were genotyped by Illumina ovine SNP50BeadChip.After quality-control filtering of SNPs, We used 49425 SNPs to estimate FST parameterbyLOSITAN package. Three strong selection signals were observed on Chromosome 10, 13 and 25, respectively. In Chromosome 10, the peak signal spans *RXFP2* gene, known as candidate responsible for horn morphology of sheep. In Chromosome 13, the peak signal spans *BMP2* gene, which involves the regulation of hair shaft formation. Furthermore, in Chromosome 25 significant selection signals spanned the region consisting of *TARBP1*, *EIF2S2*, *IRF2BP2* and an uncharacterized gene were identified. Based on previous studies, a QTL related to fibre diameter was idebtified in this region, which inferred that these genes might be associated with wool traits. This study provides promising knowledge of the genetic selection on Merino sheep, which should be applied to develop novel genetic markers for wool traits.

P5053 Genome-wide association study for tick resistance in South African Nguni cattle. Ntanganedzeni Mapholi, Azwihangwisi Maiwashe and Lucky Nedambale (Agricultural Research Council), Michael MacNeil (Delta G & Agricultural Research Council), Valentina Riggio (The Roslin Institute and Royal (Dick) School of Veterinary Studies), Oswald Matika (Roslin Institute and Royal (Dick) School of Veterinary Studies), Jeremy Taylor (University of Missouri-Columbia) and Kennedy Dzama (University of Stellenbosch)

Ticks and tick-borne diseases are major constraints on beef cattle production in tropical and subtropical countries. South African Nguni cattle are adapted to harsh environments and are tolerant to tick and tick-borne diseases. The objective of this study was to explore variation in genetic resistance to ticks in Nguni cattle genotyped with the Illumina BovineSNP50 assay. Tick counts were collected for several endemic species (*Amblyomma hebraeum, Hyalomma marginatum, Rhipicephalus appendiculatus, Rhipicephalus Boophilus (decoloratus and microplus), and Rhipicephalus evertsi evertsi*), under natural challenge conditions, over a period of twelve months from 400 Nguni cattle in three provinces of South Africa. After quality control, 41,522 SNPs were used for the genome-wide association study (GWAS), fitting the fixed effects of sex, ranch, year of birth and the first three principal components. Relationships between animals was accounted for by using the G matrix in a univariate mixed model analysis. Several SNPs were identified as being associated with tick resistance, some of which were significant across different sampling times throughout the year. One SNP (on chromosome 12, rs 60527567) was significant at the Bonferroni genome-wide ($p<0.05$) threshold, whereas seven SNPs (on chromosomes 3, 8, 9, 10, 14, 20 and 27) were significant at the suggestive threshold (i.e., one false positive in a genome scan). Further analyses will be needed to confirm these results.

P5054 Allelic difference in binding affinity to the epitopes from pathogens by SLA-DQB1 and DRB1 may result in differences in post-weaning piglet survivability. Minh thong Le, Minkyung Choi, Kyung-Tae Kim, Hunduma Dinka, Hailu Dadi and Chankyu Park (Department of Animal Biotechnology, Konkuk University)

We performed a case-control study to evaluate the polymorphisms of two porcine MHC class II genes, SLA-DQB1 and DRB1 on the post-weaning survivability of piglets. We randomly collected tissues from 388 F1 animals

from Landrace-Yorkshire crosses comprised of 201 piglets with symptoms comparable to wasting diseases or disease like phenotypes including weight loss and emaciation, diarrhea, respiratory distress and sudden death and 187 healthy piglets. We successfully typed SLA-DQB1 and DRB1 exon 2 using a genomic DNA based high resolution SLA typing. A total of 16 and 18 alleles were identified from SLA-DQB1 and DRB1 each, resulting in 37 different DQB1 and DRB1 haplotypes. The haplotype 0101:0101 showed strong association to the piglets with wasting disease like symptoms (OR=4.88, p=8.31x10-9) and the haplotype 0701:0603Q to the healthy piglets (OR=0.39, p=8.33x10-5), suggesting that these alleles might have differences to induce immune responses against infected pathogens. We also analyzed the position and effects of each SNP sites of the alleles on the post-weaning survival rate of the pigs. A total of 43 and 83 SNPs were analyzed for DQB1 and DRB1, respectively. The results showed that phenotype related SNPs were located to the critical region of the antigen biding groove of MHC class II molecules. More interestingly, in-silico simulation analysis against the database which allows the prediction of the epitope binding affinity to MHC molecules showed that the favorable allele for post-weaning survivability showed the strong biding affinity to epitopes of viral pathogens such as PRRSV and PCV2. In contrast, the binding affinity of unfavorable allele to these epitopes was much weaker. In conclusion, the prediction of epitope binding to MHC alleles using the high resolution SLA class II typing indicated that the differences of the binding affinity to specific epitope by MHC alleles may contribute to difference in post-weaning piglet survivability.

P5055 Fine mapping the QTL related with body weight in outbred chicken advanced intercross lines. Yuzhe Wang and Xiaoxiang Hu (China Agricultural University)

Indigenous chickens in China not only have characteristic of high-quality meat, but also an effective model for genetic and evolutionary selection study. However, the growth rate of indigenous chickens is much lower than commercial chicken breeds, indicates that they have not reached their full economic value. Our research aims to mapping genes related with the growth rate of chicken. In our previous study, we generate F2 intercross which between the slow growing native broiler breed, Huiyang Beard chicken and fast growing commercial broiler breed, High Quality chicken Line A, and several highly significant QTLs of body weight on chicken chromosome 1 were reported in the unique F2 population. Here we focus on the F0 generation. According to the 70x screening genome sequencing data, we get high density of SNP marker information, insert and delect position. Advanced Intercross Lines (AIL) are also produced by repeated intercrossing of F2 animals, different allele frequency loci are used to genotyping and fine mapping the QTL regions in F8 generation. Selection sweep analysis including population genetic differences and heterozygosity analysis help us narrowed the genome region to ~1Mb and obtained several candidate genes.

P5056 Association analyses between SNPs and WSSV resistance of the white Pacific shrimp *Litopenaeus vannamei*. Fuhua LI, Jingwen Liu, Yang Yu, Xiaojun Zhang and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

Litopenaeus vannamei (*L. vannamei*)is the most important cultivated shrimp species with its productionoccupied nearly 80% of the world penaeid shrimp production. WSSV is one of the most dangerous pathogen that is highly virulent in penaeid shrimp. Genetic breeding of WSSV resistant shrimp is very important for the sustainable development of shrimp aquaculture industry. Molecular markers are very useful in

accelerating the breeding process for disease resistance traits. In the present study, SNPs were discovered from the transcriptomes of *Litopenaeus vannamei* generated by Illumina sequencing, and 96,040 SNPs were predicted by SNP calling including 5,242 non-synonymous SNPs and 29,129 synonymous SNPs respectively. SNPs existed in 242 immune unigenes including genes in immune signaling pathways and immnue effectors were selected and further confirmed in a new bred variety Kehai No.1. A total of 1644 SNPs were predicted and 681 SNPs of them were used to construct a database after alignments of their transcripts with genomic sequences. Association analyses between SNPs and WSSV resistance were performed between WSSV susceptive and resistant shrimp. Loci in unigene30237 (STAM), unigene15411 (TLR) and unigene16729 (TRAF6) were significantly associated with resistance to WSSV in alleles and/or genotypes (P<0.05). Frequencies of CT genotype of unigene26970 (Hemocyanin), AA of unigene34569 (Cu/Zn SOD), CT and T allele of unigene34129-1 in resistant group were much higher than in susceptive group (P<0.05). Furthermore, SNPs in 6 ALF genes (nLvALF1-6) were analyzed and partial SNPs were selected for genotyping. Loci g.1361-T>C, g.1370-T>C, g.1419-T>A of *nLvALF1*, g.2422 A>G, g.2466 T>C, g.2529 G>A of *nLvALF2* and g.2994 T>A of *nLvALF6*were also associated with WSSV resistance. The specific haplotype CT consisted of g.1415-C>A and g.1419-T>A in *nLvALF1* had significant association with WSSV-resistant trait of Kehai No.1. These data will provide important information for selective breeding of WSSV resistant shrimp in the future.

P5057 A novel PCR RFLP for detection of Single Nucleotide Polymorphism in exon1 of *GPR54* in goats (*Capra hircus)*. Radhika G, Raghavan C, Stephy Thomas, Aravindakshan V and Thirupathy Venketachalapathy (Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, India)

Kiss peptins , the peptide products of *Kiss 1* gene act as ligands of the G-Protein Coupled Receptor 54 gene [*GPR54*] otherwise termed as *Kiss 1 R*. Several experimental studies in human and mice have confirmed the role of *Kiss 1* and *GPR54* in reproductive maturation and function. It is now proven beyond doubt that *GPR54* is required for normal functioning of hypothalamic-pituitary-gonadal axis, probably at the level of gonadotropin releasing hormone secretion. Studies on *GPR54* in goats were very few and hence an effort was made to explore the polymorphism of exon 1 of *GPR54* in two native goat breeds of Kerala, India – Malabari and Attapady Black. These two breeds showed definite difference in reproductive function as Malabari goats had the advantage of high prolificacy with higher twinning percentage. Age at first kidding was also significantly lower in Malabari goats compared to Attapady Black. Amplicon of 250 base pair enclosing exon 1 of *GPR54* was obtained from genomic DNA isolated from blood of goats. BLAST analysis of sequenced amplicons from Malabari and Attapady goats revealed a mismatch C>T 100. (Accession no: KF 533109.1, KF 533108.1) This mutation resulted in the disappearance of recognition site ACGGC for the restriction enzyme *BceA1*. Hence a new Restriction Fragment Length Polymorphism was designed using *BceA1* which resulted in three genotypes- GG [111,139] , GP [250,111,139] and PP [250]. Gene frequencies of G and P alleles were 0.75 and 0.25 in Malabari goats and 0.41 and 0.59 in Attapady Black goats. Only 10% of Malabari goats showed PP genotype whereas 81.8 % of Attapady Black goats revealed GP and PP genotypes. Thus a novel PCR RFLP assay was designed for detection of Single Nucleotide Polymorphism in 100th position of exon 1 of *GPR54 in* goats.

P5058 Continuing the Search for Genomic Regions Associated with Polycerate Traits in

Jacob and Navajo-Churro Sheep. Tracy Hadfield (Utah State University), James Kijas (CSIRO Livestock Industries) and Noelle Cockett (Utah State University)

A genome-wide association study (GWAS) is underway to identify the genetic cause for unique traits in Navajo-Churro (NC) and Jacob (J) sheep. Both breeds have polycerate (multi-horned) animals where the number of horns ranges from one to six. A split upper eyelid deformity (SUED) is also present in these breeds. Genomic DNA was collected from 37 Navajo-Churros and 106 Jacob sheep and genotyped with the Illumina Ovine Infinium HD SNP BeadChip. Horn type ranged from polled to five horns and scurs (incomplete bone growth) were also present. The polled trait has been previously mapped to the *RXFP2* gene, ovine chromosome 10 (OAR 10). In a preliminary analysis of 60 samples (NC=36, J=24) comparing polled (n = 10) and horned (n = 22), an association signal was identified on OAR10 in the region of the *RXFP2* gene. An analysis of two (n = 7) and four (n = 21) horns returned a significant region on OAR 1 (142 Mb). Ten animals in the subset demonstrated at least one eye with SUED and eight of the ten animals (80%) had at least one scur. Interestingly, the polled condition and an atypical eyelash-and-eyelid phenotype are in perfect association in cattle. We present GWAS findings based on the full set of animals that extend the previously reported preliminary analysis.

P5059 Reproductive traits for Holstein cattle. ashraf ward (TRIPOLI UNIVERISTY)

Data consisting of 2757 records from ten Holstein herds made between 2000 and 2010 were used to examine environmental factors affecting age at first calving (AFC) and calving intervals (CI) and consequently estimate genetic and phenotypic parameters and trends.The overall means and standard errors for AFC and CI were 39.4 ± 7.2 months and 487.5 ± 151.6 days respectively. The respective heritability estimates were 0.091 ± 0.05 and 0.044 ± 0.032, while the repeatability estimate for CI was 0.096 ± 0.001. The genetic trends for CI and AFC were -0.6d/yr and -0.01mo/yr respectively and were both significant (P<0.001), indicating a decrease in mean breeding value over the study period. Phenotypic trends were -0.31 mo/yr and -0.35 d/yr for AFC and CI respectively though non-significant (P>0.05). The low heritability for CI and AFC indicated that temporary environmental influences were much greater than genetic influences or permanent environmental influences on these traits.

P5060 A high resolution Copy Number Variant (CNV) scan based on Illumina's 777k chip in the autochthonous Italian Valdostana Red Pied cattle population. Alessandro Bagnato, Maria Strillacci, Fausta Schiavini and Erika Frigo (Università degli Studi di Milano), Antonia Samoré (Università degli Studi di Bologna), Raphaelle T. M. Prinsen and Maria Cozzi (Università degli Studi di Milano), Luca Fontanesi (Università degli Studi di Bologna) and Marlies Dolezal (University of Veterinary Medicine)

CNVs are an important source of genomic structural variation, recognized to cause phenotypic variation in many mammalian species. Recently CNVs have been mapped also in several cattle populations including the Brown Swiss where they have been shown to be located also in relevant gene families such as the MHC (BOLA) locus. Here we report on a high resolution CNV scan from log R ratio (LRR) data on Illumina's 777k BovineHD beadchip for 143 Valdostana Red-Pied bulls. Valdostana Red-Pied, an autochthonous Italian dual purpose cattle population, did not undergo strong selection for production traits as compared to Holstein or Brown Swiss and can deliver valuable information on structural variation and its association with complex (health) traits in a

non-cosmopolitan cattle breed. After stringent quality control and filtering (derivative LRR spread, genomic wave correction) CNVs were called across 108 bulls using the multivariate option in the copy number analysis module of SVS v8.1.0 (Golden Helix). No batch correction was necessary as all eigenvalues in a PCA were below 0.25. We identified a total of 1499 CNVs in 108 sires that were summarized to 46 CNV regions on 16 autosomes covering a total of ~3.5 Mb of the UMD3.1 autosome. This is a conservative estimate as the multivariate approach employed has higher power for intermediate to high frequency CNVs and a reduced false positive rate as compared to univariate analysis (e.g PennCNV-software) at the expense of missing potential low frequency variants. CNVs found here confirm regions identified on BTA12 (70-75 Mb) and 23 (MHC region) in the Italian Brown Swiss and in orthologous regions in Capra hircus, Ovis aries and Sus scrofa. CNVs ranged from 0.5kb to 773 Kb. Data was generated as part of the FP7 project QUANTOMICS contract n. 222664-2.

P5061 Ubiquitin B gene as biomarker in horse sperm motility expression studies. Almudena Perez-Rico (Laboratorio de Investigacion Aplicada), Francisco Crespo (Centro Militar de Cria Caballar de Avila), Lourdes Sanmartin-Sanchez (Laboratorio de Investigacion Aplicada), Alvaro De Santiago (Centro Militar de Cria Caballar de Ecija) and Jose Luis Vega-Pla (Laboratorio de Investigacion Aplicada)

The ubiquitin system has a central role in the key events in acrosome formation, nuclear condensation, intracellular membrane trafficking and paternal mitochondrial elimination. Ubiquitin is also expressed in the epididymis and preferentially binds to the defective spermatozoa.Frozen sperm straws derived from 12 stallions were randomly selected from a germplasm bank. Velocity straight Line (VSL), Velocity average Path (VAP) and Velocity Curvilinear (VCL) were measured and Straightness (STR) as VSL/VAP and Wobble (WOB) as VAP/VCL were calculated by means of the computerized ISAS® (Integrated Semen Analysis System). Thereafter, semen samples were divided into two groups (1 and 2 respectively) according to Straightness (STR) (good >72%; poor < 72%;) and to Wobble (good <66%; bad>66%). A raw semen sample (500 µl) was gently layered over the top of 1.5 ml 40% silanized silica particle solution and centrifuged at 10,000 g during 15 sec in order to separate the mature spermatozoa from other somatic cells and immature diploid spermatozoa. Spermatozoa pellets were diluted up to a concentration of 0.5 x 106 spermatozoa/ul to be used for RNA extraction. Intron-spanning mRNA specific primers for Horse Ubiquitin B (UBQ) were designed and RT-qPCR was carried out using a One-Step RT-PCR protocol. β-actin (ACTB) gene was amplified as reference gen. All relative quantification was assessed using REST software 2009, with PCR efficiencies calculated by Rotor-Gene-6000 software v.1.7. There was a regular pattern of expression for the two STR and WOB phenotypes for the UBQ gene showing a downregulation in the group 1 in both cases, indicating that UBQ is decreased in spermatozoa with good motility. The results of the present study suggest that UBQ could be used as biomarker of equine spermatozoa motility.

P5062 A high resolution Copy Number Variant (CNV) scan based on Illumina's 777k chip in the autochthonous Italian Valdostana Red Pied cattle population. Alessandro Bagnato, Maria Strillacci, Fausta Schiavini and Erika Frigo (Università degli Studi di Milano), Antonia Samoré (Università degli Studi di Bologna), Raphaelle T. M. Prinsen and Maria Cozzi (Università degli Studi di Milano), Luca Fontanesi (Università degli Studi di Bologna) and Marlies Dolezal (University of Veterinary

Medicine)

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P5063 Ubiquitin B gene as biomarker in horse sperm motility expression studies. Almudena Perez-Rico (Laboratorio de Investigacion Aplicada), Francisco Crespo (Centro Militar de Cria Caballar de Avila), Lourdes Sanmartin-Sanchez (Laboratorio de Investigacion Aplicada), Alvaro De Santiago (Centro Militar de Cria Caballar de Ecija) and Jose Luis Vega-Pla (Laboratorio de Investigacion Aplicada)

The ubiquitin system has a central role in the key events in acrosome formation, nuclear condensation, intracellular membrane trafficking and paternal mitochondrial elimination. Ubiquitin is also expressed in the epididymis and preferentially binds to the defective spermatozoa.Frozen sperm straws derived from 12 stallions were randomly selected from a germplasm bank. Velocity straight Line (VSL), Velocity average Path (VAP) and Velocity Curvilinear (VCL) were measured and Straightness (STR) as VSL/VAP and Wobble (WOB) as VAP/VCL were calculated by means of the computerized ISAS® (Integrated Semen Analysis System). Thereafter, semen samples were divided into two groups (1 and 2 respectively) according to Straightness (STR) (good >72%; poor < 72%;) and to Wobble (good <66%; bad>66%). A raw semen sample (500 µl) was gently layered over the top of 1.5 ml 40% silanized silica particle solution and centrifuged at 10,000 g during 15 sec in order to separate the mature spermatozoa from other somatic cells and immature diploid spermatozoa. Spermatozoa pellets were diluted up to a concentration of 0.5 x

106 spermatozoa/ul to be used for RNA extraction. Intron-spanning mRNA specific primers for Horse Ubiquitin B (UBQ) were designed and RT-qPCR was carried out using a One-Step RT-PCR protocol. β-actin (ACTB) gene was amplified as reference gen. All relative quantification was assessed using REST software 2009, with PCR efficiencies calculated by Rotor-Gene-6000 software v.1.7. There was a regular pattern of expression for the two STR and WOB phenotypes for the UBQ gene showing a downregulation in the group 1 in both cases, indicating that UBQ is decreased in spermatozoa with good motility. The results of the present study suggest that UBQ could be used as biomarker of equine spermatozoa motility.

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P6001 The International Swine Methylome Consortium (ISMC): Supporting epigenomics and biomedical research. Kyle Schachtschneider and Laurie Rund (University of Illinois), Ole Madsen (Wageningen University), Rod Johnson (University of Illinois), Alan Archibald (University of Edinburgh), Chankyu Park (Konkuk University), Martien Groenen (Wageningen University) and Lawrence Schook (University of Illinois)

Pigs (*Sus scrofa*) are an important biomedical model due to their anatomical, behavioral, genetic and physiological similarities with humans, as well as their availability, short generation interval and large litter size. Studies using pigs have been shown to be more predictive of therapeutic treatments in humans than rodent studies, and are currently being used to study a variety of human diseases including Huntington's, Alzheimer's and cardiovascular diseases. DNA methylation is an epigenetic mark that occurs at CpG sites throughout the genome, and altered methylation levels are associated with aberrant gene transcription. Furthermore, DNA methylation represents a link between genetics and environmental signals that has been reported to play an important role in human pathologies including cancer and neurological disorders, revealing the importance of accessing DNA methylation patterns in understanding disease development. The International Swine Methylome Consortium (ISMC) was created to produce a porcine methylome map in order to enhance studies of DNA methylation patterns and their association with the development and detection of relevant human diseases. Reduced representation bisulfite sequencing and RNAseq libraries are being used to target CpG islands and assess gene transcription for biomedically relevant tissues and developmental time points. In a preliminary exploratory study, eight tissue samples (from the adult female Duroc (2-14) utilized for the pig genome project) were analyzed. Additional samples identified for development of the swine methylome map will consist of biomedically relevant tissue samples from Yucatan, Sinclair, Minnesota and NIH mini-pig breeds, as well as domestic breeds. Breeds were chosen based on their availability and use by biomedical researchers. The tissue types and developmental time points were prioritized to ensure a high quality methylome map of broad utility for the biomedical community. These resources will facilitate future biomedical research in pigs, ensuring they remain an important human disease model.

P6002 Higher methylation of the *JAK2* promoter is associated with depressed gene expression in healthy controls and vice versa in mastitis dairy cattle. Tahir Usman, Yachun Wang, Xiao Wang, Chao Liu, Qin Zhang and Ying Yu* (Corresponding author) (China Agricultural University)

Mastitis is the most prevalent inflammatory disease of economic concern in dairy cattle. Janus Kinase 2 (*JAK2*) is extensively involved in a variety of inflammatory conditions in different species and is an integral part of JAK-STAT signaling pathway. The objective of the present study was to evaluate the methylation levels of *JAK2* promoter CpG methylation in peripheral blood cells using pyrosequencing assay and to analyze the gene expression by quantitative real time PCR in healthy control and mastitis Chinese Holstein cows. Student T test was used to analyze the differential methylation levels and mRNA expression of *JAK2* gene between healthy and mastitis cows. The results showed that out of 9 CpG sites in the *JAK2* promoter, 6 CpG sites were significantly highly methylated in healthy cows compared to the mastitis ($P<0.05$). Four active transcription factors (SRY, c-Rel, MZF1 and Sp1) were predicted to be present on the CpG sites of the bovine *JAK2* gene. The gene expression results indicated that the mRNA expression of *JAK2* was significantly higher in mastitis cows compare to that in healthy cows

(*P*<0.05). These results suggest that higher methylation in healthy control was associated with depressed gene expression of *JAK2* and vice versa in mastitis cattle. The differential methylation and gene expression of *JAK2* in healthy control and mastitis cows, and the negative correlation between methylation and expression of *JAK2* imply that DNA methylation in *JAK2* could play essential role in mastitis resistance in dairy cattle. Key words: *JAK2*; Promoter DNA methylation; mastitis; Peripheral blood cell. Acknowledgments: This project was financially supported by the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-37), the National Natural Science Foundation of China (31272420) and the National Key Technologies R & D Program (2011BAD28B02).

P6003 Allele Specific Expression in Chicken Lungs Following Avian Influenza Virus Infection Detected Using RNA-Seq. Ying Wang, Perot Saelao and Zhenhua Zhao (University of California, Davis), Blanca Lupiani and Reddy Sanjay (Texas A&M University), Susan Lamont (Iowa State University) and Huaijun Zhou (University of California, Davis)

Avian influenza virus (AIV) infection not only can cause significant economic losses to the poultry industry, but also raise a great public health threat to humans. To develop more effective intervention strategies, it is essential to elucidate molecular mechanisms of host response to AIV infection in chickens. The objective of this study was to identify allele specific differentially expressed genes associated with AIV infection in chickens. An F1-cross of two genetically distinct, highly inbred chicken lines (Fayoumi, resistant, and Leghorn, susceptible to AIV infection) was used. Three-week old chickens were inoculated with 107 EID50 of low pathogenic H5N3 AIV, and lungs were harvested 4 days post inoculation. Eight cDNA libraries (4 libraries each from infected and non-infected birds) were prepared and sequenced by Illumina HiSeq 2000, which yielded 312 million 100 bp paired-end reads. Gene expression levels of all annotated chicken genes were analyzed using the CLC Genomics Workbench. Regions identified as heterozygous were used to estimate allele specific expression by comparing the relative ratios of uniquely mapped reads containing each variant in a heterozygous sample. Allelic imbalance (AI) was identified with statistically significant deviation from an expected 50:50 ratio (Chi-square $P < 0.05$) and with a frequency abundance cut off of greater than 60%. Of 26,715 SNPs, 1,700 with AIs were observed, in which 657 and 1024 AIs were unique to infected and non-infected birds, respectively. TLR4 was up-regulated with AIV infection and one SNP on TLR4 had the AI ratio of 76:24 in infected and 24:76 in non-infected birds, in which allele from Fayoumi had higher expression than from Leghorn with AIV infection. Further investigation of the roles of these candidate genes in the regulation of host-AIV interaction can lead to new directions for the development of anti-viral drugs or vaccines in poultry.

P6004 Genome-wide linkage disequilibrium linkage analysis (LDLA) of obesity and obesity-related traits in an F2 porcine model for human obesity. Sameer Pant, Peter Karlskov-Mortensen, Susanna Cirera, Lisette Kogelman and Mette Jacobsen (University of Copenhagen), Camilla Bruun (University of Cobenhagen), Claus Jørgensen (University of Copenhagen), Theo Meuwissen (University of Life Sciences) and Haja Kadarmideen (University og Copenhagen)

Animal models of high comparative value are essential for unraveling the complex genetic architecture behind obesity. We have established an F2 resource population for the purpose of elucidating the molecular pathogenesis of obesity and obesity related diseases using an obesity prone breed and breeds selected for leanness in

the parental generation. To establish the possibility of exploiting differences in LD between breeds purebred Duroc and Yorkshire sows were crossed with Göttingen minipig boars to obtain two separate F2 intercross populations (n=285 and 277 respectively). Several obesity, metabolic and slaughter measurements were recorded from birth to slaughter (220 ± 45 days). In addition, body composition was determined at about two months of age (64 ± 11 days) via dual-energy x-ray absorptiometry (DXA) scanning. All pigs were genotyped using Illumina Porcine 60k SNP Beadchip and a combined LDLA approach was used to perform genome-wide linkage and association analysis for obesity and obesity-related traits. Subsequently bioinformatic analysis was performed to identify genes in close proximity of chromosomal positions where statistically significant QTLs were identified. Several important genes previously linked to obesity along with other novel genes were identified, that together provide novel insights that may further the current understanding of the molecular mechanisms underlying human obesity and obesity related diseases.

P6005 Clenbuterol inhibits C2C12 cell proliferation by delaying P27 degradation. Min Chen and Qingyong Meng (China Agricultural University)

Skeletal muscle development contains embryonic myogenesis and postnatal development. It has been widely accepted that Clenbuterol (CLB) enhances postnatal muscle growth mainly by promoting protein anabolism and preventing protein catabolism. But the effects of CLB on proliferation of myoblasts, which is critical for embryonic myogenesis and regeneration, have not been thoroughly studied. Here we demonstrated that CLB caused an inhibition in proliferation of C2C12 cells partly via increasing P27 protein. But P27 mRNA level was not altered after CLB administration by real-time PCR. CLB administration was found to inhibit P27 protein degradation when adding cycloheximide to the cell culture medium. Furthermore, An accumulation of P27 protein in the nucleus companied a decline in cytosolic were found in CLB-treated cells by Western blot. Finally, the regulation of P27 by CLB was not altered by adding PKA inhibitor H-89 or β2-adrenoceptors antagonist ICI to the cell culture, suggesting that the regulation of P27 by CLB was mediated in a PKA- and β2-adrenoceptor-Gas independent way. Together, our findings suggested that CLB promoted P27 nucleus accumulation, leading cellular proliferation inhibition. Our data may provide a new view of CLB treatment in the muscle growth and regeneration.

P6006 Genetic variation in microRNAs' seed regions lead to different susceptibility to HP-PRRSV infection across breeds. Jia Li and Dan Cui (State Key Laboratory for Agrobiotechnology) and Ning Li (State Key Laboratory for Agrobiotechnology,China Agriculture University)

Porcine reproductive and respiratory syndrome (PRRS) is a world-wide, intractable and most damaging contagious swine disease. It is caused by a small positive single-stranded RNA virus - porcine reproductive and respiratory syndrome virus (PRRSV). The difference in susceptibility to PRRS is observed across different pig breeds. However, little is known about the underlying mechanisms about that genetic background contributes to the different susceptibility. Herein we compared the pathogenicity (survival time, body temperature, food intake and virus load) of HP-PRRSV in Tongcheng (China domestic breed) and Landrace pig (commercial breed), and found that these two pig breeds showed distinct susceptibility to PPRS. We also performed deep transcriptome analyses of lung tissues from these two pig breeds before and after infection with HP-PRRSV. Transcriptome analyses showed that

there were 1288 genes with significantly altered expression (DEGs) at 3, 5, 7 d after inoculation in Tongcheng which is less than half of Landrace (2746) when compared to control individuals. However, the DEGs (2746) were marked larger magnitude of expression change than that of DEGs (1288) in Tongcheng. Further analyses suggested that such marked difference in the gene expression change between the two breeds might be attributed to microRNA regulation. This was supported by the different miRNA-mRNA pairs related to PRRSV infection between the two breeds and the detection of breed-specific SNPs located in a conserved seed sequence in 3`UTR region of DEGs in Tongcheng. Moreover, by integrating the co-expression network analysis, we identified six anti-PRRS genes (*BID, BTG2, SOD1, BCL-XL, MX1* and *IFIT1*) and all of them could significantly inhibit the replication of PRRSV *in vitro*. These transcriptome analyses provide an important resource for characterizing molecular mechanism of genetic variation in microRNAs or microRNA's seed sequences to difference in susceptibility to PRRS.

P6007 Overexpression of porcine *HDAC6* gene lead to genetic resistance to PRRSV. Zhiyuan song and Tianyu Lu (China Agricultural University), Kegong Tian (China Animal Disease Control Center) and Ning Li (China Agricultural University)

Porcine reproductive and respiratory syndrome virus (PRRSV) is a type of virus that causes great loss to the pig industry over the world. Great progress has been made, more need to be done before total elimination of the disease. In this research, we set out to establish a new breed of pigs that could be resistant to the virus. Histone deacetylase 6 (HDAC6) is a distinctive member of the histone deacetylase family. HDAC6 mainly locates in the cytoplasm and regulates many important biological processes including cell migration, immune synapse formation, and viral infection in cultured cells. It remains elusive whether overexpression of *HDAC6* actually can help resist virus infection in animals.

In this research, we generated transgenic pigs that overexpressed *HDAC6* and examined their antiviral ability by virus challenge both *in vivo* and *in vitro* according to standard protocols. The transgenic pigs showed the germ line transmission of the overexpression of HDAC6 proteins from the founders to the next generation. Our results demonstrated that the *HDAC6* transgenic pigs exhibited enhanced resistance to PRRSV infection both *in vitro* and *in vivo*. After PRRSV infection, virus load of the PAMs (Porcine Alveolar Macrophages) of *HDAC6*-TG pig was significantly lower than that of non-transgenic pig. In the *in vivo* experiments, *HDAC6*-TG pigs had lower virus titer, delayed respiratory symptoms and longer survival days when compared to Non-TG pigs post PRRSV infection. In summary, overexpression of porcine *HDAC6* gene could help protecting pigs from PRRSV infection by inhibiting its invasion and replication. Therefore, this strategy may spark new inspiration of antivirus in animals.

P6008 Inhibition of influenza A virus replication by influenza A virus ploymerase. Tianyu Lu and Xiaojuan Liu (State Key Laboratories for Agrobiotechnology, College of Biological Sciences, China Agricultural University), Ye Shen (Key Laboratory of Animal Epidemiology and Zoonosis,Ministry of Agriculture,College of Veterinary Medicine,China Agricultural University) and Meng Wang and Ning Li (State Key Laboratories for Agrobiotechnology, College of Biological Sciences, China Agricultural University)

Influenza A virus(IAV) can infect a wide range of animal species including humans and animals. Previous studies have shown that all gene segments of influenza virus share a common feature at their termini. Furthermore, the exogenous terminal conserved sequence was able to competitively inhibit influenza A virus

polymerase activity. And the terminal sequence play a major role in transcription, replication. Here, we developed an influenza A virus decoy system(IVDS) that transcribed the RNA flanked by the 5' and 3' UTR of IAV, serving as a competitive inhibitor to suppress the virus replication. Meanwhile, a IAV-restraining protein was expressed rapidly by the RNA ploymerase of influenza virus which further destroy the virus replication. In this study, we constructed a series of IVDS plasmids which could transcribe virus RNA-like sequence encoding reporter gene and Mx respectively. The expression of reporter gene and Mx could be detected *in vitro* when co-transfected the cells with the plasmids expressing influenza A virus polymerase subunit proteins and nucleoprotein. DF-1 cells transfected with the plasmids over-expressing Mx, expressing Mx of IVDS, transcribing the terminal sequence of IAV were infected with H5N1 respectively. Our data shown,the cells transfec ted with IVDS plasmid that transcribe Mx in negative sense flanked by regions of AIV were able to inhibit the replication of IAV more efficiently than the cells which could only transcribe the terminal regions of AIV, not so efficiently as that of overexpressing Mx protein though. Our results suggested that the IVDS plasmid could suppress AIV replication to some extent. However, the efficiency of this system needs to be improved. Based on our current data, we believe that IVDS could be a potential approach to breed genetically modified influenza A viral-resistant livestocks.

P6009 Genome-wide analysis to identify genes responsible for Ankyloglossia in Kangal Dogs. Bengi Cinar Kul (Ankara Universitesi Veteriner Fakultesi Genetik Anabilim Dali Diskapi Ankara), Naci Ocal (Kirikkale Universitesi Veteriner Fakultes Ic Hastaliklari Anabilim Dali Kirikkale), Nuket Bilgen, M. Yenal AKKURT and M. Cevat TEMIZKAN (Ankara Universitesi Veteriner Fakultesi Genetik Anabilim Dali Diskapi Ankara), Nurten Akarsu (Hacettepe Universitesi Tıp Fakultesi Tibbi Genetik Anabilim Dali Gen Haritalama Laboratuarı Sihhiye Ankara) and Okan Ertuğrul (Ankara Universitesi Veteriner Fakultesi Genetik Anabilim Dali Diskapi Ankara)

Ankyloglossia is a congenital anomaly that prevents tongue movement because of the presence of a short and thick lingual frenulum anchoring the tip of the tongue to the floor of the mouth. Evidence for a genetic basis for Ankyloglossia in animals has yet to be established, however, all previously reported Ankyloglossia cases in dogs are restricted only to the Kangal breed. This strongly suggests a hereditary basis for Ankyloglossia in dogs and by analogy also in human. In this study, the first phenotypic characterization and genotyping were performed in 5 affected and 4 non-affected Kangal dogs. For the genotypic evaluation, a genome-wide analysis was performed using 50K SNPs (Affymetrix, GeneChip Canine SNP Array, V2). SNP data were visualized using Visual Genome Studio (VIGENOS) and homozygous haplotype blocks were searched in the affected dogs under the assumption of autosomal recessive inheritance. However, no conserved homozygous blocks among all affected animals were suggested the presence of a potentially more complex mode of inheritance. At this regard a new study was designed to do gene mapping by the evaluation of 170K SNPs (Illumina, CanineHD Bead Chip array) data for more comprehensive pedigree-based analysis utilizing high-resolution SNP arrays scored in a larger sample of dogs. This study is a first attempt towards the discovery of the gene or genes responsible for Ankyloglossia in dogs in addition to being the first example of a genome-wide analysis applied to the dog genome in Turkey.*This study was supported by TUBITAK (TOVAG-109O855 and 112O844).

P6010 Gga-miR-181a and its target *MYBL1* gene were implicated in chicken Marek's disease lymphoma transformation. Ling Lian,

Xin Li, Chunfang Zhao, Lujiang Qu and Ning Yang (Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China)

Marek's disease (MD) is caused by Marek's disease virus (MDV). It is lymphoproliferative neoplastic disease of the chicken, which causes great damage to poultry health. One class of non-coding RNA, microRNA has been reported to be involved in Marek's disease lymphomagenesis. Our previous study showed that gga-miR-181a was down-regulated in MDV-induced lymphoma and its target gene *MYBL1* was predicted. In this study, we found that *MYBL1* showed a completely opposite expression pattern with gga-miR-181a in MD lymphoma. *MYBL1* gene was up-regulation in MD lymphoma than that in non-infected spleens. The subsequent experiment was performed to investigate interaction between gga-miR-181a and *MYBL1* gene by detecting mRNA and protein level of *MYBL1* in MD tumor cell line, MSB1, after miR-181a mimics or inhibitor transfection. The results showed that *MYBL1* was up-regulated in inhibitor group at 48 hours post transfection compared with negative control and mimic groups. The protein level of*MYBL1* was slightly reduced in mimics group at 96h post transfection, which verified the interaction of gga-miR-181a and *MYBL1* gene. The results concluded that both gga-miR-181a and *MYBL1* might play important roles in MD lymphoma transformation.

P6011 A genome-wide association study on copy number variation for umbilical hernia in swine. Yi Long, Ying Su, Jun Ren, HuaShui Ai, ZhiYan Zhang and Bin Yang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University), GuoRong Ruan (Fujian vocational college of Agriculture) and ShiJun Xiao, NengShui Ding and LuSheng Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University)

Umbilical hernia is one of the most common congenital defects in pigs, leading to considerable economic loss and serious animal welfare problem. To investigate whether copy number variations (CNVs) contribute to pig umbilical hernia (UH), we performed a case-control genome-wide CNV association study using the Porcine SNP60 SNP data and PennCNV algorithm in 905 pigs from Duroc, Landrace and Large White breeds. We constructed a genomic map containing 6193 CNVs pertaining to 737 CNV regions (CNVRs), and found 20 CNVs associated with the risk for umbilical hernia in the three pig breeds. Six of seven significantly associated CNVs randomly chosen from the 20 CNVs were validated using quantitative real-time PCR (qPCR). A rare CNV (CNV14:13030843-13059455) encompassing the *NUGGC* gene, was strongly associated with umbilical hernia (Permutation-corrected p=0.0015) in Duroc pigs. Notably, this CNV occurred exclusively in 7 Duroc affected individuals and SNPs surrounding the CNV did not show association signals, indicating that rare CNV may play an important role in pig complex diseases like umbilical hernia. The *NUGGC* gene has been implicated in human omphalocele and inguinal hernia. Our finding supports that *NUGGC*CNV may contribute to the pathogenesis of pig umbilical hernia.

P6012 RNA interference in transgenic cloned swine led the resistance to foot-and-mouth disease virus. Wenping Hu (China Agricultural University), Haixue Zheng (Lanzhou Veterinarian Research Institute, the Chinese Academy of Agricultural Sciences), Qiuyan Li, Yuhang Wang, Wenjie Liu, Shen Liu and Wenhai Feng (China Agricultural University), Xiangtao Liu and Xuepeng Cai (Lanzhou Veterinarian Research Institute, the Chinese Academy of

Agricultural Sciences) and Ning Li (China Agricultural University)

Foot-and-mouth disease virus (FMDV) is a RNA virus, naturally infects swine, cattle and other cloven-hoofed animals, causing an acute disease. Due to the viral high mutant rate vaccines against FMDV do not work effectively. The mechanism of RNA interference (RNAi) as a functional antiviral pathway in mammalian cells was just confirmed. Here, we generated FMDV-specific shRNA transgenic cells targeting against either nonstructural protein 2B or polymerase 3D of FMDV. The shRNA transgenic positive cells had stable shRNA expression and displayed significant lower viral production than that of the control cells after infected with FMDV ($p < 0.05$). Ten transgenic cloned swine (TGCS) and five non-transgenic cloned swine (Non-TGCS), which were produced by somatic cell nuclear transfer (SCNT), were used in FMDV challenge studies. At the challenge dose of the 100 SID50, one TGCS was completely protected while all the normal control swine (NS) developed clinical signs. Mean onset of lesions and the mean days for developing severe lesions in the other four TGCS swine were delayed than that of NS ($P < 0.05$). At the dose of 10 SID50, one TGCS recovered after showing clinical signs for two days, while all Non-TGCS developed FMD and one swine died by 5 d.p.c.. Viral RNA load in blood and tissues of TGCS was reduced in both challenge dose study. These results indicated that TGCS displayed resistance to the infection of FMDV. Immune cells including CD3+, CD4+, CD8+, CD21+, and CD172+ type cells and the production of IFN-γ were analyzed and there were no significant difference observed between TGCS and NS or Non-TGCS, suggesting that the FMDV resistance may mainly derived from the RNAi-based antiviral pathway. Our work provided a foundation for the breeding approach of anti-infectious disease in swine.

P6013 Oviduct-specific expression of human neutrophil defensin 4 in transgenic chickens using lentiviral vectors. Tongxin Liu (China Agricultral University) and Hanyu Wu, Dainan Cao, Ning Li and Xiaoxiang Hu (China Agricultural University)

Oviduct-specific expression of recombinant proteins in transgenic chickens is a promising technology for producing therapeutic proteins in eggs. Human neutrophil defensin 4 (HNP4) is a kind of antimicrobial peptides which is able to inhibit bacteria, fungoid and HIV virus. In this study, we constructed a lentiviral vector encoding an expression cassette for HNP4. Firstly antimicrobial activity of the recombinant HNP4 protein was tested at the cellular level. Then the 2.8-kb ovalbumin promoter containing both the steroid-dependent regulatory element (SDRE) and the negative regulatory element (NRE) was used to express HNP4 specifically in transgenic chickens by lentivirus-mediated approach. Genetically manipulated chickens were generated by injecting lentivral vectors encoding HNP4 gene into developing embryos (stage X). Out of the 695 injected eggs, 182 chickens successfully hatched. Ten roosters in which semen were identified as positive for transgene by PCR were mated with wild type hens. Fifteen F1 positive transgenic chickens from 1274 offsprings were obtained, which was confirmed by PCR and Southern blot. The Genome Walking indicated that a single copy of HNP4 gene was integrated into chromosome 1, 2, 3, 4 and 6 of transgenic chickens, respectively. Two transgenic F1 roosters were further mated with wild type hens, and the proportion of transgenic and non-transgenic F2 offsprings was 1:1. The egg white will be assayed by ELISA. These results indicated that the HNP4 gene was stably integrated into the chicken genome.

P6014 A Genetical Mouse Model for PRRSV Infection. Linlin Zhang, Li Li and Ning Li (China Agriculture University)

Porcine reproductive and respiratory syndrome (PRRS) is an economically devastating viral disease caused by PRRS virus (PRRSV). Antiviral treatments and effective vaccines have been hampered by the paucity of a suitable small animal model for PRRSV infection. PRRSV has been previously shown to use pig CD163 (pCD163), pig CD169 (pCD169) and simian CD151 (sCD151) as cellular receptors. Building on these findings, we set out to establish a mouse model for PRRSV infection via introducing all the three receptors into the mice genetically. To date, the transgenic mice co-expressing the three receptors have been acquired and the phenotypes of antivirus are going to be validated through virus challenge in vivo. Following that, the mice will be subjected to the passive immunization in which to clarify whether PPRSV replication can be blocked by the specific antibody. In all, our goal is to produce a mouse model that is susceptible to PRRSV 」which provides a practical platform to study the pathogenesis of PRRS in vivo and develop new antiviral strategies.

P6015 Production and immunogenicity of VP2 protein of porcine parvovirus expressed in Pichia pastoris. Chunhe Guo and Yaosheng Chen (State Key Laboratory of Biocontrol, Guangzhou Higher Education Mega Center, School of Life Sciences, Sun Yat-sen University)

Viral protein 2 (VP2) of porcine parvovirus (PPV) is the major viral structural protein and is respon-sible for eliciting neutralizing antibodies in immunized animals. In this study, we constructed and characterized a recombinant yeast vector encoding the VP2 protein, des-ignated as pGAPZ aA-VP2. The construct was confirmed by restriction enzyme digestion, PCR, and sequencing and then introduced into P. pastoris strain SMD1168 by elec-troporation. The expressed VP2 protein was analyzed by SDS-PAGE and western blot. Immunization of mice with the VP2 protein elicited a PPV-specific humoral immune response. Notably, a preparation of VP2 protein containing adjuvant induced a much better antibody response than VP2 alone. Clearly, the adjuvant strongly enhanced the immunogenicity of VP2. This study provides a foundation for the application of the VP2 protein in the clinical diagnosis of PPV and in vaccination against PPV in the future.

P6016 Avian pathogenic *Escherichia coli* (APEC) infection alters bone marrow transcriptome in broiler chickens. Hongyan Sun, Peng Liu, Lisa Nolan and Susan Lamont (Iowa State University)

Avian pathogenic *Escherichia coli* (APEC) cause colibacillosis, a disease which may manifest as septicemia, chronic respiratory disease, percarditis, and airsacculitis, resulting in significant economic loss in the poultry industry worldwide. Greader understanding of host genetic resistance to APEC will aid in the development of control strategies to reduce APEC-induced pathology. Hematopoietic cells from the bone marrow of APEC infected and uninfected chickens were used to study the transcriptome. Male broiler chicks were challenged at 4 weeks of age with APEC (or mock-challenged as controls), and bone marrow was harvested at 1 and 5 days post-infection (dpi). Lesions of liver, pericardium, and air sacs were scored on challenged birds and used to designate birds as having mild or severe pathology, representing resistant and susceptible phenotypes, respectively. Trimmed RNA sequence reads were analyzed using the R package, EdgeR, to identify differentially expressed genes. At 1 dpi, 885 differentially expressed (DE) genes (FDR<0.05) were detected between normal and susceptible birds. At 5 dpi, thousands of genes were DE between normal or resistant birds and susceptible birds. Moreover, within the same infection and pathology level, 1371 DE genes were detected in susceptible birds between 1 and 5 dpi. Analysis using the R package GOseq revealed enriched

immune pathways including NOD-like receptor signaling, Toll-like receptor signaling, Phagosome, Lysosome, and Cytokine-cytokine receptor interaction. The results of this study shed light on the host transcriptomic response associated with different pathology levels or time points after infection, as well as genes and networks associated with response to APEC. Bone marrow is an excellent tissue source for gene expression profiling in APEC infection as it provides new avenues to understand the innate immune system, as well as adaptive immune system, at the transcriptional level.

P6017 A genome-wide association study reveals the existence of major loci affecting the antibody response to avian influenza virus in chicken. Chenglong Luo, Jie Ma, Jie Wang, Hao Qu and Dingming Shu (Institute of Animal Science, Guangdong Academy of Agricultural Sciences; State Key Laboratory of Livestock and Poultry Breeding)

Avian influenza has been highly concerned because it can cause severe diseases in poultry and human since 1997. However, the genetics basis of the host immune responses against avian influenza virus (AIV) is poorly understood. In this study, the antibody levels against AIV post-immunization were measured by an enzyme-linked immunosorbent assay in the serum of 511 individuals from a commercial chicken (*Gallus gallus*) population. A genome-wide association study using 43,211 single nucleotide polymorphism markers was performed to identify the major loci affecting the immune response to AIV. This study detected four significant (P < 3.66E-6) effect single nucleotide polymorphisms, which were on chicken chromosome 1 and 2, for the antibody level against AIV. The nearest genes of these single nucleotide polymorphisms were *SAM domain, SH3 domain and nuclear localization signals 1* (*SAMSN1*), *roundabout, axon guidance receptor, homolog 2 (Drosophila)* (*ROBO2*), *ubiquitin-conjugating enzyme E2E 2*(*UBE2E2*) and *zinc finger protein 385D* (*ZNF385D*), respectively. Of these genes, the expressions of the *SAMSN1* involving in regulation of B cell activation and the *UBE2E2* involving in class 1 MHC mediated antigen processing and presentation in chicken spleens were both significantly positive correlations with the antibody level against AIV. Their correlation coefficients were 0.584 (P = 2.27E-6) and 0.594 (P = 1.38E-6), respectively. This study suggested that the chicken genome has several important loci affecting the immune response to AIV, and increased our knowledge of how to control outbreaks of avian influenza.

P6018 Deletion variant near *ZNF389* is associated with control of small ruminant lentivirus (SRLV) in multiple sheep flocks. Stephen White and Michelle Mousel (USDA-ARS Animal Disease Research), Michael Gonzalez and Lynn Herrmann-Hoesing (Dept. Veterinary Microbiology & Pathology, Washington State University), Margaret Highland and James Reynolds (USDA-ARS Animal Disease Research), Bret Taylor (USDA-ARS US Sheep Experiment Station) and Donald Knowles (USDA-ARS Animal Disease Research)

Small ruminant lentiviruses (SRLV) are macrophage-tropic viruses from the same clade as human immunodeficiency virus (HIV) that cause pneumonia, mastitis, arthritis, and poor body condition in sheep. There is no preventive vaccine and no cure for SRLV, but a recent genome-wide association study (GWAS) identified a region associated with proviral concentration. Proviral concentration is a live-animal diagnostic measure for post-infection SRLV control that has been correlated to severity of SRLV-induced lesions. To fine map this GWAS region, we tested additional variants and identified a small deletion variant near *ZNF389* that was associated with proviral concentration in 3 flocks (P<0.05). These flocks contained

Polypay, Rambouillet, and crossbred sheep from multiple locations and management conditions. One of the flocks with significant association (P<0.001) had a history of shared injection needles between sheep, high prevalence (>87%), and very high mean SRLV proviral concentration (>950 copies/ug). An overall estimate of proviral concentration, based on all 1,310 SRLV-positive animals, showed insertion homozygotes had less than half the proviral concentration of other genotypes (P<0.0001). A second study including two groups totaling 764 SRLV-negative sheep found no consistent association between this deletion and any of 13 standard production traits. Taken together, these results identify the first validated genetic marker for SRLV control post-infection, and suggest that use in selective breeding programs may not have deleterious impact on sheep production. Future research directions include testing additional breeds, management conditions, and viral subtypes, as well as identifying functional implications of the haplotype tracked by this deletion variant.

P6019 Personalized genomics in dogs - Whole genome sequencing greatly accelerates the identification of causative variants for Mendelian traits. Tosso Leeb, Vidhya Jagannathan and Cord Drögemüller (University of Bern)

The availability of a high quality canine genome reference sequence and high density SNP chips for genome-wide association studies (GWAS) opened unprecedented opportunities for canine genetic research. With the implementation of whole genome sequencing (WGS) another seminal leap in the molecular analysis of heritable traits became possible. During the last two years we have sequenced ~50 dog genomes. Together with some minimal positional information, these genome sequences enabled us to quickly identify 10 causative variants for inherited diseases including 4 variants in "new" genes that had not been functionally characterized before. These results underline the scientific potential of canine genetics to functionally annotate the mammalian genome. With more and more publicly available sequence data, it should soon become possible to identify at least a certain proportion of causative variants in individual dogs without prior mapping experiments. During the conference we will present our methodological pipeline and selected examples of our research.

P6020 Breed-specific immune responses of swine spleen infected with *Streptococcus suis* type 2 by RNA-Seq. Uma Gaur, Hua Wu and Qiao Mu (Hubei academy of agricultural sciences, Wuhan), Klaus wimmers (Leibniz Institute for Farm Animals Biology), Kui Li (Chinese Academy of Agricultural Sciences) and Shu Mei and Guisheng Liu (Hubei academy of agricultural sciences, Wuhan)

Streptococcus suis type 2 (*SS2*) is an important zoonotic pathogen. Different pig breeds have shown differential susceptibility to the pathogen infection; however, the molecular mechanisms of the susceptibility are not fully understood. With the aim of identifying the genes responsible for the infection susceptibility, two different big breeds (Enshi black and Landrace) were selected to inoculate with *SS2* and their spleen transcriptome profiles were investigated in the present study. The differentially expressed genes (DEGs) were analyzed from infected vs. control pigs in each breed, and then compared between two breeds. Enshi black pig showed more splenic DEGs than Landrace (830 vs. 611) and most of these were due to down-regulated genes (543 vs. 387). However some DEGs were uniquely expressed in one breed, while others were expressed in opposite direction in two breeds. A number of candidate genes and pathways are identified which might be involved in susceptibility to *SS2*, for example, *MMP9* and*Resistin* were only significantly expressed in Landrace. *NPG3* and *PMAP23* were up-regulated

in Landrace whereas down-regulated in Enshi black. *IGKV6* is down-regulated in Landrace but up-regulated in Enshi black. Overall, the transcriptome profiles are consistent with the clinical signs, *i.e.* the Enshi black is more susceptible to *SS2* infection than Landrace pig. This is the first study to identify the differential gene expression between indigenous and modern commercial pigs after *SS2* infection using RNA-seq. The significant DEGs in splenic profiles between two breeds suggested considerable involvement of genetic background in the susceptibility to the *SS2* infection in pigs.

P6021 Understanding the molecular mechanism of external ear innate defect by using pig as a model. Rui Qaio, Yu He, Xu Zhang, Jing Li, Jun Ren and Lu Huang (Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University)

Microtia is a complex genetic disease causing various types of outer ear malformition in humans. The genetic basis of this innate defect remains poorly understood. We have observed some individuals with different levels of auricle malformation in an Erhualian × Shaziling F2 pig population. The segregation pattern in the F2 pedigree revealed that the disorder is an autosomal recessive monogenic trait. To map the disease locus, we genotyped all 47 individuals in the pedigree using illumina porcine 60k chips and performed genome-wide association study on these individuals. The strongest signals were detected in a cluster of 50 SNPs on chromosome 18. All 11 affected pigs shared a 5.0-Mb homozygous region only on this chromosome. Recombination breakpoint analysis refined the critical region to a 2.1-Mb segment harboring 18 annotated genes. We further resequenced one affected individual and its parents using NimbleGen SeqCap EZ Designs Library for the target 2.1-Mb region and finally identified 14 candidate causal mutations. By using a broad panel of samples and concordance analysis, we illustrate that a new base insertion in exon 1 of *HOXA1* gene is the causal mutation underlying this disorder. To elucidate the effect of this mutation on other genes at the expression level, we conducted a RNA-Seq experiment using two RNA pools each containing two normal and affected embryos at day 15 of pregnancy. We identified a total of 337 differentially expressed genes and some of them are over represented in biological processes important for the development of ears. Of the 337 genes, four genes including *FGFR3*, *FGF1*, *CTCF* and *HOXC4* appeared to be strong candidates for Microtia on the basis of a set of bioinformatic analyses. Last but not the least, we have found a three-amino-acid deletion in the homodomain of HOXA1 in 4 isolated human Microtia patients.

P6022 Deletion of selectable marker genes from transgenic pigs' fibroblast cells by Cre recombinase. Xiaoling Huang, Xian Zou, Zicong Li, Dewu Liu and Zhenfang Wu (National Engineering Research Center for Breeding Swine Industry, Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou, China)

Using the piggyBac transposition-mediated gene transfer technique, we have produced transgenic cloned pigs expressing anti-porcine circovirus type 2(PCV2) *shRNA* gene and loxP-flanked selectable marker genes which were composed of a neomycin resistance (*neoR*) gene and a 2A peptide linked enhanced green fluorescent protein (*EGFP*) gene. As determined by reverse PCR, the transgene was integrated, by single copy manner, in five sites of transgenic pigs' genome. To remove the selectable marker genes from the genome of transgenic pigs, ear fibroblast cells were isolated from transgenic pigs and incubated directly with Cre recombinase in vitro. Cre enzyme-treated fibroblast cells were diluted and seeded in 96-wells plate to allow cell

colony formation. In 91 resulting cell colonies，17 showed no expression of *EGFP*. DNA from these 15 *EGFP*-negative cell colonies was analyzed by PCR and subsequent sequencing to detect the inserted transgene sequence at each integration site. The results demonstrated that loxP-flanked*neoR-EGFP* sequence was deleted from all five inserted sites. In conclusion, we successfully excised *neoR* and *EGFP* genes from the genome of fibroblast cells of transgenic pigs expressing *shRNA* interference against PCV2. The transgenic fibroblasts only carrying the PCV2-targeted *shRNA* gene could be used as nuclear donor cells to produce selectable marker-free PCV2-resistant transgenic pigs, which may allay public concerns about biological safety.

P6023 An imprinted GFP insertion was reactivated by deletion of imprinting center 2 on MMU7. Ting Gu (Huazhong Agricultral University), Aaron Bogutz and Louis Lefebvre (UBC) and Shuhong Zhao (Huazhong Agricultral University)

Most imprinted genes are located in large clusters in the genome and are regulated by a nearby imprinted center (IC), as one of the parental alleles is silenced during development. A green fluorescent protein (GFP) gene has been inserted into the distal MMU7 between the IC1 and IC2 regulated regions. This transgenic gene, called Tel7KI, was demonstrated by previous studies that it is specifically expressed from the maternal allele in post-implantation embryos and probably controlled by IC2. To make a concrete conclusion that Tel7KI gene is controlled by IC2, we bred the Tel7KI and IC2 knockout homozygotes mice (called IC2KO, produced by Michael Higgins' lab) and obtained a recombinant mouse line in which the Tel7KI and IC2KO alleles are located on the same chromosome, which is called Tel7KI-IC2KO. Our results showed that the silenced paternal Tel7KI allele is re-activated whenIC2 has been knocked out in the embryos on day 9.5 and further on. Furthermore, the GFP positive cells were more in maternal and paternal Tel7KI-IC2KO embryos on day 9.5 then the ones in maternal Tel7KI embryos of the same time point, indicating that even the active Tel7KI gene on the maternal chromosome have been repressed by the IC2 somehow. Our results confirm that imprinting of the Tel7KI transgene is regulated by IC2 and also suggest that IC2 might also limit expression of Tel7KI from the methylated maternal allele.

P6024 Differential Expression of *HIF-2α* gene and hypoxic adaptation in Chickens. Wenyu Gou, Junfei Peng, Qian Zhang, Hao Zhang and Changxin Wu (China agricultural university)

The Tibetan chicken has distribution at altitudes of 2200m-4100m in the Qinghai-Tibet Plateau for a long history. HIF2α gene (EPAS1) stimulates production of red blood cells and thus increases the concentration of hemoglobin in blood. There were some SNPs about EPAS1 in high linkage disequilibrium that correlated significantly with hemoglobin concentration. In present study, eggs of Tibetan chicken (TC) and Shouguang chicken (SG) (a lowland indigenous breed as control) were collected and incubated in a normoxia (21% O2) and hypoxic (13% O2) conditions. On day 9 (D9), 11 (D11), 16 (D16) of incubation, embryonic chorioallantoic membrane (CAM) were obtained for extracting total RNA and protein and measuring the expression of EPAS1 gene with real-time PCR and western blotting. The results showed the levels of HIF2α expression in normoxia was significantly lower than that in hypoxia on all time point at TC ($P<0.05$). However, there was no significantly difference between hypoxic and normoxic incubation of SG until developing to D16. The EPAS1 mRNA and protein expression of TC was much higher than SG in hypoxia at D9-11 ($P<0.05$), but there was no difference between them in normoxia. Until the embryos developed to D16 in hypoxia the EPAS1 expression of SG

had a dramatic increasing and catched the same level with TC. Both the breeds had the high expressions of EPAS1 under hypoxia at D16. The genomic region of the EPAS1 gene was PCR and sequenced to screen SNPs between TC and SG. There were 28 SNPs in 5000bp region upstream of transcriptional site, and 7 SNPs in all exons. It was concluded that the increasing EPAS1 gene expression on D9-11 in CAM was vital during early incubation and the Tibetan chicken could had a higher EPAS1 expression than the SG on the time under hypoxic incubation, which might improve the embryo survival and contribute to the hypoxic adaptation.

P6025 Inhibition of porcine circovirus type 2 infection in transgenic pigs by RNA interference. Wenchao Gao, Zicong Li, Xian Zou, Zhiqian Xu, Dewu Liu and Zhenfang Wu (College of Animal Science, South China Agricultural University)

Porcine circovirus type 2 (PCV2) is the primary causative agent of an emerging swine disease, postweaning multisystemic wasting syndrome (PMWS). To exploit the possibility of using RNA interference (RNAi) to against the PCV2 infection, plasmids carrying PCV2-targeted short hairpin RNAs (shRNAs) were constructed. Transfection of these plasmids into cultured pig kidney (PK15) cells inhibits the replication of PCV2 after infection. A piggyBac transposes-expressing plasmid and a piggyBac transposon plasmid containing anti-PCV2 shRNA and neo-EGFP were co-transfected into pig fetal fibroblasts and the transgenic fibroblasts from one selected cell colony were used as nuclear donor cells to produce transgenic pigs by SCNT. All live born 20 transgenic cloned piglets express strong EGFP on their noses and hooves. Anti-PCV2 shRNA also are highly expressed in various tissues of transgenic pigs, as shown by qPCR. Southern blot, inverse PCR and sequencing results demonstrated that the transgene was inserted at five sites, by single copy manner, of transgenic pigs' genome. Semen from six transgenic founder boars was used to inseminate non-transgenic sows, and 46 F1-generation piglets were produced, of which only two were EGFP-negative. The PCR and Southern blot showed that F1 transgenic pigs carry 1-5 copies of transgene. The F1 transgenic pigs and non- transgenic control pigs were inoculated with PCV2 to test their resistance to PCV2 infection. Currently the PCV2 infection test is completed, and the relevant data is under analysis.

P6026 Progesterone regulates distribution of uterine dendritic cells by increasing CCL21 expression in uterine luminal epithelia of pig. Ziyao Fan (China Agricultural University)

Progesterone plays crucial role in maintaining pregnancy, including modifying maternal immunity. Resident dendritic cells (DCs) in uterus, are not only involved in transporting harmful antigen invading into uterus to T cells in lymph nodes and driving adaptive immune responses, but also promote the process of angiogenesis in uterus. Functionally, DCs display function depending on its location in the endometrium. However, mechanism of local processes in the pregnant uterus regulating distribution of DCs remains unknown. Here, we reported that distance between every CCR7+ DCs and uterus luminal epithelia in sows injected with progesterone was significantly shorter compare to control *in vivo*. We also found that progesterone increased CCL21 expression in pig uterine luminal epithelia *in vitro* and *in vivo*, the chemokine that drives DC entry into lymphatic and blood stream. Using model of chemotaxis, DCs which isolated from pig uterus, were activated chemotaxis after being stimulated by group of pig CCL21 recombinant protein and group of progesterone treated uterus luminal epithelial cells of pig, whereas, remained immobile even after being stimulated by group of uterus luminal epithelial cells treated with

progesterone and inhibitor of progesterone receptor, group of uterus luminal epithelial cells treated with progesterone and anti-CCL21 natural antibody and control. Collectively, progesterone prevents DCs homing and reduces the susceptibility of maternal immune system against foreign antigens in pig uterus.

P6027 Efficient production of mutant library in transgenic chickens based on piggyBac transposon-mediated gene trapping. Hanyu Wu, Yingmin Sun, Dainan Cao, Tongxin Liu, Sen Wu, Ning Li and Xiaoxiang Hu (China Agricultral University)

Chickens are important animal model for biological study, and play roles in food production. Gene trapping is an advantageous technique in generating genome wide mutations, which has been widely used to study gene's function. As piggyBac (PB) transposon changes its position when transposase is expressing, we use PB as a gene trapping vector to achieve genome-wide insertional mutations in chickens. Transgenic chickens containing piggyBac transposon and transposase were produced in White Leghorn, respectively. We constructed the donor vector expressing a GFP marker gene and a Neomycin resistant gene based on PB. Then it was co-transfected with a help vector (CAG-PBase) into sub-germinal cavity of newly laid eggs to produce chimeric chickens. Six male chickens with gonadal mosaicism were screened and passaged. Twelve heterozygous individuals in the F1 generation were identified by PCR. Meanwhile, transposase chimeric chickens were produced by lentivirus method. One male chicken with gonadal mosaicism was screened and passaged. Five heterozygous individuals in the F1 generation were obtained. We will cross these two kinds of transgenic chickens when they are sexual maturity, and screen the progenies containing both transposon and transposase. Since the transposase could mediate the tansposon translocation specifically, the double-positive birds could produce offspring with various insertional positions after hybriding with the wild type chickens. We would overcome the limitations of the traditional technique in producing transgenic chickens and construct the mutant library by this method. After that, we could screen phenotypes of transgenic chickens to identify new functional genes. The study is a trial to solve the problems of poultry breeding and production, and it may provide theoretical guidance for poultry production and scientific research.

P6028 Research on differential migration model to melanoblasts development in Silkie and White Leghorn and Shouguang chicken embryos. shuxiang wang

Silky fowl is a natural mutant with hyperpigmentation of various internal tissues. Recent studies have showed that the abnormal migration of melanoblasts and the absence of barrier molecules are responsible for the hyperpigmentation in Silkie. Shouguang chicken is a local breed in china, it presents the similar black shank as in Silkie, but it has black feather, white skin and normal internal organs which is different from Silkie. White leghorn has no obvious pigmentation neither in shank nor in skin or internal organs. In this study, we used these 3 chicken models to investigated the difference of melanblasts migration and the distribution of melanocytes in chicken embryos. At the embryo days 4, 4.5 and 5, we observed abundant migration of menlanoblasts from neural crest invade into the dorsolateral and ventral path in Silkie, and very slight migration of menlanoblasts in dorsolateral path in White Leghorn as partial reported in the former studies. While in Shouguang chicken, we found the similar melanoblasts migration pattern with Silkie though they have extremely melanocytes distribution in skin and internal organs. At the embryo days 8, 10, 12, 15, 18 and 21, Silkie melanocytes showed strong replication and

migration in the dermis of the skin, muscle and internal organs, but Shouguang melanocytes did not invade into the internal tissue, whereas the melanocytes increased in hair follicle and epidermis of the shank. In White Leghorn, the melanocytes gradually disappeared, only few of them appeared in the hair follicle and skin. These developmental patterns can be explained the melanin inhibition gene (id) mutation but the melanin amplification gene (Fm) mutation. But how the melanocytes in Shouguang deposited into follicle and epidermis of leg remains interesting to be further investigated.

P6029 Hypoandrogenism reduces immunity of caponized male chicken by up-regulating the inflammatory factor*IL20RA*. Fan Shao (China Agricultural University), Jinlin Duan (none), Junying Li and Kedao Teng (China Agricultural University), Yonggang Shao (Xingjiang Agricultural University), Hongwei Li (HuiZhou University) and Changxin Wu (China Agricultural University)

Interleukin 20 receptor A (IL20RA) gene encodes a receptor subunit of IL-19, IL-20, IL-24 and IL-26, which are members of IL-10 subfamily. It was reported that these new IL-10 subfamily member cytokines were involved in immune regulation and inflammatory response. Studies on interleukin cytokines and their receptors were mainly focused on human and murine, but no on poultry. Caponized male Single Crown White Leghorns and intact males were divided into group1 (9-wk caponization and intact males) and group2 (17-wk caponization and intact males) in this study. Estrogen (E2) and Testosterone (T) concentration in serum were detected and the results showed that T content was decreased very significantly ($P<0.01$) in capons both of two groups and E2 content was increased significantly ($P<0.05$) in 9-wk capons but not 17-wk capons. Routine Blood test concluded that capons both of 9-wk and 17-wk had lower WBC and RBC ($P<0.05$). Liver microarray analyses in group1 showed *IL-20RA* had a 2.01-fold change and did not find abnormal expression on IL-10 subfamily. We analyzed expression of *IL20RA* in liver, kidney and muscle of capons and intact males of two groups by real time quantitative PCR. Statistical analysis of qPCR data in T-test showed that *IL20RA* expressed in liver and kidney, but only had trace amount expression in muscle. In both two groups, expression of IL20RA gene in capons liver was very significantly ($P<0.01$) higher than intact males. In addition, there was no significant difference of expression in kidney between the capons and the intact males. These findings suggest that hypoandrogenism up-regulate the gene expression of inflammatory cytokine receptors, which may lead to reduce organism immunity.

P6030 A transcriptomic landscape for lymphocyte count variation in the poly I:C-induced porcine peripheral blood. Haiyan Wang (Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, Huazhong Agricultural University)

Lymphocyte count is an important metric of lymphocyte phenotypes that has been reported to be related or potentially related to the individual anti-virus capacity in pigs and other mammals. But to date, besides a handful of genes and pathways, little is known about how global gene expression affects the lymphocyte count variation. In the present work, we first investigated the lymphocyte count variation after poly I:C simulation, and then compared the transcriptome between pigs with the larger and smaller differences of lymphocyte count before and after the poly I:C simulation (shortened as LOW pigs and HIGH pigs, respectively). In a tendency, lymphocyte counts of all animals were observed to decline after the poly I:C stimulation. The microarray analysis identified 1,121 transcripts (981 differentially expressed genes) in the HIGH pigs and 1,045 transcripts (904 differentially expressed genes) in the LOW pigs. We found that

many differentially expressed genes (DEGs) were involved in both innate and adaptive immune responses, but the LOW pigs had more rapid innate immune responses than that of the HIGH pigs. Inferred from our results, the activations of signaling pathways associated with cell death, cytotoxicity and apoptosis might contribute to the poly I:C-induced decrease of lymphocyte counts in the periphery. Moreover, the differential expression patterns of chemokines and FAS might provide or partially provide an interpretation for the different degree of decrease between HIGH and LOW pigs. Our study would be helpful to provide a better understanding of molecular basis for anti-virus capacity in pigs and even in other mammals.

P6031 A rapid and reliable method for detection of brachyspina syndrome carriers in Holstein cattle. Hua Li (Beijing Dairy Cattle Center), Xiao Sun (College of Animal Science and Technology, China Agricultural University), Lin ZHu (Beijing Dairy Cattle Center), Li Zhang and Zhao Fang (College of Animal Science and Technology, China Agricultural University) and Lin Liu, Qing Lv and Lv Qiao (Beijing Dairy Cattle Center)

Brachyspina syndrome (BS) is a genetic abnormality in the Holstein cattle breed that causes either early-term abortion or stillborn calves when an individual is homozygous recessive for the lethal gene. It was first observed in Denmark in 2006. Until 2012, with next-generation sequencing, the causal mutation for BS was successfully identified as a 3.3-kb deletion in the bovine fanconi anemia complementation-group 1 (*FANCI*) gene. The primary purpose of this study was to develop a rapid and reliable method to detect the recessive allele of BS and known about its distribution in the Chinese Holstein population.

A total of 269 frozen semen of Holstein bulls and 136 blood samples of Chinese Holstein cows were randomly collected in Beijing region. Genomic DNA was extracted using a standard phenol-chloroform procedure. A multiplex-PCR method was developed, of which two pairs of primers were designed. The specific primers for BS were designed based on the 3.3 kb deletion fragmemnt (GenBank NO.: AC_000178.1): 5′-GCTCAAGTAGTTAGTTGCTCCACTG-3′; 5′-ATAAATAAATAAAGCAGGATGCTGAAA-3′, and the internal primers designed based on mitochondrion gene (GenBank NO.: HQ184045.1) as a positive control were: 5′-TAAGTTAGAGATTGAGAGCC-3′; 5′-GATAAGGGTTACGAGAGGGA -3′. The multiplex-PCR was set up in a final volume of 25 μl, annealing for 30 sec at 60°C, and extension for 30 sec at 72 ℃.

For normal animals, only one DNA fragment of 269 bp was amplified, while BS carriers yielded 2 DNA fragments, i.e. 409 bp and 269 bp (Figure.1). Out of the detected Holsteins, 12 bulls and 3 cows were identified as BS carriers. Thus, the frequency of carriers was 4.5% in bulls and 2.2% in cows, respectively. As expected, no mutant homozygote was found. Furthermore, with pedigree analysis, all of the 12 carrier bulls were traced back on a common ancestor, the U.S. Holstein sire Sweet Haven Tradition, and no more remote ancestors were found.

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P6032 The differentially expressed microRNAs in tumorous tissue infected by Marek's disease virus. Han Bo, Ling Lian, Xin Li, Lujiang Qu and Ning Yang (China Agricultural University)

Marek's disease is a highly contagious T-cell lymphoid neoplasia of chickens induced by Marek's disease virus (MDV), which causes huge economic losses to the poultry industry. The microRNAs (miRNAs) are small non-coding RNAs, which regulate transcriptional and post-transcriptional gene expression, playing an

important role in transformation of tumor and form of cancer. In our previous study, we identified 187 known miRNAs in MDV-infected samples by Solexa deep sequencing. The current study further verified differential expression of 11 miRNAs, including gga-miR-122, gga-miR-130a, gga-miR-181b, gga-miR-2964, gga-miR-146c-3p, gga-miR-155, gga-miR-199-5p, gga-miR-103-3p, gga-miR-107-3p, gga-miR-29b-3p, and gga-let-7b among MDV-infected whole spleen (tumorous spleen), MD lymphoma from liver and non-infected spleen by qPCR. The results showed that two miRNAs, gga-miR-146c-3p and gga-miR-29b-3p were up-regulated and eight miRNAs, including gga-miR-130a, gga-miR-181b, gga-miR-2964, gga-miR-155, gga-miR-199-5p, gga-miR-103-3p, gga-miR-107-3p, and gga-let-7b were down-regulated in MDV-infected samples compared with non-infected spleens. The further study is on-going to verify target genes of miRNAs and investigate the function of miRNA and their targets.

P6033 The growth, reproduction and disease resistance of transgenic sheep overexpressing Toll-like receptor 4. Yuchang Yao (College of Animal Science and Technology, Northeast Agricultural University, Harbin, P.R. China.), Hongbing Han (Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China.), Xuting Song (College of Animal Science and Technology, Northeast Agricultural University, Harbin, P.R. China.), Shoulong Deng (State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.), Zhixian Wang (Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China.), Minghai Lu (College of Animal Science and Technology, Northeast Agricultural University, Harbin, P.R. China.), Hai Bai (Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China.), Sumei Wang and Wu Li (College of Animal Science and Technology, Northeast Agricultural University, Harbin, P.R. China.) and Zhengxing Lian (Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China.)

Toll-like receptor 4 (*TLR4*) is critical for the recognition of lipopolysaccharide/endotoxin from Gram-negative bacteria by different host cells. Because of the scientific importance and potential economic value, the transgenic sheep overexpressing *TLR4* were produced by microinjection to improve disease resistance. Materials and methods RNA was extracted from sheep spleens and the *TLR4* cDNA sequence was amplified using Reverse Transcript-PCR. The PCR products were connected to the vector to generate*TLR4* expression vector pIRES2-TLR4. Fertilized eggs were surgically recovered by flushing both oviducts at 72 h after CIDR removal. Zygotes were microinjected linearized pIRES2-TLR4 vector in vitro. Results 1. The first generation of transgenic sheep (3 male and 4 female) was produced by microinjection, with the overall integration frequency being 1.97% of the zygotes injected and 25.93% of the offspring born. The immunocytochemical results revealed that *TRL4* was overexpressed in transgenic individuals. No differences were found in growth performances between transgenic and non-transgenic sheep at 3, 100, 300 and 600 d of age ($p > 0.05$). 2. The values of basic semen quality were similar between *TLR4* transgenic and non-transgenic rams. Most spermatozoa of transgenic and non-transgenic rams showed common morphometric and ultrastructural characteristics. The successful transgene transmission via sperm of *TLR4* transgenic rams was confirmed by birth of 66 positive lambs acquired from artificial insemination. 3. The

survival rate of positive lambs born from the founder *TLR4* transgenic ram was higher than that of negative lambs at weaning (90.91% vs 86.18%), although the difference was not statistically significant (p=0.230). The influence of overexpressing *TLR4* on resistance against brucellosis was assessed in naturally infected female group. The transgenic ewes (n=17), compared with the negative ewes (n=32), showed a lower infection rate (29.41% vs 46.88%), although the difference was not statistically significant (p=0.190). Now, experimental infections of the *TLR4* transgenic sheep are performing with a variety of bacteria.

P6034 Porcine GBP1 and GBP2 mediate antiviral effect against porcine H3N2 and PRRSV. Liangliang Fu (College of Animal Science, Huazhong Agricultural University, Wuhan, 430070, P R China)

Experimental evidence has indicated that the p65 family of Interferon-inducible guanylate-binding proteins (GBPs), which is up-regulated by interferon gamma, is important for host immune defense against various pathogens. p65 family of Interferon-inducible guanylate-binding proteins (GBPs) coordinate a potent oxidative and vesicular trafficking program to prevent the infected host. In this study, we investigated the effect of porcine GBP1 and GBP2 genes on porcine H3N2 and PRRSV replication using MDCK cells and Marc-145 cells. The main results are as follows: 1) The open reading frames (ORF), encoding porcine GBP1 and GBP2, were amplified from their cDNA clones and subcloned into lentiviral expression vector. Overexpression of GBP1 and GBP2 by transfection of lentiviral vector respectively carrying GBP1 and GBP2 gene significantly increased expression of GBP1 and GBP2 protein. 2) Quantitative real-time PCR (Q-RT-PCR) analyses showed that the viral copy number was significantly lower in overexpressing porcine GBP1 and GBP2 cells than that in the control group at 36h post-infection with porcine H3N2 and PRRSV. 3) Immunofluorescence was used to detect the porcine H3N2 and PRRSV protein, the results showed that in comparison to the control group, expression of viral protein in cells overexpressed porcine GBP1 and GBP2 were significantly reduced at 36h post-infection with porcine H3N2 and PRRSV. 4) Western blot was used to detect M protein of porcine H3N2 and N protein of PRRSV. The expression of viral M and N protein in the overexpressed porcine GBP1 and GBP2 cells were lower than that in the control cells at 36 h post-infection with porcine H3N2 and PRRSV. These results demonstrated that the porcine GBP1 and GBP2 mediated antiviral effect against porcine H3N2 and PRRSV and likely play a broader role in host resistance to viral infection.

P6035 Species identification by mtDNA D-loop among four cyprinid fishes. Linhe Bian and Shengguo Zhao (Gansu Agriculture University)

In order to identify genetic variation and species identification between the cyprinid fishes, mtDNA D-loop was selected as a marker to determine mtDNA D-loop nucleotide sequence of four common cyprinid fishes (*Ctenopharyngodoni dellus*;*Hypophthalmichthys molitri*; *Cyprinus carpio*; *Carassius cuvieri*) in the study. The nucleotide composition A+T (averagely 71.4%) are much more abundant than G+C (averagely 28.6%) in the four kinds of fish. 132 polymorphic sites(22 singleton variable sites and 110 parsimony informative sites) was detected in 108 fishes . Based on the research, we defined 57 haplotypes (H1-H57) in these fishes. Haplotype diversity and nucleotide diversity were the highest in *Carassius cuvieri*(haplotype diversity: 1.000±0.017, nucleotide diversity: 0.03593) and the lowest in *Ctenopharyngodoni dellus* (haplotype diversity: 0.499±0.071, nucleotide diversity: 0.00817). The four kinds of fish also could be identified clearly by 53

characteristic nucleotides. According to the genetic variation analysis we can include that the haplotypes were different among four fish populations. No common haplotype was shared between them. Characteristic nucleotides as a method used to identify four kinds of fish. Marking mtDNA D-loop can provide the germplasm resources conservation of fish and the authenticity of fish products.

P6036 Identification of Genes Related to LPS by RNA-Seq in Spleen of Ducks. bing deng, ting yu, zhiping ran, shengqiang ye, lixia wang, yu yang, weiwei tong, liu wu, hua zhou and ping gong (Wuhan Institute of Animal Science and Veterinary Medicine, Wuhan Academy of Agricultural Science &Technology)

LPS which is the major component in the outer cell wall of Gram-negative bacteria,coulddamage the immune system and threaten the health of livestock and poultry.However, there isvery little information about the genes and pathways involved in the immune response ofduckling by LPS. To elucidate the genes involved in the spleen of 7 days duckling treated byLPS, spleen RNA of duckling were analyzed by RNA-Seq in LPS treated and control group.Results had shown that there were 11095 and 10836 genes in LPS treated and control grouprespectively, and every one of those genes was more than 10 clean reads in RNA-Seq data.Among these genes, there are 89 differentially expressed genes(Log2Ratio≥1,P≤0.01,FDR≤0.001) which shows 67 up-regulated and 22 down-regulated genes comparedlibrary-treat to library-control. Pathway analysis had shown that some immune system relatedsignaling pathway such as Hematopoietic cell lineage, Toll-like receptor signaling pathway, Tcell receptor signaling pathway, Complement and coagulation cascades, Antigen processingand presentation, Chemokine signaling pathway had joined in this progress. In order toconfirm the RNA-Seq results, we detected the expression of CCL4, LBP, CD71 and STEAP3by Real time-PCR, and the results had shown that the expression of four genes wereconsistent with the RNA-Seq results. Our experimental results give new information of therelated genes involved in the immune response of spleen of ducks after LPS treatment.

P6037 MicroRNA expression profiling in chicken cecum following *Campylobacter jejuni* infection by Solexa sequencing. Xiaoyi Liu, liying Liu, maozhi zhang, jinzhong Wang, huicui wang and xianyao Li

Campylobacter jejuni (*C. jejuni*) is one of major foodborne pathogen that cause human diarrhea by consuming *C. jejuni* contaminated chicken products. Genetic background plays an important role in the response to *C. jejuni* infection. MicroRNAs (miRNAs) play an integral role in many different biological processes including bacteria and virus infection in chicken. To investigate the role of chicken miRNAs in the response to *C. jejuni* infection, three-day old SPF leghorn chickens were selected and randomly divided into two groups, 4 chickens in each group. One group was infected with *C. jejuni* and the other was mock infected with PBS. The cecum was collected at 8 hours post infection (hpi) for RNA isolation. Two RNA pools were made from each group (two samples in one pool) and used for Solexa sequencing. The significantly expressed microRNAs between infected and non-infected chickens at 8 hpi were identified. Further functional analysis of the target genes of those significantly expressed miRNAs was performed. As a result, a total of 16746387, 16663306, 19862054 and 16465067 reads were annotated in four libraries, respectively. There were 423943, 370108, 297619, 449905 unique sRNAs identified in four libraries, respectively. There were 106919, 83918, 67690, 106739 unique sRNAs were annotated. There were 36 miRNAs differentially expressed between infected and non-infected group including 25 up-regulated miRNAs and 11

down-regulated miRNAs (P<0.05). There were 7257 putative target genes for those 36 differentially expressed miRNAs predicted by miRanda software. Functional analysis of those target genes were annotated through DAVID analysis. There were significantly enriched 353 GO BP terms associated with miRNAs regulated genes (P<0.05). Fifteen pathways were significantly enriched for those target genes (P <0.05). This is the first large-scale identification and characterization of miRNAs in the response to *C. jejuni* infection in chicken cecum. The result herein will lay the foundation for the further study of regulatory mechanism of miRNAs in the response to *C. jejuni* infection.

P6038 Development and blood parameters analysis of transgenic sheep for TLR2 over-expression. Wuqi Jiang (Beijing Key laboratory of Animal Genetic improvement, College of Animal Science and Technology, China Agricultural University, Beijing, China; Shenyang Agricultural University, Shenyang, China), Zhengxing Lian (Beijing Key laboratory of Animal Genetic improvement, College of Animal Science and Technology,China Agricultural University), Shoulong Deng (Institute of Zoology, Chinese Academy of Sciences, Beijing, China), Hongtao Zhang (Beijing Key laboratory of Animal Genetic improvement, College of Animal Science and Technology,China Agricultural University), Juncai Fu (College of Animal Science and Technology, China Agricultural University, Beijing, China) and Hongbing Han (Beijing Key laboratory of Animal Genetic improvement, College of Animal Science and Technology,China Agricultural University)

Most gram-positive bacteria, such as *Staphylococcus*, *Streptococcus*, *Bacillus*, *Listeria*, and *pneumococcus*, are pathogens causing a wide range of infections and diseases. Toll-like receptor 2(TLR2) is a transmembrane protein capable of recognizing conserved structures of these pathogens. In order to obtain TLR2 transgenic sheep for disease-resistant breeding research, our group constructed a *Capra hircus* TLR2 over-expression vector, produced transgenic ovine embryos by microinjection and transplanted to the receptors. The lambs were measured by PCR and Southern blotting to identify the integration of exogenous genes. The body weight, body slope length, body height and chest circumference at the age of 0, 1, 2, 7 and 15 months were measured. Some blood routine parameters including red blood cell(RBC), hemoglobin(HGB), hematocrit(HCT), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC), platelet count(PLT) and white blood cell count(WBC) at 20 months were measured. These development and blood parameters were analyzed. The results showed that the integration efficiency was 9.28%. Real-time PCR results confirmed that the transgenic sheep over-expressing TLR2 expressed more TLR2 mRNA than wild-type sheep. There was a significant difference between the weight of TLR2-transgenic sheep at the age of 1 months and that of wild-type sheep (9.62±3.37kg vs 5.85±1.80kg，P<0.05), and that of TLR2-transgenic sheep were 1.64 times of that of wild-type sheep. The weight of TLR2-transgenic sheep at the age of 7 months was higher than that of wild-type sheep (43.01±6.10kg vs 32.89±8.66kg，P>0.05), but there was no significance between them. The results of blood routine examination showed that, TLR2-transgenic sheep were as normal as wild-type sheep. So we draw the conclusion that, there is no adverse effect of TLR2-over-expressin on sheep, and TLR2-transgenic sheep perform faster growing trends at the age of 1 month.

P6039 The role of host TDP2 in FMDV life cycle. Wenjie Liu, Yuhang Wang and Ning Li (China Agricultural University)

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed ungulates. Foot-and-mouth disease virus (FMDV), the causative agent of FMD, is a positive RNA virus belonging to the Picornaviridae family. The genome RNA of FMDV has a length of about 8.5nt, featuring by an about 22aa peptide named VPg linking to its 5' end through a unique tyrosyl-RNA phosphodiester bond. In infected cells, the 5' tyrosyl-RNA phosphodiester bond can be cleaved by a VPg unlinkase, which turns out to be TDP2. FMDV replicated at membrane vesicles with replicase proteins binding on them, and those membrane vesicles are mostly derived from endoplasmic reticulum (ER) and/or Golgi compartment. By this way, TDP2 are separated from FMDV RNAs. In my current research, I want to have TDP2 and viral RNA interact during virus infection. To this end, exogenous TDP2 are stably expressed in Endoplasmic reticulum lumen of BHK-21 cells, colocalized with ERp57. The insertion of TDP2 expression vector into chromosome and the expression pattern of exogenous TDP2 were verified. Four hours after challenged with FMDV serotype O virus (MOI=0.01), viral RNA were detected by q-PCR. Compared with control BHK-21 cells, viral RNA level of cells expressing EGFP in Endoplasmic reticulum lumen is almost the same, but the level of BHK-21 cells expressing TDP2 showed a reduction of 64.4%, the copy number from 3.08×107 to 1.09×107 , the difference being significant (p=0.0001 in t test). As shown in my preliminary result, VPg plays an important role in FMDV life cycle and TDP2 can affect FMDV replication in host cells.

P6040 Increased numbers of functional NK cells in pigs with Severe Combined Immune Deficiency (SCID) caused by natural mutations in the Artemis gene. Ellis Powell, Joan Cunnick, Susie Knetter, Emily Waide and Jack Dekkers (Iowa State University)

The SCID pigs at Iowa State University lack B-cells and most T-cells, but possess Natural Killer (NK) cells. This phenotype is caused by homozygosity or compound heterozygosity of two recently discovered mutations in the Artemis gene. Our group is characterizing NK cell function in the SCID context. Interestingly, two human tumor cell lines, PANC-1 and A375-SM, survived after injection into SCID pigs (Basel et al. 2012). From this result, two important questions arise. First, whether NK cells from SCID pigs can recognize human tumor cells, and second, whether they are being activated. NK cells are activated by interleukin (IL)-2 (produced by T-cells) or by IL-12 plus IL-18 (produced by macrophages and dendritic cells; likely present in SCIDs). We first determined whether normal porcine NK cells can recognize and kill human tumor cells. After activation with IL-2 *in vitro*, non-SCID porcine NK cells (CD16+ SWC3a-) could kill PANC-1, A375-SM cells, and K562 cells (another human tumor line). We used the K562 cells to measure killing activity from non-SCID and SCID piglet NK cells isolated at approximately 4, 10, 21, and 28 days of age. SCID pigs had higher NK cell concentrations in whole blood (P-value < 0.0001) across all days. NK cells constituted 40.2% of the peripheral blood mononuclear cell population in SCID pigs compared to 5.1% in non-SCIDs. Cells from both sources required activation with either IL-2 or IL-12/IL-18 to kill K562 cells *in vitro*. However, no significant differences between SCID and Non-SCID killing activity per NK cell were found. Thus, our current hypothesis is that the SCID NK cells are not being activated *in vivo* and suggests the SCID pig is a valuable model for immunogenetics and cancer.

P6041 Physiological Differences between Overexpression of TLR4 Transgenic and Non-transgenic Sheep on Acute Stage of the Brucellosis. Zhixian Wang, Hai Bai, Rui Hu, Hongbing Han and Zhengxing Lian (China Agricultural University)

Toll-like receptor 4 is a crucial signal transducer for LPS, the major component of Gram-negative bacteria outer cell membrane. *Brucella melitensis* is an intracellular pathogen that uses a crafty strategy to invade and proliferate within host cells. In this study, effects of *TLR4* overexpression under *B. melitensis* infected were evaluate in vivo. 10 transgenic sheep which about 2.5 times *TLR4* expression compared with wild type sheep and 10 wild type sheep that were the similar body condition, age, healthy, were picked into two groups of different dosage of innoculation(106 and 108 CFU) of the *B.melitensis* 16M, infected by way of conjunctival infections. The body weight changes, blood samples for bacterial culture, clinical hematology and immune responses to brucellosis (microagglutination) at day 28 were monitored, to slaughter sheep and take terminal organs and lymph nodus for isolation of bacteria and observing by pathological sections at day 28. The results of microagglutination, RBPT and the terminal tissue burden showed the transgenic and non-transgenic group had each 6 sheep infected at day 28. The weight changes (1.175kg and 1.433kg), necropsy and organ weight, blood samples for bacterial culture were no significant differences between the two groups. Tissue sections showed that transgenic individuals launched inflammation response more fiercely and more infiltration of neutrophils in the spleen, that suggested transgenic sheep were more sensitive and inflammatory reaction were severer. In the results of blood routine examination for brucellosis individuals, RBC (6.17*1012/L), HGB (69.2g/L), HCT (21.05%) and RDW (13.37%) of wild type were lower than that of transgenic sheep (7.9*1012/L, 90g/L, 28.2%, 14.35%)($P<0.05$) and that suggested the non-transgenic sheep were anaemia. Between overexpression of *TLR4* transgenic and non-transgenic sheep on acute stage of the brucellosis, there were no significant pathological differences but the non-transgenic sheep were anaemia from the clinical hematology and transgenic sheep had been more inflammatory reaction than non-transgenic sheep from the results of tissue sections.

P6042 MicroRNA-1s induces myotubes atrorhy via suppressing insulin like growth factor 1(Igf-1). Kuo Zhang

Insulin like growth factor 1(Igf-1) influences the skeletal muscle size, which depends upon a dynamic balance between anabolic and catabolic processes. Cancer *cachexia* is characterized by skeletal muscle wasting that is mainly supported by hypercatabolism. This atrophy has been suggested to depend on impaired IGF-1 signal transduction pathway. In various cancer, miR-17-92, a polycistronic microRNA cluster, is overexpressed .but mir-1s, a member of the mir-17-92 cluster always don't play a role in accounting for cancer cell growth. Here we demonstrate that Igf-1 was a direct target of miR-1s through binding to its 3'-untranslated region. Overexpression of miR-1s suppressed the expression of Igf-1 in skeletal muscle progenitor cells (C2C12), and prevented Akt phosphorylation.. These influences of mir-1s could be rescued by the inhibitor of the PI3K/Akt pathway. At the same time, mir-1s could induce C2C12 arrested at G1 phase via Igf-1 inactivation. Because the IGF-1/PI3K/Akt pathway mediate hypertrophy and atrophy, We also observed ectopicexpression of miR-1s prevented Akt-mediated inhibition of two ubiquitin–protein ligases (E3) called MAFbx/Atrogin-1 and MuRF-1 upregulation. So this microRNA caused dramatic atrophy of myotubes. These effects of mir-1s were all reversed by treatment ofIgf-1 Thus, mir-1s play a critical role in the development of muscle atrophy, and inhibition of mir-1s is an attractive approach to combat cachexia induced by cancer.

P6043 Identification and characterization of miRNAs in lung tissues of pigs differing in the

resistance to PCV2 infection. liyuan wang (Shandong Agricultural university) and Yanping Li, Pengfei Wang and Yunliang Jiang (Shandong Agricultural University)

Porcine circovirus type 2 (PCV2) is known to be associated with post-weaning multi-systemic wasting syndrome (PMWS), a recently described disease of young pigs. Differences between breeds have been regarded as a primary aspect in the susceptibility of pigs to PCV2 virus. miRNAs are known to play diverse and complex roles in viral infections. To discover the impact of PCV2 infection on the cellular miRNAome and the susceptibility of different breeds, especially in Chinese indigenous pigs, Illumina deep sequencing was used to construct small RNA expression profiles from the lung tissues of the Laiwu pigs and Yorkshire × Landrace (YL) pigs infected with PCV2. A total of 24 cellular miRNAs were significantly differentially expressed: for Laiwu pigs, 18 miRNAs were up-regulated and 4 miRNAs were down-regulated after PCV2 infection, and for the YL pigs, the number is 5 and 2, respectively. The differentially expressed miRNAs were further analyzed using bioinformatics, including target genes prediction, GO annotation and KEGG pathway analysis. Seven genes including SLC39A8, ADAM11, MMP16, DPYSL2, and DHX15 participating in metabolic process, multicellular organism process, organelle, enzyme regulator activity, MAPK signaling pathway and Axon guidance, were identified as the targeted genes for 5 significantly differentially expressed miRNAs. Overall, the present study had revealed that a group of miRNAs were expressed differentially in different breeds. However, whether the expression of a subset of these miRNAs is altered in PCV2 infected cells and whether the targeted genes were truly regulated by these miRNAs need to be clarified.

P6044 A SNP in *CD14* gene causes an aberrant splice variant associated with dairy cow mastitis. Xiuge Wang, Jinming Huang, Changfa Wang, Zhihua Ju, Yan Zhang, Jifeng Zhong and Yundong Gao (Shandong Academy of Agricultural Sciences)

Bovine mastitisis an immune response process under a variety of immune cells. Macrophages that compose of first line of defense play a leading and sentinel effects against foreign invading pathogens. Once invaders are detected, then the immune cells release cytokines that direct migration of polymorphonuclear neutrophil leukocytes (PMN) into the area of inflammation to kill foreign pathogens. CD14 (cluster of differentiation 14) is expressed mainly by macrophages and neutrophils, and acts as a co-receptor binding bacterial lipopolysaccharide (LPS). In the study, we found a new splicing variant named as *CD14-AS* in PMN, characterized by a region deletion from the c. 143 nt to c. 579 nt. Moreover, a single nucleotide polymorphism (SNP) (c. 523 A>G) located in the branch site (BS) region of the pre-mRNA splicing conserved elements was identified and predicted existing the relationship between the SNP and the production of the *CD14-AS* splicing variant using bioinformatics methods. Next experiment, we will verify the function of *CD14* and *CD14-AS* transcripts on mastitis caused by *Escherichia Coli* in dairy cows, analyze the association between the SNP and mastitis related indicators of genomic breeding value by Illumina BovineSNP50K BeadChip and further confirm that the SNP can cause production of the *CD14-AS* aberrant splice variant through constructing *pSPL3* exon capturing vector and transfecting cells.

P6045 *IARS* mutation causes prenatal death in Japanese black cattle. Takashi Hirano (Tokyo University of Agriculture), Tamako Matsuhashi and Kenji Takeda (Gifu Prefectural Livestock Research Institute), Hiromi Hara (Tokyo University of Agriculture), Naohiko Kobayashi

and Kita Kazuo (Gifu Prefectural Livestock Research Institute), Yoshikazu Sugimoto (Shirakawa Institute of Animal Genetics) and Kei Hanzawa (Tokyo University of Agriculture)

IARS (isoleucyl-tRNA synthetase) catalyzes the aminoacylation of tRNAIle with isoleucine. In Japanese black cattle, *IARS* c.235G>C (p.V79L) was a causative mutation for a recessive disease that was characterized with a lower birth weight, weakness and poor sucking, called IARS disorder. However, the gestation period of affected animal was normal or slightly long. It implies intrauterine growth retardation, and the *IARS* mutation may cause also a prenatal death. To search that, we analysed *IARS* genotypes in animals from carrier x carrier, and association between genotypes of bull and dam in artificial-insemination (AI) and period until next AI.

The postnatal mortality in animals (~ 8 months old) from carrier (G/C) x carrier (dead/alive; 34/207, 16.4%) was higher than that in animals from carrier x normal (G/G) (27/563, 4.8%) and normal x normal (12/260, 4.6%) (p<0.001). It was suggested that the mortality of *IARS* mutant homozygous animal was high. *IARS* genotypes in 61 animals from carrier x carrier were 20 normal, 34 carrier and 7 affected animal (C/C) (expected number; 15, 30 and 15, respectively). Affected animal was significantly few in the population (p<0.05), and it was suggested that more than half of affected embryo died prenatally. Furthermore, 11,580 AI data indicated that frequency of re-insemination after carrier x carrier insemination was significantly high in 61-140 days (after carrier x carrier AI; 15.7%, and after other; 10.1%, p<0.001). The period was consistent with the stage that embryo begin to rapidly increase length and weight. These things suggest that *IARS* mutation homozygote may cause the fetal or embryonic death as well as the calf death.

P6046 Host Responses to Equine Arteritis Virus are Associated with Alleles of *CXCL16*. Ernest Bailey, Y. Go, J. Eberth, K. Shuck, F. Cook, P. Timoney and U.B.R. Balasuriya (University of Kentucky)

Previously, genome wide association studies demonstrated that a dominant gene on horse chromosome 11 (ECA 11) determined whether or not CD3+T cells can be infected in vitro with Equine Arteritis Virus (EAV). Here we report evidence that the trait is caused by variation in the gene *CXCL16*. A 30X Next Gen whole genome sequence was obtained for a susceptible Thoroughbred, a susceptible Standardbred and a resistant Thoroughbred based on the ability of their CD3+T cells to be infected, in vitro. Genes within the implicated region, i.e. bounded by ECA11:49M and ECA11:51M, were compared among the three horses. Twelve missense mutations were found among 8 genes. One of the genes, *CXCL16*, was of particular interest because 4 missense mutations occurred within the first exon, altering the amino acids at positions 40, 50, 51 and 53 of the mature CXCL16 protein. Two alleles were observed. The first sequence was identical to the reference genome for equine *CXCL16* and associated with resistance. The second sequence included all 4 variants and was associated with susceptibility. The susceptibility allele showed nearly complete association with the CD3+T cell susceptibility trait. Two other genes in the implicated region, *SHBG* and *NLRP1*, were excluded as candidates because the distributions of their variants were not associated with the trait. The *CXCL16* susceptibility variant was also strongly associated with development of a carrier state with shedding of virus in semen of mature stallions (P<0.000001). The presence of the variant was subsequently investigated among exotic equids, namely zebras, onagers, asses and the Przewalski's horse. The non-horse equids had the *CXCL16* variants associated with susceptibility while the Przewalskii's horse had the resistance genotype.

P6047 99 Lives cat genome sequencing initiative.. Leslie Lyons (University of Missouri - Columbia, College of Veterinary Medicine) and Erica Creighton and Barbara Gandolfi (University of Missouri, College of Veterinary Medicine)

Precise determination of the genome of a species is important to understanding disease and for the development of diagnostic and screening tests that will improve health. The genome has been well sequenced in many species, iincluding humans, mice, cattle, pigs and dogs. Unfortunately, only one cat has a publically available genome sequence. Cinnamon was an Abyssinian cat whose genome was sequenced in 2007. The Broad Institute produced a ~1.9x coverage genome sequence, Hill's Pet Food, Inc. along with AngenCourt then added another ~1.1x coverage, which was combined to produce an approximately 2.0x Sanger-based cat genome sequence. The Genome Institute at Washington University has added 11x of NextGen sequencing, including data from Riche 454 and illumina HiSeq. To enhance the variant detection for the domestic cat and to promote the detection of variants causing diseases and traits, a public and collaborative effort has developed to sequence additional cats to support the cat genome project. To date, 2 Birmans, 2 random breds, and five trios of cats are in the sequencing pipeline (n = 19). The cats are being sequenced using 2 PCR-free libraries at 350 bp and 550 bp using 100 bp paired – end reads from illumina HiSeq instruments. Data is being managed and analyzed by Maverics Biomics, which is overlaying the data on the UCSC browser of cat assembly Ver6.2 and providing variant calls. Besides Missouri, other contributors include UC Davis (N. Pedersen), Iowa State (M. Rothschild), Texas A&M (W. Murphy), University of Helsinki (H. Lohi), and Cornell University (R. Todhunter). Funding for the project has been provided by the Winn Feline Foundation and Zoetis. Any interested researcher who can provide either funding or a cat sequence is welcome to join the initiative to benefit from the data of all cats. Data is currently available on ~15 cats. These cats segregate for ~12 unidentified Mendelian diseases and traits.

P6048 Proinflammatory cytokines expression in milk somatic cells of goats infected with CAE virus. Emilia Bagnicka and Justyna Jarczak (Institute of Genetics and Animal Breeding), Jaroslaw Kaba and Michal Czopowicz (Warsaw University of Life Sciences) and Jozef Krzyzewski, Danuta Sloniewska, Bozena Pyzel and Lech Zwierzchowski (Institute of Genetics and Animal Breeding)

The aim of study was to evaluate the impact of caprine arthritis-encephalitis (CAE) infection on the expression of immune system genes in goat milk somatic cells (SC). Twenty six dairy goats were divided by breed and parity into two groups: control – free from virus infection (N=13) and experimental – infected with CAE virus (N=13). Each group consisted of 7 Polish White Improved (PWI) and 6 Polish Fawn Improved (PFI) goats. The milk samples were taken four times during lactation on days 7, 30, 120 and 240. Total RNA was isolated from SC and expression of Ilα, Ilβ, INFα, INFβ, INFγ, TNF, IL-6, IL-10, IL-16 and IL-18 genes was measured with qRT-PCR using cyclophilin A (PPM) as a reference gene. A qualitative and quantitative assessment of the isolated RNA was conducted using a NanoDrop (NanoDrop, USA) spectrophotometer.
The expression of the IL-2, IL-4 and IL-12 genes was not found indicating that they are not constitutively expressed in milk cells and do not participate in the defense of the udder against CAE virus infection. Moreover, there were no differences in expression of Ilα, Ilβ, INFα, INFβ, TNF, IL-6, IL-10, and IL-16 genes between groups. Their expression did not undergo any fluctuations during lactation. Thus, they are

produced constitutively in milk somatic cells. The expression of INFγ and IL-18 genes did not changed during lactation in milk cells derived from virus-free animals. However, the differences in these gene expressions were found in milk cells derived from infected animals. The expression of INFγ was the lowest while of IL-18 was the highest at the end of lactation.

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P6049 Unravelling the genetic basis of cerebellar abiotrophy in Australian working kelpie dogs. Annie Pan, Rosanne Taylor, Peter Williamson and Claire Wade (University of Sydney)

The Australian Kelpie is a dog breed developed for livestock herding. Cerebellar abiotrophy (CA) is a neurodegenerative disorder which results in early onset ataxia, and was first documented in Australian Kelpie (AK) and Australian Working Kelpie (AWK) dog breeds in 1989. The cerebellum controls movement and coordination and is affected in CA by a loss or failure of development of Purkinje and granular cells, but with a degree of variation in severity. The present study involved a genome-wide association analysis of 30 CA affected, 35 related unaffected dogs, 12 unrelated AWK presumed to be unaffected, and 22 unaffected AK,using the Illumina high-density 172K canine SNP array. The data was filtered using PLINK software as follows: minor allele frequency > 15%, SNP call rate > 90%, and individual missing call rate < 10%. Six associated regions on five chromosomes were identified: CFA 3 (praw = 2.81E-09), CFA 22 (praw = 3.55E-08), CFA 34 (praw = 5.17E-05), CFA 35 (praw = 2.46E-11) and CFA X (praw = 4.50E-10). The results reveal CA in the AWK to be a complex Mendelian disorder with an incomplete penetrance. Some of the identified loci contain candidate genes that are known to be associated with cerebellar ataxia in humans. A small number of dogs identified as CA affected remain unexplained in this analysis. Further work is underway to search and validate functional DNA changes in the identified regions using genome sequence information collected from preliminary high throughput sequencing using the Illumina HiSeq 2000 platform.

P6050 Statistical evaluation of scrapie susceptibility in some Italian sheep breeds. Carla Sebastiani, Ludovica Curcio, Marcella Ciullo, Carmen Maresca and Annalisa Dettori (Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy), Emiliano Lasagna (Faculty of Agriculture, University of Perugia, Italy) and Massimo Biagetti (Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy)

The aim of this work was to evaluate the probability to find genotypes susceptibles/resistant to scrapie in 8 italian sheep breeds (Sarda, Merinizzata, Merinos, Bergamasca, Massese, Appenninica, Fabrianese, Comisana) on the basis of genotype and allele frequencies. Statistical analysis was included to express Odds Ratio (OR), with 95% confidence interval (CI), as a measure of association between breed and frequencies of susceptible and resistant alleles and genotypes. Data with *P*-value ≤0.05 were considered statistically significant. The probability to have ARR resistant allele for each breed was calculated. Five breeds had a higher probability to present ARR allele with OR>1 respect to all other breeds; in particular, Sarda (OR 1.26; CI 1.20-1.32), Merinos (OR 1.14; CI 1.05-1.24), Massese (OR 2.14; CI 1.94-2.36), Comisana (OR 2.29; CI 1.69-3.11), and Appenninica (OR 1.09; CI 0.87-1.38). *P*-values were statistically significant for all breeds except for Appenninica. On the other hand, calculating OR on genotype frequencies and analyzing the behavior of the breeds respect to susceptible genotypes, it results

that Sarda, Merinos, Massese, and Comisana breeds show a lower probability to have a sensitive genotype than other breeds (OR<1, *P*-value≤0.05); in detail, Sarda (OR 0.66; CI 0.62-0.72), Merinos (OR 0.79; CI 0.70-0.89), Massese (OR 0.34; CI 0.29-0.40), and Comisana (OR 0.31; CI 0.17-0.56). Two breeds, Fabrianese and Appenninica, have OR<1, but *P*-value was not statistically significant. Bergamasca breed have OR>1 with *P*-value not statistically significant. The results show that Sarda, Comisana, Massese and Merinos breeds have a low probability to be affected by scrapie than the other breeds analyzed and so they could be classifiable as resistant breeds. As regards to the other breeds, they have amedium to high probability to be susceptible to scrapie.

P6051 Gut microbiota composition in swine: genetic parameters and links with immunity traits. Jordi Estelle, Nuria Mach and Yuliaxis Ramayo-Caldas (INRA, UMR1313 GABI), Joel Dore (INRA, UMR1319 MICALIS), Yvon Billon (INRA, UE1372 GenESI), Marie-Jose Mercat (IFIP-BIOPORC), Catherine Larzul (INRA, UMR1313 GABI), Patricia Lepage (INRA, UMR1319 MICALIS) and Claire Rogel-Gaillard (INRA, UMR1313 GABI)

The intestinal microbiome plays a major role in host's physiology and homeostasis. It participates in the immunological barrier against infections, helps to develop and mature the immune system, and contributes to extract nutrients and energy from food. Despite large scale studies in human, little is known on gut microbiota composition and potential associations with individual traits in livestock species. The objective of this study was to estimate the genetic parameters of the gut microbiota composition and analyze its links with immunity traits in French Large White pigs. A cohort of 60 days old piglets was assessed for fecal microbiota composition by pyrosequencing the 16S rRNA gene. First results on 299 piglets showed a predominance of *Prevotella* followed by*Oscillibacter*, *Dialister*, *Roseburia* and *Treponema*. Among a set of 63 genera, 7 had low (0.10.4) heritabilities for abundance variations. At the genetic level, the relative abundance of *Prevotella*, *Oribacterium*, *Selenomonas*, *Dialister* and *Megasphaera* were found positively correlated with each other and tended to be negatively correlated to other genera. Finally, regularized canonical correlations (rCCA) and sparse Partial Least Squares (sPLS) analyzes highlighted both positive and negative correlations between various immunity traits (e.g. monocytes, eosinophils, platelets) and genera such as *Prevotella*, *Roseburia* and *Dialister*. In this report we demonstrate for the first time that the gut microbiota composition in swine is influenced by the genetics of the host. In addition, we have found covariations between microbiota composition and immunity traits. These results pave the way for studying the microbiota as a new component of phenotype construction in pigs. Microbiota parameters together with zootechnical and immunity traits will help to better decipher the driving forces that shape animal performances and robustness.

P6052 The associations between the single-nucleotide polymorphisms of *APOBEC3F* gene and the pigs' susceptibility to PRRSV. Chunhua Meng, Qianming Zhu, Huili Wang, Jingxin Li, Yonghao Qiao, Xiaodan Man and shaoxian Cao (Jiangsu Academy of Agricultural Sciences)

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important infectious diseases. Apolipoprotein B mRNA editing enzyme catalytic polypeptide - like 3F (APOBEC3F)⌋†a natural immune factor in the host cells⌋†can effectively prevent endogenous and exogenous virus replications in human. In this study, pig *APOBEC3F* gene was amplified and sequenced to screen SNPs, and the susceptibility to PRRSV was evaluated by

porcine alveolar macrophages (PAMs) infectious model *in vitro*. The associations of polymorphism with pig susceptibility to PRRSV were analyzed in 9 populations including 193 samples. Sequencing results showed that there were 5 SNPs, g.337 T/G, g.341 G/A and g.343 T/C in exon 6, g.16 A/G in intron 3 and g.5 G/A in exon 8. Two genotypes GG (0.79/0.80) and GA (0.21/0.20) were found in Jiangquhai and Meishan pig populations, but only genotype GG was detected in other populations at 341 locus, and three genotypes GG (0.50/0.60), GA (0.33/0.20) and AA (0.17/0.20) were found in Dingyuan and Meishan pig populations, two genotypes GG (0.60) and GA (0.40) were found in Erhualian pig population, but only genotype GG was detected in the other populations at locus 5 of exon 8. Association analysis showed that the relative amount of PRRSV in PAMs with GG genotype was significantly higher than that of GA genotype at locus 341 of exon 6 at 18h after infection, the relative amount of PRRSV in PAMs with AA genotype was significantly higher than that of GG and GA genotypes at locus 5 of exon 8 at 12 h after infection. The A allele in the locus 341 of the exon 6 and the G allele in the locus 5 of exon 8 were more conducive to PRRSV-resistance, which laid a foundation for further study of *APOBEC3F* gene as an assisted selection marker to PRRSV resistant breeding.

P6053 A mixture of viral siRNA and miRNA in acute infection of the DNA virus white spot syndrome virus (WSSV). Chengzhang Liu, Fuhua Li, Yumiao Sun, Xiaojun Zhang and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

Both siRNA and miRNA play vital roles in virus-host interactions. Shrimp white spot syndrome virus (WSSV) is a major pathogen in shrimp aquaculture, which has a large double strand DNA genome and was reported to express siRNA of vp28 and high density of miRNAs. Yet the expression profiles of siRNA and miRNA have not been compared for any animal DNA virus before. Here, by sRNA sequencing of infected Chinese Shrimp (*Fenneropenaeus chinensis*) in acute and latent stages, we found that WSSV produces a mixture of abundant sRNA during acute infection. Genomic analysis suggested that the majority of these sRNAs are siRNAs. The WSSV sRNAs showed dramatic positional and strand-specific expression, with a hotspot located around 150kb on the minus strand. Viral genes producing the most abundant sRNAs include *wssv326*, *wsv277* and *wsv360*. Homology was found between viral and host sRNA, indicating their interaction via the RNA interfering (RNAi) mechanism. By separating miRNA from siRNA, we identified 12 viral miRNAs including 10 novel ones. RT-PCR assay showed that most the viral miRNAs' expression kept increasing till 48 hours post infection and some miRNAs were expressed differently between the stomach and the lymphoid (Oka) organ. By integrating sRNA and mRNA transcriptome data, we identified primary miRNA transcripts for 10 of the 12 WSSV miRNAs, and predicted both viral and host target genes of WSSV miRNAs. Enrichment analysis of target genes indicated that viral miRNAs may alter immune related processes such as chemokine signaling pathway, Ras signaling pathway, melanogenesis and phagocytosis, indicating their involvement in acute infection stage. This study provided the first integrated transcriptome study of siRNA, miRNA and mRNA of a DNA virus, and presented a comprehensive regulatory network between WSSV miRNAs and their target genes.

P6054 Silver nano particles synthesis by bioremediation bacteria *Bacillus subtilis* on the immune gene expression and their competency in controlling vibriosis in shrimp *Litopenaeus vannamei*. Elayaraja Sivaramasamy, Shihao Li, Jingwen Liu, Fuhua Li and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

The application experiment was conducted to investigate the silver nano particles (AgNPs) synthesis ofbioremediation bacteria isolated from shrimp *Litopenaeus vannamei* intestines. Molecular identification of the isolates showed it as a strain of *Bacillus subtilis*. The silver resistant strain was exposed to 1mM concentration of AgNO3 it was found to have the ability to form silver nanoparticles extracellularly at room temperature within 24 hrs. Bio synthesized AgNPs were characterized as UV-visible spectrum of the supernatant of cell culture showed an absorbance peak of AgNPs at ~ 420 nm. The average size of the synthesized AgNPs was found to be in the range of 5 - 30 nm with a spherical shape were ascertained by Transmission Electron Micrography (TEM). The bio synthesized silver nanoparticles were found to inhibit *Vibrio* pathogens viz., *Vibrio parahaemolyticus* and *Vibrio* spp. and this antibacterial effect were better than that of *B. subtilis* and pure silvers solution as proved by disc diffusion assay. The bio synthesized nanoparticles were then tested for the shrimp *L. vannamei* challenged on the *Vibrio* pathogens for 60 days. Subsequently, real-time PCR was employed to determine the mRNA levels of prophenoloxidase (proPO), anti lipopolysaccharide factor (ALF), peroxinectin (PE), superoxide dismutase (SOD), 18S, lipopolysaccharide and β -1,3-glucan-binding protein (LGBP) and serine protein (SP). The expression of all immune related genes studied was significantly up-regulated in the shrimp fed biosynthesized AgNPs diets compared to the other treatment. The shrimps fed with biosynthesized silver nanoparticles exhibited higher survival, associated with haemocyte counts and histological examines of *L. vannamei* which is on par with that of control. This investigation demonstrated the effectiveness of the bio synthesized AgNPs as a new tool in combating against shrimp pathogens.

P6055 Lack of cytosolic carboxypeptidase 1 leads to subfertility due to the reduced number of antral follicles in *pcd3J-/-*females. Ning Song, Nameun Kim, Rui Xiao, Haiin Jo, Minkyeung Choi, Hojun Choi and Min-Hee Kang (Department of Animal Biotechnology, Konkuk University), Zhao-Jia Ge (Reproductive Medicine Center, Henan Provincial People's Hospital) and Chankyu Park (Department of Animal Biotechnology, Konkuk University)

Females homozygous for the Purkinje cell degeneration mutation (*pcd*) are fertile, although the success rate is much lower than in the wild type. We performed detailed analysis of reproductive abnormalities of *pcd* females. The number of oocytes produced following exogenous gonadotropin treatment was much lower in *pcd3J-/-* females than in *pcd3J+/+* females. Histological analyses and follicle counting of 4-and 8-week-old *pcd3J-/-*ovaries showed an increase in the number of secondary follicles and a decrease in the number of antral follicles, indicating that AGTPBP1/ CCP1plays an important role in the development of secondary follicles into antral follicles. Consistent with a previous analysis of the *pcd* cerebellum, *pcd3J-/-* ovaries also showeda clear increase in the level of polyglutamylation. Gene expression analysis showed that both oocytes and cumulus cells express *CCP1*. However, *Ccp4* and *Agbl4/CCP6*, which can compensate the function of CCP1, were not expressed in mouse ovaries. Failure of microtubule deglutamylation did not affect the structure and function of the meiotic spindle in properly aligning chromosomes in the center of the nucleus during meiosis in *pcd3J-/-* females. We also showed that the pituitary-derived growth-related endocrine system functions normally in *pcd3J-/-* mice. The results of this study provide insight into additional functions of CCP1, which cannot be fully explained by the side chain deglutamylation of microtubules alone.

P6056 Detection of BLV Infection and Molecular Characterization of Bovine leukemia virus isolates in Philippine. Palati Mairepati, Ayumu Ohno and Shin-nosuke Takeshima (RIKEN), Claro Mingala (Philippine Carabao Center) and Misao Onuma and Yoko Aida (RIKEN)

Bovine leukaemia virus (BLV) is an oncogenic virus, and the etiological agent of enzootic bovine leukosis, which is the most common neoplastic disease of cattle. However, there is lack of comprehensive studies of BLV presences in Philippine and the genetic characteristics of Philippine BLV isolates is still unknown. Objective: to detect BLV infection, and then to address phylogenetic characteristics of the Philippine BLV isolates by partial sequencing of the *env* gene. Study design: Blood samples were taken from 1117 cattle from different farms in 5 Islands of Philippine. The BLV proviral load was measured using BLV CoCoMo-qPCR. BLV presences were detected by CoCoMo-qPCR and nested PCR. Partial *env* gp51 sequences of 43 samples which shows BLV positive by both CoCoMo-qPCR and nested PCR were used for genetic characterization of BLV isolates in Philippine. Phylogenetic analysis were performed in the BLV positive samples by amplifying 423 bp *evn* gene sequences by Nested PCR. Results: Out of 1117 samples screened, 9.7% (108/1117) samples were detected as BLV positive by CoCoMo-qPCR and 4.8% (54/1117) samples were BLV positive by Nested PCR. Among the five islands screened, Luzon island showed the highest BLV infection. Phylogenetic analysis based on 423bp fragment of *env* gene revealed that Philippine BLV isolates clustered into either genotype 1 or genotype 6. A number of amino acid substitutions were found in the sequences of BLV isolates studied. substitutions encompassed mainly in the CD4+ epitope, second neutralizing domain and B-epitope.

P6057 Feline Amyloidosis: new genomic and proteomic approaches for an old and current disorder. Maria Longeri (Universit à degli Studi di Milano - DIVET), Anne Thomas (Antagene), Giuseppe Sironi, Gabriella Tedeschi, Stefano Marelli, Michele Polli and Luana Crescenti (Universit à degli Studi di Milano - DIVET) and Erica Creighton and Leslie Lyons (University of Missouri - Columbia)

Amyloidosis is a disorder wherein amyloid proteins are abnormally deposited in organs, causing secondary health complications. In humans, eight genes cause different forms of amyloidosis. In Abyssinian / Somali and Siamese / Oriental cats, juvenile amyloidosis has been reported as familial. After being a significant health concern in Abyssinians in the recent past, the disease is now emerging in Siamese / Orientals worldwide, however with different pathogenesis. Abyssinians tend to succumb to renal disease while Siamese tend to succumb to hepatic disease. As the pathogenesis of feline amyloidosis is unknown, the onset of symptoms unpredictable, and biopsy confirmation of disease unreliable, a genomic and proteomic approach was designed to clarify the mechanisms and to find the genetic basis of the disease. Genomic DNA from 22 Abyssinian / Somali and 23 Siamese / Oriental, all with *post mortem* positive reports for amyloidosis, ages ≤ than 6 years and an equal number of controls with *post mortem* negative reports, ages ≥ than 7 years, was extracted from tissues of cats bred in Europe, USA and Australia. To inspect population stratification of cats from the different countries, 148 unlinked SNP markers will be genotyped using Sequenom MassARRAY technology to further support the selection of controls and the combined analysis of the cats from different regions. A preliminary genome-wide association will be performed using the Illumina feline 63K array on 48 samples to identify the regions harboring causative genes. Moreover, to characterize the main proteic components, samples from massive deposits and

corresponding normal tissues have been digested. A mass spectrometric strategy for in-depth protein sequencing has been applied, shotgun proteomics, using the ThermoFisher LTQ Orbitrap Velos ETD equipped with Nano HPLC Ultimate 3000, which is ideally suited for discovery-driven profiling and can achieve the identification of different proteins from a biological sample.

P6058 Association of *AGER* gene polymorphisms with susceptibility to scrapie in goats.. Simone Peletto, Claudia Boin, Paola Modesto, Silvia Colussi, Maria Maniaci, Stefano Turini, Maria Mazza and PierLuigi Acutis (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta)

Genetic selection towards resistance is a promising approach to control scrapie in goats. Previously, we found a linkage between scrapie positivity and an indel polymorphism of the *SPRN* gene, coding for the prion-like Shadoo protein. Now we have investigated the genetic variability of the Receptor for the Advanced Glycation End Product (*AGER*) gene and its implication in susceptibility/resistance to caprine scrapie.Analyses were carried out on 92 goats (29 scrapie positive and 63 negative controls) from different scrapie outbreaks. Two overlapping PCRs were set up based on homologous bovine sequences to amplify the entire *AGER* gene (~ 3 Kb). Allele and genotype frequencies were calculated for each polymorphism. Their associations with the scrapie status were assessed performing the Chi-Square test and were considered statistically significant for p AGER genetic variability showed the occurrence of 10 SNPs and one indel polymorphism. The SNP at position 416 (A→G) in the gene ORF showed a higher frequency of the A allele in cases than in controls (p=0.029). This result was confirmed also by genotype analysis: grouped A/G and A/A genotypes were significantly associated with scrapie positivity (p=0.011). Moreover, the deletion of a GTGTGT motif at position 989 was significantly associated with scrapie when genotypes were considered in the analysis (p=0.020).This study showed that the allele 416-A and the A/G and A/A genotypes of the caprine *AGER* gene are related to susceptibility to scrapie. Furthermore, the allele carrying the GTGTGT deletion of the 989-indel polymorphism was mostly present in scrapie positive goats. Our results demonstrate a correlation between the *AGER* gene and susceptibility to scrapie in goats thus providing an ancillary target to the prion protein gene for scrapie selection. Moreover, our findings support the idea that *AGER* is involved in the mechanism of neuronal dysfunction associated with prion diseases.

P6059 Identification of chromosomal regions influencing cortisol responses in sheep. Niel Karrow (University of Guelph), Sameer Pant (University of Copenhagen) and Qiumei You, Gordon Vander Voort, Laila Schenkel, Jim Wilton, Laura Cain and Flavio Schenkel (University of Guelph)

Activation of the hypothalamic-pituitary-adrenal axis (HPAA) and subsequent glucocorticoid production during stress has a significant impact on behavior, metabolism and immune function. Moreover, dysregulation of the HPAA can negatively influence both human health and animal health and production. Animal studies have demonstrated considerable variation in stress-induced cortisol responses, and the phenotype appears to be moderately-to-highly heritable. Therefore, the objective of the current study was to identify key genetic determinants in sheep that putatively influence the cortisol response to endotoxin-induced stress - a model of acute bacterial infection. The ovine50K BeadChip containing 54,241 single nucleotide polymorphisms (SNPs) was used to genotype 75 sheep with high (HCR) and low cortisol responses (LCR) (38 HCR and 37 LCR). A total

of 18 SNPs were identified to be significantly associated with the cortisol levels in this population. These SNPs were grouped into 8 chromosomal regions based on proximity on the same chromosome and important genes such as *CD14*, *ITGAM*, *SNX2* and *ITGAL* were found to reside within the identified QTLs. A candidate gene approach was subsequently used with 3 novel SNPs in *SNX2*, and SNP (ss# 974768716) was found to significantly associated with the cortisol response phenotype (p=0.0004).

P6060 GO annotation and WGCNA clustering of RNAseq data in response to Porcine Reproductive and Respiratory Syndrome (PRRS). Martine Schroyen, Christopher Eisley and Eric Fritz-Waters (Iowa State University), Igseo Choi (ARS, USDA), James Koltes (Iowa State University), Nick Boddicker (Genesus, Inc.), James Reecy (Iowa State University), Joan Lunney (ARS, USDA) and Susan Carpenter, Peng Liu, Jack Dekkers and Christopher Tuggle (Iowa State University)

As part of the PRRS Host Genetics Consortium (PHGC), a region on SSC4 surrounding SNP marker WUR10000125 (WUR) has been identified to be strongly associated with both weight gain and PRRS viral load. Subsequently, an RNAseq experiment was performed on globin reduced wholeblood RNA samples collected on 0, 4, 7, 10 and 14 days post infection (dpi) of eight littermate pairs. Each pair contained a pig with a favorable (AB) and a pig with an unfavorable (AA) WUR genotype. After data normalization, removing lowly expressed genes, and model adjustments for pre- and post-globin reduction RNA Integrity Number, and 5'-3' read skewness, 8,997 annotated transcripts were retained. Analyzing transcripts with a false discovery rate (FDR) of <0.05 for each dpi compared to day 0 showed enrichment of GO terms (p<0.05) for inflammatory response in each comparison, with higher enrichment scores and more specific terms, such as regulation of NFκB, cytokine and chemokine activity, at 4 and 7 dpi. At 10 and 14 dpi, GO term enrichment indicated a switch to DNA damage response, cell cycle checkpoints, and DNA replication. However, few enriched GO terms were seen in genes with significant WUR genotype effects (FDR<0.05) for any individual day or across days. To elucidate regulatory networks differing between samples based on WUR genotype, a weighted gene co-expression network analysis (WGCNA) was performed. For all dpi - day 0 comparisons, this analysis identified gene clusters whose averaged expression pattern was significantly correlated with WUR genotype across all pigs, with the strongest correlations at 4 dpi. The module with the highest correlation with WUR genotype (0.70, p=0.01) displayed weakly enriched GO terms (p<0.1) for B, T and NK cell signaling pathways, which may indicate the existence of co-regulation networks affected by SSC4 WUR genotype. Acknowledgements: PHGC for samples and PIC/Genus for funding the RNA sequencing.

P6061 Pigmentary Chorioretinopathy in the Chinese Crested dog: Whole genome re-sequencing to reveal candidate loci.. Merina Shrestha, Agnese Viluma and Shumaila Sayyab (Swedish University of Agricultural Sciences), Marcin Kierczak and Leif Andersson (Uppsala University), Kristina Narfström (University of Missouri-Columbia) and Göran Andersson (Swedish University of Agricultural Sciences)

Pigmentary chorioretinopathy is an eye disease described in the Chinese Crested Dog that appears to be inherited in an autosomal recessive mode. The morphological characteristics include degenerative changes in the choroidal structures and in the retinal pigment epithelium (RPE), followed by progressive degeneration of rods and cones. With a genome-wide association study, we have previously identified a 300 kb region with two SNPs highly associated on chromosome 8 (*Praw* 8.4 x 10-6 and 1.2 x 10-5) and a region on

chromosome 5, where the top associated SNP *(Praw* = 3.1 x 10-5) is located in an intron of a gene that has been implicated in several diseases including inherited eye diseases. Re-sequencing of all known exons from these two genes did not reveal any obvious causative mutation for the disease. Here we present a different approach using whole genome sequencing of two unrelated sib-pairs where one parent was an obligate carrier and the other was affected by pigmentary chorioretinopathy. Each sib-pair, consisted of one affected and one healthy individual. For each of the four dogs included in the study, a 200 bp fragment library was constructed and sequenced on the Ion Proton™ System using two Ion PI Chips. The reads were aligned to the canine reference sequence (CanFam3.1) using TMAP included in the Torrent Suite Software. The sequencing resulted in a mean coverage of 8X (6X after duplicate removal) for each dog. GATK and SAMtools were used to call SNPs and INDELs and ANNOVAR was used to functionally annotate sequence variants in Ensembl genes. We will present the results from this ongoing study.

P6062 Frequencies of candidate gene variants for ovine lentivirus susceptibility in German sheep breeds. Gesine Lühken, Marwa Eltanany and Susanne Hübner (Department of Animal Breeding and Genetics, Justus Liebig University of Gießen)

Variants in or near the genes *CCR5*, *TMEM154* and *ZNF389* have been reported to be associated with the serological status or the provirus concentration of ovine lentivirus (OvLV) in U.S. sheep. They aim of the current project was to test if the allele frequencies of those gene variants are in accordance with the known OvLV susceptibility level of some sheep breeds reared in Germany.
32 unrelated sheep each of 3 sheep breeds known to be less susceptible for OvLV (German Grey Heath, Merinoland, Suffolk) and of 3 sheep breeds known to be highly susceptible for this disease (Cameroon, East Friesian Milk, Texel) were genotyped. Insertion-deletion polymorphisms in the *CCR5* promoter ("aatg") and near *ZNF389* ("at") were determined by fragment length analysis. A nucleotide substitution causing an amino acid substitution in *TMEM154* (E35K) was genotyped by allele-specific PCR.
In the groups of less susceptible and highly susceptible sheep breeds, the mean frequency of the allele considered to confer a lower OvLV susceptibility was 14.9 and 5.8 % (*CCR5* deletion), 75.4 and 21.1 % (*TMEM154* 35K), and 46.3 and 54.6 % (*ZNF389*insertion), respectively. For *CCR5* and *TMEM154*, but not for *ZNF389*, the results are mainly in accordance with the known breed differences in OvLV susceptibility. However, the frequency of the CCR5 deletion in German Grey Heath (7 %) was in a similar range as East Friesian Milk and Texel. Additionally, the frequency of *TMEM154* 35K in Suffolk (43.5 %) was lower than expected from the published frequency in this breed and the known OvLV susceptibility level. This may be compensated by the highest frequency for K at position 35 in*TMEM154* (98.4 %) in German Grey Heath and the highest frequency for the *CCR5* deletion (21.9 %) in Suffolk. The frequency of the *ZNF389* insertion which has been shown before to be associated with lower OvLV provirus concentration unexpectedly was highest in Texel (77.6 %). Altogether, these results warrant further study.

P6063 The elastin gene promoter in draught horses affected with chronic progressive lymphedema. Kirsten De Keyser, Anneleen Stinckens, Tom Luyten and Nadine Buys (KU Leuven)

Several draught horse breeds suffer from chronic progressive lymphedema (CPL). In the Belgian Draught Horse, a genetic susceptibility for CPL was demonstrated. Until now, molecular genetic research in other affected breeds did not reveal

any CPL associated polymorphisms. Clinical CPL signs are related to marked quantitative and qualitative dermal and perilymphatic elastin alterations (histology of skin). Therefore, the elastin gene (ELN), which has not been investigated in chronic lymphedema affected horses or men, is a good candidate gene for CPL. In men, ELN transcription initiation is mediated by multiple transcription activation sites, suggestive for a complex transcriptional mechanism. The promoter region in the horse is largely unknown (www.enseml.org, ENSECAG 00000011106). However, ELN is known to be highly conserved between species. Therefore, based on the human ELN promoter sequence (www.ncbi.nlm.nih.gov, J05453.1), we searched for the homologous region in the equine genome using a blast algorithm (www.ensembl.org). We performed an in silico analysis to determine homologous transcription activation sites, and sequenced the expected equine promoter in Belgian Draught Horses and Thoroughbred Horses. The equine promoter region was expected at -2.260bp to the start of exon 1, analogous to the human promoter sequence from NCBI. Homologous transcription activation sites from in silico analysis were confirmed by sequence analysis in both Belgian Draught and Thoroughbred Horses. We demonstrated polymorphisms in the Belgian Draught Horse ELN promoter region compared to the equine ensebml reference genome, but those mutations were not typical for this CPL susceptible breed, as they also occurred in Thoroughbred Horses.

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P7001 Construction of lentiviral vector-mediated RNA interference targeting *Pit-1* gene of pigs*(Sus scrofa)*. Haiguang Mao, Yifei Shen,Jing Peng and Ningying Xu (Zhejiang university)

Pituitary-specific Transcription Factor *(Pit-1)* is one of the important transcription factors of GH, PRL and THS gene. *Pit-1* plays an important role in animals' growth, development and reproduction via regulating the expression of these genes. Through blocking the expression of target genes in post-transcriptional level, RNA interference can make the targeting gene expression partly or completely lost. Lentivirus-mediated RNAi is currently the most effective means of the interference, which can achieve highly silencing and maintain target gene silencing for a longer period of time.Two pairs of shRNA sequences targeting *Pit-1* gene were designed and synthesized. By annealing and digesting, the double-stranded shRNA was inserted into the lentiviral vector pHBLV-U6-ZSGreen. After transformation of *E.coli* competent cells, screening of positive colonies and amplifying operation, the plasmids were extracted for DNA sequencing. Then the correct recombinant lentiviral vectors and lentiviral packaging plasmids were transfected in 293T cells. The supernatant that contains the virus particles was concentrated, and the titer of viral particles was determined by Real-time PCR.Finally,restriction enzyme digestion and sequence analysis demonstrated that the recombinant pHBLV-sh*Pit-1* vector was successfully constructed.

P7002 Transgenic expression of fat2 and fat1 gene significantly increase polyunsaturated fatty acids content in pigs. Fei Tang, Xiaoyan He, Zicong Li , Dewu Liu and Zhenfang Wu (South China Agriculture University)

Polyunsaturated fatty acids (PUFAs) are good for human health. Increasing the PUFAs content in our foods, such as pork, will help to improve our health. ω-3 and ω-6 PUFAs are two type of important PUFAs, which are synthesized by two key enzymes, *fat1* and *fat2*. The purpose of this study was to use the transgenic technology to produce pork that is rich in the polyunsaturated fatty acids. First, we cloned the *fat2* and *fat1* gene from *C. elegans*, and constructed a vector carrying a *CMV* promoter-driving *fat2-fat1* fusion gene, and the *Neo-EGFP* fusion gene. After transfection of the vector into Laiwu pig's fetal fibroblast cells and selection with G418, we collected the transgenic cells and measured the PUFAs content with GC-MS. PUFAs content in transgenic cells is significantly higher than that in wild-type control cells. Using the transgenic cells as nuclear donors, we produced 5 transgenic Laiwu pigs expressing *EGFP* by somatic cell nuclear transfer (SCNT). PCR showed that all transgenic pigs carry the *fat2-fat1* transgene, and *fat2-fat1*is expressed in the ear and tail tissues of transgenic pigs, as demonstrated by RT-PCR. We euthasized one of the survived transgene pigs at the age of 120 days to measure the PUFAs content in muscle and fat tissues. The result showed that the content of ω-3PUFAs and ω-*6* PUFAs in the muscle and fat tissues of transgenic pigs were significantly higher than that in wild-type control pigs.

P7003 A surrogate reporter with dual-reporter genes for effcient enrichment of genetic modified cells. Chonghua Ren, Kun Xu, Zhongtian Liu and Zhiying Zhang (Northwest A&F University)

Zinc-finger nucleases (ZFNs), TAL-effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR) /CRISPR-associated (Cas) systems are powerful tools for mammalian genome engineering. However, wild-type and genetic modified cells induced by these programmable nucleases are often phenotypically indistinguishable, hampering isolation of mutant cells. Hyojin Kim

et al. designed a surrogate reporter system based on the fluorescent proteins. However, this method requires the instrument flow cytometry, which is not always available in some labs. Here we introduced a novel surrogate reporter system with dual-reporter genes, puromycin resistant and fluorescent genes, which can be used for the efficient enrichment of genetic modified cells either by drug resistance selection or by flow cytometry isolation.The surrogate reporter plasmids contain two fluorescent genes, RFP and GFP, and a puromycin resistant gene (RPG: RFP, PuroR, GFP). We constructed both the NHEJ-RPG reporter and SSA-RPG reporter plasmids with different length of direct repeats. The target sequence was inserted in the N-terminus of puromycin resistant gene, leading the disruption of the open reading frame. The functional PuroR and GFP expression could be induced either by the programmed nuclease triggered NHEJ or SSA DNA repairing. We found the SSA-RPG reporter with a higher repair efficiency than the NHEJ-RPG, and the minimal length of direct repeats required for high efficient SSA-mediated repairing is 200bp.We used the optimized surrogate reporter for enrichment of the genetic modified cells, and the results demonstrated that CRISPR/Cas9-driven mutations were increased by~30 times detected by the T7E1 assay after the puromycin selection. The dual-reporter system is also available for ZFNs and TALENs-mediated genetic mutation enrichment.

P7004 Knockout of *Xist* gene by TALEN to improve pig cloning efficiency. dan Wu (South China Agriculture University), cong Li (National Engineering Research Center for Breeding Swine Industry Guangzhou, China), juan Yuan and Hua Huang (South China Agriculture University), wu Liu (National Engineering Research Center for Breeding Swine Industry Guangzhou, China, South China Agriculture University) and zhenfang Wu (National Engineering Research Center for Breeding Swine Industry Guangzhou, China)

Somatic cell nuclear transfer (SCNT) has been developed to clone sheep, mice, cattle, pigs and other mammals. However, the efficiency of SCNT is currently still very low. The SCNT efficiency in pigs is about 1-2%. Studies on mice showed that *Xist* gene is abnormally expressed in cloned embryos and inhibition of *Xist* expression in donor cells or in early cloned embryos can significantly increase SCNT efficiency nearly by 10 folds. We have previously showed that *Xist* expression level in cloned porcine blastocysts is higher than that in IVF-derived blastocysts, and injection of anti-*xist* siRNA into cloned porcine embryos can increase their developmental potential in vitro. The purpose of this study was to test whether depression of *Xist* in donor cells also can increase the cloning efficiency in pigs. We designed and constructed two TALEN plasmids targeting to the porcine *Xist* gene. Each targeting plasmid was transfected into porcine fibroblast cells, and the targeting site on *Xist* gene was analyzed by PCR and sequencing. The results showed that both TALEN plasmids cause mutation on the targeting site of *Xist* gene at a rate over 10%. This result suggests that the TALEN plasmids constructed in the present study can be used to produce *Xist*-null porcine somatic cells, which can be used as donor cells to test whether inhibition of *Xist* expression can increase the SCNT efficiency in pigs. Currently, we are using the *Xist*-targeted TALEN plasmids to tansfect and select *Xist*-knockout pig fibroblast cell colonies.

P7005 Study on the expression of *Cdc20* during *Cashmere Goat* oocyte maturation in vitro. Biao Wang (Inner Mongolia University), Yunxia Li (Inner Mongolia Saikexing Reproductive Biotechnology Co. Ltd), Yanglin Chen and Fang Xu (Inner Mongolia University) and Xihe Li and Yanfeng Dai (Inner Mongolia University and Inner Mongolia Saikexing Reproductive Biotechnology Co. Ltd)

The anaphase-promoting complex or cyclosomes (APC/Cs) is an ubiquitin ligase which controls kinds of cells division transition from metaphase to anaphase. *Cdc20* activate APC/Cs when spindles and chromosomes connect correctly in metaphase, then chromosomes begin separating. This is a very important mechanism which make sure cell cycles finish normally in mitosis. And also, it plays a similar role in meiosis. In this study, we analysis the expression of *Cdc20* in *Cashmere Goat* oocyte during its maturation in vitro. We construct Myc-*Cdc20* vector, then we overexpress *Cdc20* in the oocyte by microinjection after transcripting Myc-*Cdc20* into mRNA. Beside, we detect the expression of *Cdc20* in GV, GVBD, MI and MII stage oocytes by western blot. Firstly, we ascertain the ratio of maturation and the time to GVBD, MI and MII in vitro. The results show that the maturation rate is 75% in our culture condition, which going to GVBD stage needs 8h, then 18h go to MI, about 24 h 75% of these oocytes discharge first polar body. Then we microinject Myc-*Cdc20* mRNA into GV stage oocytes and culture it in vitro. 10h later, we can detect the expression of mRNA of Myc-*Cdc20*. However, the time and ratio of oocyte maturation are no differences compared with control. In addition, we detect the expression of *Cdc20* of GV, GVBD, MI and MII stage oocytes following cultured in vitro by western blot. We can see that *CDC20* protein is expressed in *Cashmere Goat* oocyte all the times of GV to MII stage. Taking all above-mantioned into consideration, we can conclude that overexpression of *Cdc20* alone has no effect on the oocytes maturation of *Cashmere Goat* in vitro, but it should be an important fator which ensure meiosis complete successfully.

P7006 RNA interference targeting foot-and-mouth disease virus replication in epithelial cells of transgenic goat. Wenting Li , Shimeng Kang, Hongbing Han and Zhengxing Lian

It is well-known that RNA interference (RNAi) targeting Foot-and-mouth disease virus (FMDV) genome could protect experimental animals (e.g. suckling mice), but no report showed whether RNAi-mediated transgenic goat, mainly infected host species, could resist FMDV replication. Principal Findings: Through prokaryotic microinjection, shRNA which highly inhibited FMDV-3D expression in vitro was employed and 61 goats were produced. Out of these, 3 transgenic goats were selected as transgenic group (Tg) detected by southern blotting and even-aged 3 non-transgenic goats (NTg) as control. Primary tongue epithelial cells were isolated and pan-cytokeratin positive rate were 83.3% by immunofluorescence. Targeted shRNA expression in transgenic goat tongue epithelial cells was identified by northern blotting, in situ hybridization and quantified by qPCR. Next, virus imitation challenge in tongue epithelial cells with psicheck2 which inserted FMDV-3D sequence confirmed that shRNA had stable inhibiting effect on FMDV-3D expression (siRNA inhibitor as reference). Based on inoculating tongue epithelial cells with 10,100 and 1000 TCID50 FMDV, we then observed the change of viral titers in supernatant and virus copy numbers in cells. We found shRNA significantly inhibited virus replication and RNA synthesis that inhibition rate reached 92.13%±3.95% at 48h. Inflammation cytokines (including IL-6, TNF-α and TGF-β1) expressions significantly down-regulated post-challenge 48h normalized to 0h. And, Tg remained higher expression than NTg that resulted from virus replication was interfered in Tg cells and heavy virions decayed NTg cell. Furthermore, we inferred FMDV was recognized by toll-like receptor 7 (TLR7) in cells based on the TLR7 and TRAF6 qPCR results. Conclusions: RNAi mediated transgenic goats tongue epithelial cell targeting viral 3D gene were able to resist FMDV infection.

P7007 Alteration of fucosylated protein profile in the cattle milk against *Helicobacter pylori* infection. Ran Zhang (China Agricultural University), Zhanna Bugaytsova and Anna Shevtsova (Umea University), Yunping Dai (China Agricultural University), Olena Rakhimova (Umea University), Yaofeng Zhao (China Agricultural University), Thomas Boren (Umea University), Lennart Hammarström (Karolinska Institute) and Ning Li (China Agricultural University)

Human milk is extremely rich in oligosaccharides and glycoproteins that are synthesized by a variety of glycosyltransferases in the lactating mammary gland. Increasing data suggest that some of these oligosaccharides and glycoproteins, especially the fucosylated glycans, have a great influence on colonization of pathogenic microorganisms. Lewis b blood group antigen is a type of glycoproteins synthesized by the α-1, 3/4-fucosyltransferase encoded by the *FUT3* gene, which acts as an important receptor to mediate the access of *H.pylori* to gastric epithelial cells. In this study, two transgenic cattle with the human *FUT3* gene were generated using the pBC1 vector under control of a mammary gland specific promoter. The integration of the transgene in the cattle genome was confirmed by PCR, Southern blot and FISH mapping. The *FUT3* transgene and Lewis b antigen were both detected in mammary epithelial cells and milk by Western blot, respectively. The inhibitory activity against *H. pylori* binding to HSA-Leb by transgenic whey was observed using the RIA assay, with the highest inhibitory capacity of up to 50 times better than that of normal whey. In addition, transgenic whey also showed efficient clearance of *H. pylori* bacterial cells in binding to human gastric tissue sections. Interestingly, inhibitory capacity of both transgenic colostrum and normal colostrum are very similar in inhibition activity but slightly lower than the inhibitory capacity of the transgenic whey, which suggests that the transgenicity is not an active part of colostrum milk, but instead makes a difference in whey milk. Our data provide a new concept and feasibility of production of humanized cattle milk for healthcare and anti-microbial purposes.

P7008 Knockout of Xist gene by CRISPR/CAS9 to improve the efficiency of somatic cell nuclear transfer in pigs. Sheng Ni (South China agricultural university) and Zicong Li, Zhihua Huang, Yujuan Yuan, Dewu Liu and Zhenfang Wu (South China Agricultural University)

Somatic cell nuclear transfer (SCNT) has been used to produce cloned sheep, mice, cattle, pigs and other mammals. However, the efficiency of SCNT is currently still very low. The SCNT efficiency in pigs is about 1-2%. Studies on mice showed that Xist gene is abnormally expressed in cloned embryos and inhibition of Xist expression in donor cells or in early cloned embryos can increase SCNT efficiency nearly by 10 folds. The purpose of this study was to test whether depression of Xist in donor cells also can increase the cloning efficiency in pigs. We designed and constructed four CRISPR/CAS9 plasmid systems targeting to the porcine Xist gene's important functional regions. Each targeting plasmid was transfected into porcine fibroblast cells, the targeting site on Xist gene was analyzed by PCR and sequencing. The results showed that all four CRISPR/CAS9 plasmid systems cause mutation on the targeting site of Xist gene at a rate over 40%. This result suggests that the four CRISPR/CAS9 plasmids constructed in the present study can be used to produce Xist-null porcine somatic cells, which can be used as donor cells to test whether inhibition of Xist expression can increase the SCNT efficiency in pigs. Currently, we are using the Xist-targeted CRISPR/CAS9 plasmids to tansfect and select Xist-knockout pig fibroblast cell colonies.

P7009 Analysis of F1 generation of transgenic pigs that specifically express phytase - glucanase - xylanase in their salivary glands. Dehua Wang, Xiangwei Zhang, Zicong Li, Dewu Liu and Zhenfang Wu (South China Agricultural University)

To increase feed utilization efficiency and reduce manure pollution to environment, we have produced transgenic cloned pigs carrying a phytase gene, a glucanase gene and a xylanase gene, which were linked by a *2A* peptide and controlled by a salivary glands-specific promoter. The transgenic pigs also carry a *CMV* promoter controlled *neo-2A-EGFP* selectable marker gene. Reverse *PCR* and sequencing result showed that the transgene construct was inserted by a single copy manner at the chromosome 7 in all transgenic founder *(F0)* pigs. Green fluorescence was observed on the transgenic pigs, and high activity of phytase, glucanase and xylanase was detected in the saliva collected from transgenic pigs. The transgenic founder pigs have a significantly higher feed digestibility and a significantly lower phosphorus and nitrogen emission in their manure, as compared to non-transgenic pigs. To assess whether the transgene is stably transmitted and expressed in transgenic pigs, a transgenic founder boar was used to mate with a wild-type sow and produced 11 offsprings. Six of them inherited all the transgenes inserted at chromosome 7, as identified by PCR and southern blot. These six *F1* transgenic pigs also expressed high level of phytase, glucanase and xylanase in their saliva. However, only two of the six *F1* transgenic pigs expressed *EGFP*. These results suggest that the exogenous genes can be stably transmitted from *F0* to *F1* generation, and three enzyme genes are stably expressed in salivary glands of *F0* and *F1* transgenic pigs. However, the *EGFP* selectable marker gene might be silenced in some of the *F1* transgenic pigs. The DNA methylation status of promoter driving *EGFP* expression in *F1* transgenic pigs should be examined in the coming studies.

P7010 Optimization of the sgRNA structure of the *Streptococcus thermophilus* CIRSPR/Cas9 system for efficient genome editing in eukaryotes. Kun Xu, Lijun Guo, Zhongtian Liu and Zhiying Zhang (Northwest A&F University)

The CRISPR/Cas system has recently emerged as a powerful tool for genome engineering, but most of the works reported thus far have focused on the system derived from a single *Streptococcus pyogenes* species, and few studies were conducted to investigate the structure of the chimeric single guiding RNA (sgRNA). Here, we would like to report the optimization of the sgRNA structure derived from the *Streptococcus thermophilus* CRISPR/Cas9 (StCas9) in yeast and its high genome engineering efficiency in mammalian cells. In order to investigate the *Streptococcus thermophilus* CRISPR/Cas system in yeast, we initially designed a yeast cell assay system, a short chimeric RNA (scRNA) according Jinek's research and a long chimeric RNA (sgRNA.WT) by joining the *Streptococcus thermophilus* CRISPR3/Cas tracrRNA and crRNA. The sgRNA.WT succeeded while the scRNA failed to guide the StCas9 activity in yeast. Then, we conducted an exhaustive and systematic investigation of the sgRNA structure, including the minimal length of tracrRNA, Loop structure, Match II region, Bulge motif, the minimal length of guide sequence, tolerance of mismatches and target sequence preference. Results suggested the full length of tracrRNA, a MatchII region of 4-5 base pairs, the Bulge motif, and a minimal guide sequence length of 19 nt are required for the StCas9 activity. A simple shorter chimeric RNA was finally designed, which was similar to that used in *Streptococcus pyogenes* CRISPR/Cas9 system. Coupled with the codon humanized StCas9, the optimized sgRNA design achieved over 12% and 40% targeting efficiencies on reporters in yeast and human

293T cells respectively. Further study with human *AAVS1*, *CCR5* and mouse *IGF2*, *ROSA26* loci demonstrated efficient StCas9 activities in both surrogate reporter assay and chromosome targeting. Our works provide important insight into the sequence and structural requirements necessary to the sgRNA design for guiding the targeted and efficient eukaryotic gene editing using the typeII CRISPR/Cas system.

P7011 Efficient production of transgenic pigs by cytoplasmic injection of *piggyBac* transposase based p*m*GENIE-3 plasmids. Fang Zeng and Zicong Li (South China Agricultural University), Junsong Shi (Guangdong Wen's Research Institute) and Dewu Liu, Chong Wang and Zhenfang Wu (South China Agricultural University)

Production of transgenic animals involves the introduction of a foreign gene, the transgene into the genome of animals. Pronuclear microinjection (PNI)-mediated gene transfer is the predominant method used to produce transgenic animals. However, this technique does not always result in germline transgenic offspring and has a low efficiency for livestock. Alternate approaches such as somatic cell nuclear transfer using transgenic fibroblasts do not show an increase in success rate compared to PNI, while viral-based transgenesis is hampered by issues regarding transgene size and biosafety considerations. We have recently described highly successful transgenic experiments with mice using a *piggyBac* transposase-based vector, p*m*GENIE-3. This construct, a single and self-inactivating plasmid contains all the transpositional elements necessary for successful gene transfer. In this series of experiments we have employed cytoplasmic injection of p*m*GENIE-3 for transgene delivery into pig zygotes. More than 8.00% of the injected embryos developed into transgenic animals containing monogenic and often single transgenes in their genome. This level by far exceeds PNI transgenesis rates of approximately 2.00% previously reported in the literature. In summary, here we have described a method that is not only easy to implement, but also demonstrated the highest efficiency rate for non-viral livestock transgenesis.

P7012 Effects of diets and breeds on the small intestinal expression of three monosaccharide transporters in chicken. Jin Cheng, Yitong Yuan, Rong Luo, Lihuan Zhang, Zhiwei Zhu and huifeng Li (Shanxi Agricultural University)

Three transporters, *SGLT1*, *GLUT2* and *GLUT5* (Na+-dependent glucose and galactose transporter 1, Na+-independent glucose, galactose, and fructose transporter 2 and 5) were closely related to the monosaccharide absorption in chicken small intestine. In order to study the expression changes of these genes and their effects on growth traits, 4 treatments（two commercial diets for broiler and layer were fed separately to Aviagen and Leghorn birds）were constructed by using orthogonal experimental design. The mRNA expression of *SGLT1*, *GLUT2* and *GLUT5* were determined by real-time PCR and the traits including body weight, the small intestine weight and length were measured at d1, d3, d7, d14 and d28. The results: (1) the differences of the mRNA expression of *SGLT1*, *GLUT2* and *GLUT5* were significant (P<0.01) at differently developmental stages. (2) The mRNA expression curves have a peak before d7, then slowly declined and stabilized at a certain level. The mRNA expression differences among these 4 treatments mainly occurred at early period, and the changes on Aviagen seems happened earlier than Leghorn. (3) Compare to Leghorn, Aviagen birds were more sensitive to diets, for the expression of these genes were affected by diets on Aviagen treatments (P<0.05). (4) With the same diets, the *GLUT5* was differentially expressed between Aviagen and Leghorn. (5) Negative correlations were obtained between the mRNA expression of

these genes and the body weight, the small intestine weight and length.

P7013 Effects of cow-derived recombinant human lactoferrin on osteoblast growth and bone status in a piglet model. Qiuling Li, Jie Zhao and Wenping Hu (China Agricultural University), Jianwu Wang and Tian Yu (Wuxi Kingenew Biotechnology Company) and Yunping Dai, Qingyong Meng and Ning Li (China Agricultural University)

Lactoferrin (LF) is an 80-kDa iron binding glycoprotein with many biological effects such as antimicrobial and immunomodulatory activities. Recently, scientists showed that LF is also a regulator of bone cell activity, suggesting its therapeutic effect on postmenopausal bone loss. However, little is known about the effects of recombinant human LF (rhLF) supplementation on bone status in young healthy infants. Our laboratory has produced transgenic cow harboring a BAC of hLF gene and secreting rhLF at the concentration of 3.4 g/L in the milk.We found that osteoblast cell proliferation and differentiation were significantly promoted in vitro, when rhLF supplementation at concentration of 200 and 400 μg/ml. Furthermore, rhLF rapidly induced phosphorylation of ERK1/2 in human osteoblast cells. In order to investigate the effects of rhLF on bone status in vivo, piglet, a valuable model for human infants study, were supplemented with rhLF milk for 30 days. Serum calcium, dual-energy X-ray absorptiometry (DEXA) and three-point bending biomechanical test showed that concentration of serum calcium, bone mineral density, bone mineral content and bone strength of tibia were increased by 20.33%, 14.81%, 28.57%, and 38.39% by rhLF milk supplementation. In addition, the overall metabolite profiling in plasma upon rhLF milk supplementation was performed. For a total of 321 metabolites, rhLF milk supplementation resulted in significant alterations. The plasma metabolomic profiles in piglets fed with rhLF milk had higher levels of essential amino acids, energy metabolites, and antioxidants. In particular, levels of branched chain amino acids that promote skeletal muscle growth and lysine that promote calcium absorbing were increased by rhLF milk supplementation. Hydroxyproline and pro-hydroxy-pro, the markers of bone collagen degradation, were decreased. In conclusion, these findings suggested that rhLF could have a beneficial effect on neonate bone health by modulating bone formation.

P7014 The construction of mammary gland expression vector *pBC-AANAT* and functional verification. Changjiu He, Jing Wang, Kuanfeng Zhu, Yao Fu, Yile Song and Guoshi Liu (College of Animal Science and Technology, China Agricultural University)

Arylalkylamine N-acetyltransferase (AANAT) is a crucial rate-limiting enzyme for melatonin biosynthesis. In mammals, AANAT transformed 5-hydroxytryptamine (5-HT) to N-acetylserotonin, the precursors of melatonin. In this reaserch, we successfully cloned the gene of sheep (*Ovis*) *AANAT* and constructed it into mammary gland expression vector *pBC1* using single enzyme digestion method. The reconstructed vector then transfected into goat mammary epithelial cell for cell culture tested. Total RNA was extracted from sheep pineal gland by using the TRIzol reagent and reverse transcribed into cDNA. The complete coding sequence (CDS) of *AANAT* were amplified using the premier: Forward-CCGCTCGAGCCACCATGTCCACG CCGAGC; Reverse-CCGCTCGAGCGGTCAGCGGTCAC TGTTCC. Restriction site *XhoI* was introduced in forward and reverse primer and kozak seuqence-GCCACC was introduced in forward primer. The PCR products then subcloned into pMD 19-T simple vector. Though analysis with DNA sequencing, the CDS of *AANAT* include 618 bases in tatal and code 206 amino-acids.

Comparison with previously published sequence showed 99.84% homologies in nucleotide sequence and 100% in amino acid sequence respectively. The *AANAT*fragments in 19-T simple vector then digested with *XhoI* and constructed into mammary gland expession vector *pBC1*. To verify the availability of *pBC1-AANAT*, we transfected it into goat mammary epithelial cells, the cells were collected for qRT-PCR after 24h, compared with control group, the ralative mRNA amounts of *AANAT* was significantly increased by 62.34 ±7.52 times; We also detected the melatonin contents of cell cuture medium by HLPC, the result show a markedly rise of melatonin level (45.31 ± 2.32ng/ml *vs* 36.14±1.82ng/ml), which indicate the transgenic cell gained the ability to synthesis more melatonin. As described above, *pBC-AANAT* is available for transgenic animal production or melatonin function research.

P7015 Production of transgenic pigs expressing double fluorescence genes by injection of lentivirus into 2-cell embryos. xiaoyu Chen, Zhiwei Zhu, Fuxian Yu, Jing Huang, Xiaorui Hu and Jianzhi Pan (Institute of Animal Husbandry and Veterinary, Zhejiang Academy of Agricultural Sciences)

Production of transgenic pig is time-consuming and expensive work because of the inefficiency to introduce exogenous gene into genome either using pronuclear injection or nuclear transfer methods. Recently, lentiviral vector has been demonstrated to be a better tool to generate transgenic animals. The objective of this study was to produce transgenic pigs using lentiviral vector which can express two fluorescent protein genes, DsRed and Venus simultaneously. A recombinant lentivirus containing DsRed and Venus genes was injected into perivitelline space of 2-cell porcine embryos and injected embryos were transplanted to two recipients with a number of 16 embryos per sow. One recipient gave birth to 3 piglets and two funders were identified by PCR amplification of transgenes. The percentage of funders in litter was as high as 66.6%. A PCR-positive female funder was naturally mated with a wild type boar and gave birth to 2 litters totaling 23 piglets, among which 11 F1 piglets were identified as transgenic individuals by PCR detection for transgene with a positive rate of 47.8%. The mRNAs of the two exogenous fluorescent protein genes were detected in all of the tested tissues separated from PCR-positive F1 piglets. The expression of two fluorescent proteins was also visible under fluorescence microscope using frozen tissue sections prepared from the transgenic F1 piglets. In addition, the foreign genes integrated in genomes of F1 piglets were analyzed by southern blotting. The result revealed that there were 3 to 5 copies of transgene including in the host genome. This is the first report of generating transgenic pigs simultaneously expressing two reporter genes by lentivirus-mediated transgenesis. These results indicate that injection of lentivirus into 2-cell embryos is an efficient method to deliver multiple exogenous genes to animals.

P7016 Construction of gene modified porcine fetal fibroblast via CRISPR-Cas9 system. Guanglei Li and Xinyun Li (Huazhong Agricultural University), Chuxin Liu and Yong Li (BGI-Shenzhen) and Changzhi Zhao, Changchun Li and Shuhong Zhao (Huazhong Agricultural University)

In mammals, the target genes can be genetically modified or transcriptionally modulated easily using these nucleases, especially using CRISPR technology. Although many genes have been knockout successfully using CRISPR approach, there were few reports on gene knock-in by this technology. We tried to test the possibility of gene knock-in using CRISPR technology in the pig in this study. Lactoferrin encoded by *LTF* gene is one of the important components in milk. It plays important roles in defensing against the

bacterial infection of new born piglets. In order to make a transgenic pig with high level of Lactoferrin in milk specifically, we tried to integrate the *LTF* CDS into the stop codon of *CSN1S1* gene blanked by a short peptide adaptor of 2A. By far, we designed a guide RNA (gRNA) which contains the target sequence of the *CSN1S1* gene. This gRNA is under control of the promoter of U6 and it was cotransfected into the fibroblast of pig with *Cas9* vector. The efficiency of CRISPR was detected using NHEJ method. According to our results, the splice efficiency is about 10% when vectors were transfected using lipo2000, while it can be raised to 40% when transfected using nucleofaction method. The recombination vector was constructed, which contains the LTF CDs flanked by the homolgous arms of *CSN1S1* gene. In order to increase the integrate efficiency, the DTA element was inserted into the recombination vector. Finally, we cotransfected three vectors into the porcine fetal fibroblast using nucleofaction method, and we obtained the positive cell clones after selection. Therefore, the CRISPR technology can be used for gene knock in in the pig.

P7017 Structure and function analysis of recombinant human BSSL in milk of transgenic mice and transgenic cloned cattle. Yuhang wang, wenjie liu and Ning Li (China Agricultural University)

Bile salt-stimulated lipase (BSSL) is an enzyme produced by the adult pancreas and breast milk, aiding in the digestion of fats. BSSL have broad substrate specificity and are capable of hydrolyzing triacylglycerides, esters of cholesterol and lipid soluble vitamins. BSSL present within milk may compensate for the low levels of other TG-digesting enzymes and aid newborns in lipid absorption. We had constructed human *BSSL* expression vector which specifically expresses BSSL in the mammary gland of transgenic mice and cattle at a high level. The transgenic mice can express recombinant human BSSL at a level of 0.98 mg/ml and the mean lipase activity is about 810,000U/L. By feeding experiment, we found that BSSL overexpression in the mouse milk can improve the survive rate and growth rate of premature pups in the neonatal period while has a side effect on the growth of term pups. We are probing into the molecular mechanism of the phenomenon. By somatic cell nuclear transfer (SCNT), we produced transgenic cloned cattle, which specific express BSSL in their milk. The recombinant human BSSL was purified from the cow milk by heparin-Sepharose chromatography and Superdex 200, purity about 99% and activity about 1408.0 umol min-1 mg-1. Compare with native human BSSL, the recombinant human BSSL has a relatively low level of glycosylation and smaller molecular weight. But there are little difference in the lipase activity and other physiological characteristics between the native and recombinant ones. In the future, we will do further comparative physiological and function studies with the native human enzyme to ascertain that the bovine enzyme has the properties making it a candidate for future therapy of fat malabsorption for physiological reasons, e.g. premature babies; e.g. cystic fibrosis.

P7018 Establishment of Chicken Genetic Engineering Technology. Fei Gao and Sen Wu (China Agricultural University)

The chicken has historically been an important model vertebrate organism in the fields of developmental biology and immunology. Gene targeting by homologous recombination has been used successfully to modify the genomes of a variety of species. However, chicken have not been available to genome editing since short of germline competent cells access to genetic engineering. With germline-competent advantages over virus mediated transgene method, PGC (Primodial germ cell) based transgene strategy is promising to generate

gene-modified chicken in a more customized way when combined with simple and efficient CRISPR-Cas gene-targeting technology. Here we describe targeting chicken ovalbumin gene by CRISPR-Cas based homologous recombination in primordial germ cells to efficiently establish fully transgenic chickens carrying the exogenous protein. Meanwhile, we will apply CRISPR-Cas system through PGCs based transgene technique to produce novel trait-customized chicken, enable genetic analysis of embryo development and establish oviduct bioreactor for humanized proteins production in chicken.

P7019 Effects on skeletal muscle of muscle-specific Smad6 knockout. Wen Chang ,Fei Chang ,Rui Fang and Ning Li (China Agricultural University)

The function of myostatin on skeletal muscle is well-defined. Increased myostatin expression can induce muscle wasting and atrophy, whereas myostatin knockout leads to remarkable increase in muscle mass. Recently,another member of the transforming growth factor beta (TGF-β)-bone morphogenetic proteins(BMPs), has been proved to be a positive factor in adult muscle maintenance and growth.BMP signaling,acting through Smad1, Smad5 and Smad8(Smad1/5/8),is the fundamental hypertrophic signal in mice.Activation of BMPs induces muscle growth and blocks atrophy,while inhibition of the BMP pathway leads to muscle atrophy and abolishes the hypertrophic phenotype of myostatin-deficient mice.Since Smad6 appears to be specific inhibitor of BMP signaling,we want to enhance the activity of BMP signaling through muscle-specific knockout of Smad6 in mice,expecting increase of skeletal muscle mass.To generate muscle-specific Smad6 knockout mice,we used the Cre-loxP recombination system in which expression of Cre recombinase is directed specifically to skeletal muscle cells by the promoter of the myosin light chain 1/3(MLC).These MLC-Cre transgenic mice were crossed with mice containing loxP sites floxed both Smad6 alleles(Smad6fl/fl) to create functional Smad6 knockout mice(Smad6-/-).We will further analyse the phenotype of the Smad6-/- mice and the signal pathways. Our works will provide new strategies for treating muscle-wasting disorders and for enhancing livestock muscle mass.

P7020 Genome editing of the bovine beta lactoglobulin locus using zinc finger nucleases (ZFNs) and transcription activator-like enzyme nucleases (TALENs). Stefan Wagner and Jingwei Wei (AgResearch), Dan Lu (China Agricultural University), David Wells (AgResearch), Daniel Carlson and Scott Fahrenkrug (Recombinetics) and Goetz Laible (AgResearch)

The recent development of genome editing tools in the form of nucleases that can be custom designed to target essentially any site within the genome now allows for the efficient and precise introduction of genetic change into livestock genomes. Most studies have so far focused on the introduction of random mutations triggered by the error-prone repair mechanism in cultured cells and the use of nuclear transfer to generate animals with edited genotypes. To circumvent the intrinsic uncertainties of random mutations and the inefficiencies of nuclear transfer we have been focusing our efforts on the introduction of specific genetic changes by homology-driven repair directly in IVF embryos. Initially, we injected ZFN-encoding mRNA or DNA into bovine zygotes to verify their activity. Following development to blastocysts, we analysed the embryos for ZFN-induced random mutations which we detected in 30%-80% of embryos. Next, in order to more precisely change the sequence of the BLG locus, we co-injected ZFNs with DNA oligonucleotides as homologous repair templates to enable homology-driven repair. We used two oligonucleotides that were designed to introduce a 5 bp insertion and a 9 bp deletion,

both changes disrupting the BLG gene. Analysis of the injected blastocysts showed that the oligonucleotides indeed induced targeted changes in approximately 30% of blastocysts. More recently, using TALENs targeting different parts of the BLG locus, we have shown that TALENs/DNA oligonucleotide co-injections also enable precise targeted gene editing events of the BLG locus in 50%-60% of injected blastocysts. Taken together, our results show that ZFN and TALENs co-injections with oligonucleotides are an efficient way to introgress specific allelic variants into the bovine genome.

P7021 The application of high level expressed recombinant lysozyme in transgenic pig milk. Mengxu Ge (China Agricultural University), Fangfang Wu (Yunnan Agricultural University) and Ning Li (China Agricultural University)

Lysozyme, also called muramidase, is an antibacterial factor for innate immunity. In human milk its concentration is about 0.4 mg/mL, which is 6100 times higher than that in sow milk. The lysozyme plays an important role in killing bacteria, modulating the inflammatory response, influencing the composition of intestinal microbiota population. Recombinant human lysozyme (rhLZ) expressed in sow's milk may regulate the microflora in the gastrointestinal tract and enhance anti-diarrhea ability of piglets.In this study, we used mammary gland high-level expression vector pBAC-hLF-hLZ-Neo which was constructed and tested in transgenic mouse model in our lab. Through transfecting porcine fetal fibroblasts with this vector, we generated transgenic cloned pigs via somatic cell nuclear transfer (SCNT). Quantitative real-time PCR was performed to examine copy number of transgene. In total, 6 piglets were selected and the copy number ranged from 1 to 2. The average concentration of rhLZ was 1.6768±0.4167 g/L in the milk, which was 25000-fold higher than that of the native pig lysozyme. In vitro, it was demonstrated that rhLZ in milk of transgenic pigs can exhibit the same antibacterial activity as naive hLZ protein. Antibacterial test revealed that rhLZ can inhibit the growth of K88, Staphylococcus aureus, which are the pathogenic bacteria caused piglets diarrhea. Together, we successfully generated high-level expression rhLZ transgenic cloned pigs. This work is an initial step in breeding pigs for diarrhea resistance by specifically expressing human lysozyme in mammary gland tissue to benefit piglet health and improve their ability to resist bacterial infections.

P7022 Protection against bacterial infection by expression of bioactive human lysozyme in transgenic cloned pigs. Guoqin Sang and Ran Zhang (China Agricultural University)

Lysozyme serves as part of the defense mechanism against bacterial infection or digestion of intestinal bacteria. However, the expression level of lysozyme is very high in human breast milk whereas it is extremely low in pig milk. Therefore, we would like to produce hLY transgenic pigs to improve the health and also protect against pathogen infection. In this study, a modified human lysozyme (hLY) BAC with neomycin resistance gene was transfected into porcine fibroblast cell lines and two transgenic cloned pigs were generated by somatic cell nuclear transfer (SCNT). In addition, a total of 21 F1 transgenic piglets were born by mating with wide-type pigs, 30% of which were hLY positive for further analysis. The copy numbers of hLY were determined by quantitative real-time PCR and it showed single copy of the transgene in all the transgenic pigs. The hLY mRNA level was expressed in several tissues detected by qPCR, such as intestine, lymph gland and so on, suggesting the expression profile of transgenic pigs in similar to that of humans. The gastrointestinal microbial analysis was assayed and the results showed that the number of beneficial bacteria Bifidobacteria was significantly increased ($p<0.01$), while the

number of the harmful bacteria E.coli was slightly reduced compared to that of wide-type pigs. hLY activity of transgenic pigs was detected using the gel diffusion assay, with the highest antibacterial activity as good as that of 0.15mg of the hLY standard in milk samples. In summary, the generation of hLY transgenic cloned pigs could modulate the intestinal bacteria and improve the intestinal health, which will be beneficial to the pig breeding industry as well as the improvement of better economic efficiency.

P7023 Recombinant Human Lysozyme expressed in transgenic chicken can promote the growth of bifidobacterium in the intestine and improve the growth of postnatal chicken. Hai Wang, Ling Lian and Zhengxing Lian (China Agricultural University)

Lysozyme is often used as a feed additive and acts as an antimicrobial protein that enhances immune function and defends against pathogenic bacteria in chicken. In this study, we genetically added the lentiviral expression vector of recombinant human lysozyme (rhLZ) gene to chicken embryo through blastoderm and investigated whether the presence of recombinant human lysozyme can influence the growth traits or intestinal microbiota and mophology in chicken. We generated 194 transgenic chicken identified by Southern blot with a positive transgenic rate for 24%. The average concentration of rhLZ was 29.90±6.50 ug/ml in the egg white. We also recorded and analyzed the growth traits of the transgenic and the non-transgenic. We found that the 6-week shank length (♂:6.31±0.56cm versus 5.93±0.45cm,$p<0.01$; ♀: 5.08±1.37cm versus 4.23±1.94cm,$p<0.05$), 6-week weight (♂:0.33±0.07kg versus 0.28±0.05kg,$p<0.01$; ♀: 0.30±0.05kg versus 0.26±0.06kg,$p<0.01$) and 18-week weight (♂:1.28±0.26kg versus 1.12±0.23kg,$p<0.05$; ♀: 0.92±0.17kg versus 0.82±0.16kg,$p<0.01$) of those transgenic significantly increased. But the hatching rate (0.66±0.22 versus 0.64±0.24,$p>0.05$) and the healthy chick rate (0.65±0.22 versus 0.63±0.25,$p>0.05$) had no significant difference. Five types of bacteria were cultured and analyzed to detect the impact of rhLZ on gut microbiota.The number ofbifidobacterium in the intestine of those transgenic was significantly increased (4.83×107±0.66×106CFU versus 1.46×107±0.13×106CFU,$p<0.05$). The results of the growth traits and intestinal microbiota and morphology demonstrated that rhLZ transgenic chicken can promote the growth of bifidobacterium in the intestine and improve the growth of postnatal chicken.

P7024 Suppression of avian influenza virus by transgnenic chickens via Mx protein and RNA interference. Qing Wen Meng, Wei Wang, Jin Tian, Zai Ping Zhang and Hong Yan Chen (Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences)

Avian influenza (AI) is a highly infectious disease caused by avian influenza virus (AIV), and poses critical threat to both global poultry production and human health. The highly genetic mutation rate and host adaptability of AIV present great challenge for the control and prevention by drug or vaccine. One novel anti AIV strategy in poultry is to genetically modify chickens by transgenic techniques. In this study, we generated transgenic chickens that constitutively express the anti-AI protein chMx and RNAis suppressing influenza virus polymerase. The recombinant lentiviruses were injected into the subgerminal cavity of freshly laid eggs. The founder (F0) bird was mated with wild-type hens to produce transgenic progeny and maintained from F1 to F3 generation. Transgenic birds were identified by PCR, Southern Blot, and Western Blot in all three generations. The insertion site of transgene was mapped to chicken chromosome 2 by Tail-PCR. Highly pathogenic avian influenza virus (HPAIV) infection experiments on F2 generation TG/WT

chickens of same breed showed that TG chickens could not resist the HPAIV lethal attacks; however they showed increased survival time. Infection experiments by lower dose of virus showed that the pathologic degree of lung tissue lesions, the virus titers and the expression levels of the IFN-α and TNF-α in TG chicken were significantly lower than WT chicken. These data proves that transgenic chickens are capable of suppressing avian influenza virus. On the basis of laboratory studies, the transgenic chickens have been approved to be conducted in middle sized experiments in Heilongjiang Province by the genetically modified organism biosafety committee of the Ministry of Agriculture. This study established transgenic chicken production technology platform, laid important foundations for developmental biology, avian natural immunity and poultry oviduct bioreactor to produce pharmaceutical or health care protein.

P7025 Generation of the skeletal muscle hypertrophy pig model with the *Fbxo40* gene Loss of function. Yunlong Zou, Yiqing Hu and Ning Li (China Agricultural University)

The IGF1 pathway is an important one known to influence body weight and muscle size, and Fbxo40, a member of SCF E3 ligase complex, directly ubiquitinates IRS1,blocking the IGF1R/IRS1/PI3K/Akt pathway. By knockdown of Fbxo40, IRS1 can be rescued. Fbxo40 is muscle specific in expression and is up regulated during muscle differentiation. The *Fbxo40* knocked-out mice exhibit heavier body weight and skeletal muscle hypertrophy, so we consider it an excellent candidate gene for improving muscle mass in livestock and inhibiting muscle atrophy in patients. The function is rarely explored in pig though it is well defined in mouse. So we want to define the pathway of Fbxo40 and evaluate the value of the gene for agricultural and biomedical application.
Recently the Type II CRISPR/Cas system has been exploited to develop RNA-guided endonucleases to enable targeted genome editing, which is scalable, affordable, and easy to engineer. And the system has been successfully used to modify genomes in cultured human cells and various species including zebrafish, mouse, rat, pig, plants and so on.
With the help of CRISPR-Cas9 system, We want to generate a hypertrophy pig model with the *Fbxo40* gene loss of function. Several gRNAs targeting the functional domains of the *Fbxo40* gene have been designed. The Cas9 plasmids have been transfected into pig fibroblasts via electroporation. We have used T7EN1 cleavage assay and sequencing to test the efficiency of gRNAs. Different gRNAs exhibit different efficiency in inducing double-strand break (DSB), which may result from the diverse chromatin state of different part in the single gene. To precisely modify the gene, single-stranded oligonucleotides (ssODN) and double-stranded plasmids have used as donor for homologous recombination respectively. By inducing DSB, CRISPR-Cas9 can improve the efficiency of homologous recombination dramatically.

P7026 Efficient production of transgenic sheep overexpressing of *TLR4* by the microinjection of *in vivo* pronuclear embryos. Yan Li (China Agricultural University), Shoulong Deng (State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China) and Guoshi Liu, Hongbing Han and Zhengxing Lian (China Agricultural University)

The main objective of this study was to produce highly disease-resistant transgenic sheep by using *in vivo* pronuclear embryos obtained from superovulation. In this study,13 donor ewes were subjected to the superovulatory treatment in the breeding season and 16 ewes superovulated in the nonbreeding season,using endoscopic surgery to complete the artificial insemination.After the linearized vectors which were highly optimized were microinjected,3 to 5 embryos were

transplanted to each recipient sheep.After dealing with the superovulation,13 donor ewes were flushed out 207 zygotes in the breeding season, and in the nonbreeding season,16 donor ewes were flushed out 301 zygotes,there were no significant differences between them in average ovulation rate (15.92±9.89 versus 18.81±10.47,P＞0.05).The rate of pronuclear embryos and 2-cell were 100% and 0% respectively both in the breeding and nonbreeding season.The rate of viable embryos(96.15±9.39% versus 79.32±33.08%,P＜0.05) was significantly higher in breeding season compared with nonbreeding season.In total, 91 recipient sheep were transplanted and 45 lambs were born,the lambing rate was 49.45%(45/91).The Southern blot result revealed that the positive rate was 22.22%(10/45);Real-time PCR result revealed that the expression level of *TLR4* was remarkably higher in the transgenic sheep than that in the non-transgenic group（P＜0.05）.This experiment preliminarily set up an efficient system of producing high-quality *in vivo* pronuclear embryos, it can be concluded that the high-quality *in vivo* pronuclear embryos through optimizeing the structure of exogenous gene will greatly enhance the success rate of transgenosis,and it can be produced in different seasons.

P7027 A mammalian model for Laron syndrome produced via Cas9 targeted disruption of the porcine growth hormone receptor gene. Fang Li, Dan Cui and Ning Li (China Agriculture University)

Laron syndrome is a congenital disorder characterized by marked short stature associated with normal or high serum growth hormone (GH) and low serum insulin-like growth factor-1 (IGF-I) levels, which fail to rise after exogenous GH administration. The disease is due to mutations in the *GHR* gene, including all or parts of exons 3, 4, 5 and 6. Many aspects of GHR dysfunction remain unknown because of ethical and practical limitations in studying humans. However, to date, the sex-linked and the Laron mouse has been reported, but these models are not an ideal paradigm for the human disorder. Thus it is necessary to create a suitable mammalian model for this disease. We devised to generate a pig bearing a disrupted of *GHR* gene through CRISPR-Cas9 system. We had designed 10 target sites tested in the pig fibroblast, and the T7 endonuclease I mutation detection assay showed that five target sites were efficient. Next, we plan to use these five sites to make a deletion more than 2.8Kb in the exon10 to create an intracellular signaling domain dysfunction. Moreover, if this model can be built , it will be a useful model in the study of many unresolved aspects of GH -IGF-I function, such as aging, longevity, tumorigenesis, and diabetes and its complication.

P7028 Generation of muscle-hypertrophy porcine model by knocking out striated muscle-specific E3 ubiquitin ligases. Yiqing Hu, Yunlong Zou and Ning Li (China agricultural University)

The muscular size depends on the balance of synthesizing and degradation of proteins. In various cases of muscle atrophy , the E3 ubiquitin ligases trigger the protein breaking down, which is initial in the process of atrophy. By knocking out the muscle-specific E3 ubiquitin ligases , the body weight and muscular weight can be increased in mouse. Whether the phenomena could be repeated in porcine, has not been proved. Using the newly developed gene targeting technology CRISPR/Cas 9, we screened several target sites. The highest efficiency of the target sites is 59%. By cotransfecting a single strand oligo DNA(ssODN) of 129 nt as a homology-directed repair(HDR) template, the genome can be precisely repaired. After a series of trials, we defined the efficient target site and ssODN, with which the interesting genes were disrupt. We are extremely looking forward to see

that the knocking-out contributes to the muscle hypertrophy in livestocks, which would promote the agricaltrual economic benefit.

P7029 TALEN-mediated insertion of the human lysozyme gene on bovine beta-casein gene locus in fibroblasts. Benli Wang, Fangrong Ding, Yunping Dai and Ning Li (China Agricultural University)

Lysozyme is a natural broad spectrum antimicrobial factors, its concentration is about 0.4mg/mL in human milk, which is 3000 times higher than that in bovine milk. The lysozyme in breast plays an important role in increasing immunity and reducing intestines disease of infants. Using the transgenic technology to express the human lysozyme in bovine milk is a very promising method to make the bovine milk more suits for human. However, there are some disadvantages in traditional transgenic research. Such as, low expression level of recombinant protein affected by position effect and the potential risk to animal because of random integration. Targeting the human lysozyme gene on bovine casein gene locus which expressed specifically and efficiently in mammary gland could overcome the disadvantage of traditional transgenic research, which is a very promising prospect in future transgenic animal for mammary bioreactor research. The limited capacity of current bioreactors has led the biopharmaceutical industry to investigate alternative protein expression systems. The milk of transgenic cattle may provide an attractive vehicle for large-scale production of biopharmaceuticals. Recent studies found that a precisely placed double-strand break induced by engineered transcription activator–like effector nucleases (TALENs)stimulated the integration of exogenous DNA stretches into a pre-determined genomic location, resulting in high-efficiency site-specific gene addition. Here we show in bovine fetal fibroblasts that targeting TALENs to the endogenous β-casein(CSN2) locus stimulates human lysozyme gene addition by homology-directed repair, resulting in hLYZ knock-in of approximately 8.7% of TALEN-treated bovine fetal fibroblasts (BFFs). Gene-targeted fibroblast cell clones were screened by junction PCR amplification and Southern blot analysis. Our findings open a unique avenue for the creation of transgenic cows from genetic engineering by providing a available techniques to produce pharmaceutical proteins in milk.

P7030 Follistatin positively regulates skeletal muscle development in pig through Smad2 and Akt signalings. Fei Chang, Rui Fang and Ning Li (State Key Laboratory for Agro-biotechnology, college of life sciences, China Agricultural University)

Follistatin(Fst), as a potent antagonist of several TGF-β superfamily members, plays a significant role in skeletal muscle development. It has been proved that overexpression of Fst in mice, monkey or fish could induce skeletal muscle hypertrophy. Fst administration could also alleviate muscle wasting symptoms in mdx mice by increasing muscle mass and strength. In spite of its huge potential in breeding of agricultural animals and in treating of muscle-pathological animal models, the role of Fst has not yet been clearly clarified in pigs. Herein, we generated transgenic pigs in which *Fst315* was specifically expressed in muscles. These pigs showed increased lean meat proportion and reduced fat accumulation. Muscle fiber hypertrophy was obvious in transgenic pigs. And the phosphorylated levels of Smad2 and Akt are down- and up-regulated, respectively. In addition, no abnormal phenotypes were observed in cardiac muscles and reproduction. Our results suggested that follistatin promotes skeletal muscle development in pigs through muscle fiber hypertrophy.

P7031 Effect of different cell-penetrating

peptides in porcine primary cells and the obtain of the marker free pigs. Zhaolin Sun, Qianqian Kang, Qiuyan Li, Ming Wang, Zhiyuan Zou, Wenping Hu, Yiqing Hu, Yurui Zhang, Shuangyu Ma, Yunlong Zou, Tan Tan, Linyuan Ma, Jingyao Chen, Wei Zhang, Mengxu Ge, Zhengzhi Cui, Rui Zhao, Fangrong Ding, Ran Zhang, Yunping Dai, Sen Wu and Ning Li (State key lab for agro-biotechnology, college of life sciences, China Agricultural University)

Cell-penetrating peptides (CPPs), including naturally occurring or synthetic, have been increasingly utilized to deliver various cargos such as DNA plasmid, oligonucleotides, siRNA, polypeptides and proteins both in *vitro* and in *vivo*. However, there is no such report in pig primary cells. The basic research and biomedical application of transgene pigs will be increased, depending on the availability of technologies for efficient genetic modification of pig primary cells. Here, we reported three CPPs can efficiently deliver the active Cre recombinase protein into pig primary cells and the mechanisms of internalization of CPPs in pig primary cells is ATP and temperature dependent endocytosis pathway. The rate-limiting step is endosome escape of TAT-Cre and R9-Cre, but not the height of CPP5-Cre concentration.The three CPPs-Cre proteins can enter the pig primary cells and subsequently perform recombination with different efficiency, the recombination efficacy of the 10uM CPP5-Cre protein is virtually 100%, but recombination efficiency of the TAT-Cre and R9-Cre are relatively low. Both HA2 and chloroquine whichenhanced endosome escape can improve the recombination efficiency of the TAT-Cre. Furthermore, we successfully obtained marker-free transgenic pigs using the CPP5-Cre and TAT-Cre protein transduction method. In summary, we expanded the CPPs research into pig transgenics and established simple and efficient methods to remove the marker gene in transgenic pigs. This technology provides an important technical basis for the modification of livestock genomes which are important for both biomedical and agricultural applications.

P7032 A simple and efficiency method for transgenic pigs marker free using PTDs-cre recombination protein. Qianqian Kang , Zhaolin Sun and Ning Li (State Key Lab for agro-biotechnology, college of life sciences, China Agricultural University)

The transgenic farm animals are very important in the research of agriculture and biomedicine. Using cell transfection and nuclear transfer technology in large animals, we can carry out precise genetic modification, such as knock-out and knock-in. However, the method of operation process needs to be introduced selectable genes, which may has side effects and overstate the public about biological safety. In this study, we investigate the possibility of deleting the marker gene by using a cell-permeable TAT-Cre recombinase which has successfully deleted marker gene in sheep. Here we generate a porcine fibroblast cell line that stable integrated a report construct which express GFP when recombination happened. Using this report cell line as nuclear donors we produce reconstructed embryos in nuclear transfer. The application of TAT-Cre protein may effectively remove the marker gene and make the embryo turn green. In addition, we also find other PTD-Cre recombinase, R9-Cre and Cpp5-Cre, can be used in marker free and especially the Cpp5-Cre which may has very high efficiency.

P7033 Depletion of conventional mature B cells and compromised specific antibody response in bovine immunoglobulin (Ig) μ heavy-chain transgenic mice. Min Zhang and Xueqian Cheng (China Agricultural University)

In this study, we introduced the bovine immunoglobulin (Ig) μ heavy-chain gene (the orphaned μ gene on BTA11) into mouse germline cells. Bovine IgM was highly expressed in

selected transgenic lines, and it largely inhibited rearrangements of the endogenous immunoglobulin heavy chain (IgH) genes in these lines. The forced expression of bovine IgM resulted in reduced numbers of pro- and pre-B cells but increased the number of immature B cells in the transgenic mice. Bovine IgM-expressing B cells can migrate from the bone marrow to the spleen, but most of the cells are arrested at the T1 transitional B cell stage, leading to significantly reduced numbers of T2 transitional and mature B cells in the spleen. Although the serum concentrations of endogenous IgM and IgG in the transgenic mice were significantly decreased, the IgA levels were slightly increased compared to the WT mice. The bovine IgM level in the serum was only one-tenth to one-fifth of that of endogenous mouse IgM, suggesting that most of the serum Igs were contributed by endogenous IgH gene-expressing B cells. These transgenic mice also exhibited a lower frequency of unique complementarity determining region 3 (CDR3) sequences in their VH repertoire but exhibited an increased frequency of unique CDR3 in their Vλ repertoire and no change in their Vκ repertoire. Compared to the WT mice, the transgenic mice had a significantly higher percentage of mouse IgM-expressing B cells that expressed λ chains. Finally, we showed that the transgenic mice were deficient in a specific antibody response to antigen stimulation.

P7034 Molecular and functional analysis of the duck immunoglobulin genes. Xiaoxing Guan (China Agricultural University)

Because of economic importance in agriculture and its immunological characters, the duck, as a representative species of water fowls, has gradually attarcted attentions of many immunologists. Ducks are usually asymptomatic carriers of influenza viruses, serving as the natural reservoir. Although immunoglobulins (Igs) play a key role in animal immune system, but the genomic structure of the duck Ig gene locus and the mechanisms by which the duck Ig repertoire is created are only partially revealed. To address these issues more clearly, we screened the Ig gene containing BAC clones from a BAC genomic library constructed using a hybrid strain of Pekin duck. The obtained BAC clones were fully sequenced to delineate the genomic structure of the duck Ig gene loci, based on which the immunoglobulin diversity generating mechanisms are also analyzed.

P7035 Breed Specific Mitochondrial Haplotypes Influence Metabolic Traits in Porcine Transmitochondrial Cybrids. Guanghui Yu, Jianhui Tian and Jingdong Yin (China Agricultural University), Carl Pinkert (Auburn University) and Qiuyan Li and Xingbo Zhao (China Agricultural University)

In farm animals, mitochondrial DNA mutations exist widely across breeds and individuals with strong correlations and relevance to economic traits. In order to test the mtDNA effects on livestock breeds and economic traits, three porcine breed-specific transmitochondrial cybrids were generated by fusion of Lantang cells devoid of mitochondrial DNA with denucleated cytoplasmy of Large White, Lantang and Xiang pigs with the respective haplotypes. These cybrid cells were analyzed for mitochondrial genome sequencing and biochemical traits of ATP content and susceptibility to reactive oxygen species (ROS). Lantang and Xiang mitochondrial genomes were highly homologous with only 17 polymorphic sites, and differed from the Large White with 202 and 197 mutations respectively. Lantang and Xiang cybrids exhibited similar levels on ROS ($P>0.05$) and significant difference on ATP content ($P<0.05$), but differed from that of the Large White, which generated greater ROS production ($P<0.05$) and less ATP content ($P<0.05$). The results of this study show that functional differences exist between breed-specific cybrid cells which differ in

mitochondrial genomic background. In conclusion, conplastic transmitochondrial cybrids provide the first direct evidence on pig biochemical traits linking breed-specific mitochondrial genome haplotypes, which will provide potential selection of the mtDNA genotype in animal breeding programs.

P7036 Breed Specific Mitochondrial Haplotypes Influence Metabolic Traits in Porcine Transmitochondrial Cybrids. Guanghui Yu, Jianhui Tian and Jingdong Yin (China Agricultural University), Carl Pinkert (Auburn University) and Qiuyan Li and Xingbo Zhao (China Agricultural University)

In farm animals, mitochondrial DNA mutations exist widely across breeds and individuals with strong correlations and relevance to economic traits. In order to test the mtDNA effects on livestock breeds and economic traits, three porcine breed-specific transmitochondrial cybrids were generated by fusion of Lantang cells devoid of mitochondrial DNA with denucleated cytoplasmy of Large White, Lantang and Xiang pigs with the respective haplotypes. These cybrid cells were analyzed for mitochondrial genome sequencing and biochemical traits of ATP content and susceptibility to reactive oxygen species (ROS). Lantang and Xiang mitochondrial genomes were highly homologous with only 17 polymorphic sites, and differed from the Large White with 202 and 197 mutations respectively. Lantang and Xiang cybrids exhibited similar levels on ROS ($P>0.05$) and significant difference on ATP content ($P<0.05$), but differed from that of the Large White, which generated greater ROS production ($P<0.05$) and less ATP content ($P<0.05$). The results of this study show that functional differences exist between breed-specific cybrid cells which differ in mitochondrial genomic background. In conclusion, conplastic transmitochondrial cybrids provide the first direct evidence on pig biochemical traits linking breed-specific mitochondrial genome haplotypes, which will provide potential selection of the mtDNA genotype in animal breeding programs.

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P8001 Identification and validation of copy number variation in Nelore breed and possible association with meat tenderness.. Vinicius Henrique Silva (Animal Biotechnology Laboratory, Animal Science Department, University of São Paulo (USP)/ Luiz de Queiroz College of Agriculture (ESALQ)), Luciana Correia de Almeida Regitano (Brazilian Agricultural Research Corporation (EMBRAPA) Southeast Livestock), Fábio Pertille (Animal Biotechnology Laboratory, Animal Science Department, University of São Paulo (USP)/ Luiz de Queiroz College of Agriculture (ESALQ)), Poliana Fernanda Giachetto (Brazilian Agricultural Research Corporation (EMBRAPA) Agricultural Informatics) and Luiz Lehmann Coutinho (Animal Biotechnology Laboratory, Animal Science Department, University of São Paulo (USP)/ Luiz de Queiroz College of Agriculture (ESALQ))

Brazil is the largest beef exporter in the world with cattle industry relying mainly in Zebu Nelore breed (*Bos taurus indicus*). Highly adapted to tropical climates, Nelore has a disadvantage regarding meat tenderness when compared to *Bos taurus taurus* breeds. Meat tenderness has been associated with polymorphisms in cattle. One genetic class of structural polymorphism, called copy number variation (CNV) has been increasingly studied. CNVs are regions in genome (> 50bp) which differ in copy number mainly due to gain and/or loss events. This study performed a CNV genome-wide analysis using Illumina Beadchip Bovine HD in 723 bulls, 30 of them population ancestors. To call CNVs and compile CNV regions (CNVRs), respectively PennCNV and CNVRuler were used. A total of 2,650 CNVRs were found, that represents 6.4% of *Bos taurus* genome (170.56 Mb). The CNVRs length average was 64.4 kb ranging from 5 kb to 4.3 Mb. To validate our CNVRs, qPCR was performed in 30 population ancestors bulls for 9 CNVRs. All regions were validated. A total of 1,156 CNVRs (43.62%) were overlapped by 2,750 genes. DAVID gene enrichment analysis showed CNVRs related to immunity, olfactory receptors genes and Guanosine triphosphate (GTP) related genes. The GTP related genes have known relationship with skeletal muscle physiology and morphology. Previously quantitative trait loci (QTLs) mapped for meat tenderness, some specific to Nelore breed, overlapped with CNVRs. Further, several genes previously associated with meat tenderness also overlapped CNVRs. Comparison with previously published reports revealed that 1,388 CNVRs (52.37%) found in Nelore were also described in other breed. A copy number state concordance (loss, normal and gain) between qPCR and SNP-array results, to each validated CNVR, ranged from 46% to 86% from tested samples. The Nelore Zebu CNVRs have a potential to emerge as new molecular markers for meat tenderness.

P8002 Abundance, arrangement, and function of structural elements in the chicken promoters. Hideaki Abe and Neil Gemmell (University of Otago)

Eukaryotic promoters are regions containing the regulatory elements necessary to control gene transcription. Much evidence has emerged showing that structural and/or contextual changes in regulatory elements can critically affect cis-regulatory activity. As cis-regulatory divergence appears to be a consequence of natural selection, it is meaningful to examine the abundance and distribution patterns of these regulatory elements between divergent species. When compared with mammals, chicken (Gallus gallus) has distinctive genome composition and sufficient genomic information to make it a good model for the exploration of promoter structure and evolution. Here we report the characterization of the chicken promoter, focusing on several structural motifs that may be involved in transcription regulation. Chicken genes contained a considerable number of short

tandem repeats (STRs) in their promoter regions. The STR motif frequencies were similar between human and chicken promoters, while the total number of STR in the chicken promoters accounted for approximately only 40% of that detected in human promoters. Unlike other STR periods, trinucleotide repeats showed a biased distribution in chicken promoters. Nearly half of the trinucleotide repeats found in promoters partly or entirely overlapped with CpG islands and the number of repeat units was significantly lower than those of other repeat units. Correlation analysis between GO categories and structural elements indicates motif-specific constraints acting on gene expression. Despite the low density of STRs in the chicken genome, chicken promoter regions share some, but not all, of the structural features observed in mammalian promoters. The non-random distribution of STRs and GC-rich elements among promoter regions hints that different levels of selection pressures have acted on distinct promoter regions in a lineage-specific manner.

P8003 High throughput physical mapping of the horse genomes using single molecule nanochannel arrays and its integration with the horse genome sequence assemblies. BIN LIU (Center of Systematic Genomics, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, CHINA), Aladaer Qi (CSG/XJIEG/CAS, China), Alex Hastie2 (BioNano Genomics, Inc.), Gemenggul Muhantay (Tarim University), Saki Chan (BioNano Genomics, Inc.), Xue Liang, Wanyu Wei, Mingqiang Wang, Jianchao Wang, Liang Dong, Jinjin Liu and Mengjie Qiu (CSG/XJIEG/CAS), Lingyan Li (Beijing Vocational College of Agriculture), An Li (Xinjiang Vocational College of Agriculture), Hongbin Zhang (Texas A&M University), Songnian Hu (Beijing Institute of Genomics, Beijing, China) and Han Cao (BioNano Genomics, Inc.)

Horse represents a unique species among the domesticated animals that have had multiple levels of interactions with human being coincided with the evolution and civilization of modern human. Therefore horse is in a unique position for the study of human physiology and disease. Whole genome survey of larger complex genomes by physical and genetic mapping is essential for study of complex quantitative traits, which is out of reach from the current high throughput genome sequencing technologies alone. In this context, we have constructed the first horse whole genome physical map from a female offspring of Thoroughbred horse using a next-generation high throughput single molecule genome mapping platform Iris from BioNano Genomics. The initial assembling of the genome map has a 62x genome coverage of single molecules in minimum size over 150 kb and the assembled genome has an N50 of 650 kb with the longest contig near 2 Mb, 5 % also of the assembled genome were not covered by the current horse reference genome sequence. The integration of this map with the sequenced horse genomes showed a good correlation with a much higher contiguity with the sequenced horse genome assemblies. The genome map boosted the combined super-scaffolds to an unprecedented large contig size and closed the gaps in the sequence assemblies. Further analysis of the next-gen horse genome physical map and the comparison with different equids should reveal other structural features with importance to equine evolution and biology. Construction of the horse genome map with the Irys technology has paved the way for finishing its genome assemblies from earlier genome sequence production and for generating a more comprehensive reference sequence map to accelerate the discovery of genetic and genomic structural variations, thus potentially opening a new tool for horse breeding and livestock genetic improvement.

P8004 Identification and close of a gap

covering the last three exons of an active porcine Y-linked oral-facial-digital syndrome 1 gene (*OFD1Y*). Jianfei Pan (Gansu Agricultural University) and Jianlin Han (CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS))

Genotyping data of Illumina's porcine SNP60 v2 BeadChip revealed heterogeneous genotypes in two linked SNPs of ASGA0080809 (SSCX:11,345,780 bp) and H3GA0051525 (SSCX:11,360,767 bp) among all males in A/G but homozygous genotypes among all females in G/G within two exons of X-linked oral-facial-digital syndrome 1 gene (*OFD1X*). Although eight transcript variants (X1 to X8) of porcine *OFD1X* gene were predicted, all of them were mapped within a single chromosomal region ranging from 11,315,153 to 11,369,244 bp (Sscrofa10.2). A homologous copy of this gene was therefore hypothesized to be located on porcine Y-chromosome. Alignment of the eight variants against the GenBank database for porcine ESTs detected two sets of mRNA sequences with the first set identical to these predicted transcripts based on *OFD1X* genomic sequence while the second set different by 3-5% nucleotides. Further alignment of the two sets of mRNA sequences against the porcine whole genome shotgun contigs recognized their homologies to two groups of contigs generated from a male Wuzhishan mini-pig. There were three contigs identical to the second set of mRNA sequences but there was a gap covering the last three exons of *OFD1X* gene. A pair of primers was thus designed based on the homologous region of *OFD1X* gene to close this gap spanning around 1 kb. The new PCR products confirmed that there was a male specific *OFD1* gene (*OFD1Y*) with a single band while females produced an additional band. The sequencing data was sufficient to close this gap of *OFD1Y*. The available mRNA sequences suggested that porcine *OFD1Y* was active. This first complete porcine *OFD1Y* genomic sequence offered an opportunity to improve the mapping of pseudo-autosomal region and to examine the mechanism of concerted evolution of complicated genes, *e.g.* the *OFD1* gene with more than 20 exons over 55 kb, on both X- and Y-chromosomes.

P8005 Comprehensive analysis of structural variants in 13 pig genomes by next-generation sequencing. pengju zhao and huimin kang (China Agricultural University)

Next-generation sequencing (NGS) technologies have become powerful tools for the characterization of structural variants (SVs) in mammalian genomes. A comprehensive perceive of SVs is necessary to the deeper analysis such as evolution analysis, presence/absence variation (PAV) analysis and selection signatures. However, there is less overall knowledge shedding light on the accurate catalogs of SVs in pigs than in most other animals, especially the sequence-based identification of SVs, such as inversions, tandem duplications and translocations. In this study, the genomes of 13 pigs from different Chinese and Western breeds were sequenced using the Illumina paired-end sequencing technology. A total of 47,062 putative SVs were identified by integrated read pairs and split-read analysis. The majority of identified SVs were deletions (65.78%), and the fewest were inter-chromosomal translocations (1.85%) to each pig. The size distribution of SVs mostly ranged from 50bp to 1kb, and the inter-chromosomal translocations significantly appeared between chr6 and chr12. In the correlation analysis of chromosome variations, there was a significant correlation among SNPs, deletions and insertions. Based on the SVs specific to the Chinese pig breeds, we also found a complete concentrated region of SVs with the length of 35M in the chromosome X (65M to 100M). The genes located in this specific region

mostly enriched Gene Ontology (GO) terms related to physical development, immune response, copperion transport and homeostasis, and nervous system. In conclusion, the comprehensive analysis of SVs herein is expected to provide a valuable resource for further investigations on the pig genomes.

P8006 Genome sequences of Mongolian horse and Przewalski's wild horse. Jinlong Huang, Yiping Zhao, Wunierfu Shiraigol, Bei Li and Dongyi Bai (Inner Mongolia Agricultural University), Weixing Ye (Shanghai Personal Biotechnology Limited Company), Dorjsuren Daidiikhuu, Lihua Yang, Burenqiqige Jin, Qinan Zhao, Yahan Gao, Jing Wu, Wuyundalai Bao, Anaer Li, Yuhong Zhang, Haige Han, Haitang Bai, Yanqing Bao and Lele Zhao (Inner Mongolia Agricultural University), Zhengxiao Zhai and Wenjing Zhao (Shanghai Jiaotong University), Zikui Sun (Shanghai Personal Biotechnology Limited Company), Yan Zhang (Virginia Tech), He Meng (Shanghai Jiaotong University) and Manglai Dugarjaviin (Inner Mongolia Agricultural University)

Mongolian horse (*Equus caballus*) is an ancient breed and Przewalski's wild horse (*Equus przewalskii*) is the only wild horse species survived in the world. Here, using next generation sequencing technology, we generated and de novo assembled quality genomes sequences for a male Mongolian horse and a male Przewalski's wild horse ("wild horse" hereafter), with about 91-fold and 93-fold coverage, respectively. To improve gene prediction accuracy, the RNA-seq was performed using the 454 FLX+ platform for eight types of tissue samples from another Mongolian horse, we found 1134 new gene loci those were not detected in current horse genomic. Portion of Y chromosome from Mongolian horse (2M bp) and wild horse assemblies (3M bp) were also sequenced and de novo assembled. Karyotypic diversification is more prominent in *Equus* species than in other mammals. In this work, we confirmed a Robertsonian translocation event through the wild horse's chromosomes 23 and 24, which contained sequences that were highly homologous with those on the domestic horse's chromosome 5. The four main types of rearrangement, insertion of unknown origin, inserted duplication, inversion, and relocation, are not evenly distributed on all the chromosomes, and some chromosomes, such as the X chromosome, contain more rearrangements than others, and the number of inversions is far less than the number of insertions and relocations in the horse genome. Furthermore, we discovered the percentages of LINE_L1 and LTR_ERV1 are significantly increased in rearrangement regions. We believe these data will be benefit to uncovering the genetic mechanisms of chromosomal evolution and species differentiation for *Equus* species.

P8007 Balancing selection at the sheep beta globin locus and its possible formation mechanism. Xihong Wang and Yu Jiang (College of Animal Science and Technology, Northwest A&F University) and James Kijas and Brian Dalrymple (Animal, Food and Health Sciences,CSIRO)

Domestic sheep (*Ovis aries*) can be classified into two groups with significantly different resistance to hypoxic environments, which is determined by two allelic beta globin haplotypes: haplotypes A and B. Sheep haplotype A is very similar to the goat beta globin locus, but haplotype B lacks four globin genes including the beta C gene which encodes a high oxygen affinity beta globin. To identify the origin of haplotype B, we surveyed the beta globin locus using re-sequencing data from 70 domestic sheep from 42 breeds and 5 North American wild sheep (3 *Ovis Canadensis* and 2 *Ovis dalli*). 34 of the domestic sheep were homozygous B haplotype and 9 were homozygous A haplotype. Interestingly, all of the 5 wild sheep were

homozygous B haplotype, indicating that haplotype B existed before the divergence of American wild sheep and domestic sheep around 2 to 3 million years ago. Sequence comparisons identified an ~30 kb deletion in haplotype B relative to haplotype A and an ~40 kb flanking sequence which had only ~87% identity with the goat syntenic sequence, far lower than the average sequence similarity (~97%) between sheep and goat. Phylogenetic analysis of mammalian beta globin loci showed that the divergent section of the sheep haplotype B was remarkably distinct from the beta globin loci in goat, chiru, cattle and yak, but still grouped with the *Ruminantia*. The distinct sequence is hard to explain by fast sequence divergence, because the majority of the distinct sequence is intergenic and expected to be under neutral selection. In conclusion, we hypothesize that the distinct 40 kb sequence may have been horizontally transferred from an unidentified non-bovid ruminant species. The divergent sequence does not recombine with the haplotype A sequence and appears to have been maintained in the sheep lineage for millions of years by balancing selection.

P8008 Sequencing the genome of all Lagomorph species: LaGomiCs, an international collaborative initiative. Luca Fontanesi (Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna), Paulo Alves (CIBIO-InBIO, University of Porto), Federica Di Palma (The Broad Institute of MIT and Harvard), Paul Flicek (European Molecular Biology Laboratory, European Bioinformatics Institute), Andrew Smith (School of Life Sciences, Arizona State University) and Carl-Gustav Thulin (Swedish University of Agricultural Sciences)

Lagomorphs are a distinct lineage of mammals divided in two families, the pikas (Ochotonidae), and the rabbits, hares and jackrabbits (Leporidae), totaling approximately 90 living species. The pika family is composed of approximately 30 species of small egg-shaped mammals. Among the Leporidae the genus *Lepus* (hares) and *Sylvilagus* (cottontails) include the larger number of species. Other leporids are represented by several unique forms, for example the Riverine rabbit (*Bunolagus monticularis*) in South Africa; the hispid hare (*Caprolagus hispidus*) of the Terai region of India and Nepal; the black Amami Island rabbit (*Pentalagus furnessi*) that occupies isolated islands in the south of Japan; the Annamite striped rabbit (*Nesolagus timminsi*) of southeast Asia; and the Volcano rabbit (*Romerolagus diazi*) that lives at high elevations on volcanoes in Mexico. The only domesticated species of the order is the European rabbit (*Oryctolagus cuniculus*). The Lagomorph Genomics Consortium (LaGomiCs) was born from a cooperative initiative between the European COST Action TD1101 "A Collaborative European Network on Rabbit Genome Biology – RGB-Net" and the World Lagomorph Society (WLS), in collaboration with the IUCN/SSC Lagomorph Specialist Group. The main scientific aim of the LaGomiCs is to provide an international research framework whose final objective is the sequencing of the genome of all extant and a few extinct Lagomorph species. LaGomiCs assembles about 20 groups of scientists interested in many different biological questions focused on Lagomorph species. A White Paper has been prepared and a priority list of species to be sequenced has been produced, including all available genomic data on lagomorph species. The sequencing of the genome of all species of this order will provide a tremendous amount of information useful to address a large number of biological problems not only related to lagomorphs but also for all mammals.

P8009 Population sequencing reveals breed and sub-species specific CNVs in cattle. Derek Bickhart (USDA ARS), Lingyang Xu (University of Maryland), Jana Hutchison, John Cole and

Steven Schroeder (USDA ARS), Jiuzhou Song (University of Maryland), Tad Sonstegard and Curtis Van Tassell (USDA ARS), Jose Garcia (UNESP) and George Liu (USDA ARS)

Structural and functional impacts of copy number variations (CNVs) on livestock genomes are not yet well understood. In this study, we have identified 1991 CNV regions (CNVRs) using population-scale sequencing data generated from 76 cattle of 8 breeds (Holstein, Angus, Jersey, Limousin, Romagnola, Brahman, Gir and Nelore). Individual genome sequence coverage ranged from 4 to 30 folds, with a mean of 11.8 folds. A total of 3.6% (102.0 Mb) of the cattle genome is predicted to be copy number variable, representing a substantial increase over our previous estimates (~2%). We validated this dataset with aCGH and qPCR, achieving a validation rate of 81% and 88%, respectively. Hundreds of CNVs were found to be breed specific or differentially variable across breeds: the *RICTOR* gene, a subunit of the mTORC2 complex that detects hormone signals related to cell growth, was found to be duplicated within dairy breeds. Duplications of the *PNPLA3* gene were found within several Angus individuals, suggesting that enhanced lipid catabolism may have been selected for within the beef breeds. Additionally, we found that clusters of the *PRP* and *PAG* genes are duplicated in all sequenced animals, suggesting that overdominance may play a role in the diversity of these fertility related genes. Further CNV analyses revealed the population structures of current taurine and indicine breeds and detected hundreds of candidate positively selected CNVs near important functional genes. Our CNV results provided a glimpse of geographic adaptation and human selection during cattle domestication, breed formation, and recent genetic improvement. This population-scale survey of CNVs identifies key regions of the cattle genome that are subject to variation and will further efforts to genotype and track large-scale structural variants in cattle.

[Coauthors also include Robert D. Schnabel, Jeremy F. Taylor, Harris A. Lewin]

P8010 What have we learned from the bovine Y-chromosome?. Wansheng Liu (Department of Animal Science Center for Reproductive Biology and Health (CRBH) College of Agricultural Sciences The Pennsylvania State University)

The mammalian sex chromosomes evolved from an ordinary pair of autosome during evolution. Unlike the X-chromosome that is highly conserved, the Y-chromosome is poorly conserved among mammalian lineages largely because of the lineage-dependent degeneration of the Y- chromosome. Here, I review recent advances in our understanding of the structure and gene content of the bovine Y-chromosome (BTAY). Structurally, BTAY is one of the smallest chromosomes in the genome (~50 Mb) and is composed of a pseudoautosomal region (PAR, 5%) and a male-specific region (MSY, 95%). Approximately 20 genes map to PAR. The bovine MSY is featured with an X-degenerate (Xd, ~2.5 Mb) and a Y-amplified (Ya, ~35 Mb) region with a Y-transitional region (Yt, ~5 Mb) in between. During the bovid evolution, a lineage-specific 'autosome-to-Y' transposition event resulted in three bovid-specific Y-chromosome gene families, PRAMEY, ZNF280BY and ZNF280AY. A total of 28 protein-coding genes/families are present in MSY, 12 of which are single copy genes located in Xd, and remaining 16 are multicopy gene families located in Yt and Ya. Unlike the primate Y-chromosomes in which variable size of palindrome sequences are present, the bovine Ya contains ~80 palindrome-like repeat units (RUs) with an sequence similarity of ≥98.5% between the inverted repeats, each RU is ~420 Kb in size. There are four extensively amplified gene families, including TSPY, HSFY, ZNF280AY and ZNF280BY, in the Ya region with copy numbers ranging from 80 to 236 for each gene family. Together, these gene families have a total of

~1270 genes, made the bovine MSY gene density the highest in the genome. In addition, 367 non-coding RNA families (ncRNAs) were also identified on BTAY. Transcriptome analysis revealed that 95% of the BTAY genes/ncRNAs are expressed predominantly in testis and may involve in spermatogenesis and male fertility.

P8011 Genome sequencing and phylogenetic analysis of Pacific white shrimp, *Litopenaeus vannamei*. Jianhai Xiang, Xiaojun Zhang, Fuhua Li, Jianbo Yuan, Yang Yu and Chengzhang Liu (Institute of Oceanology, Chinese Academy of Sciences)

Penaeid shrimps are most economically important marine aquaculture species in the world and China. Genetic information, especially whole genome sequence is necessary to better understand the biological essence, and to promote the domestication and genetically improvement in shrimp. However, the research on shrimp genome is still very limited. Both genomes of the Pacific white shrimp *Litopenaeus vannamei* and Chinese shrimp *Fenneropenaeus chinensis* have been sequenced and assembled recently. Using Next Generation Sequencing (NGS) technologies, different insert-size pair-end and mate-pair shotgun libraries were constructed, a total of 786.13 GB and 475.17GB data were generated, covered 302 X and 250 X genome size of the two important cultured species of shrimp respectively. The bioinformatics assembly is a tremendous challenge because of the approximately 80% repetitive sequences in the shrimp genome. A high-density genetic map of *L. vannamei* has been developed, on which 6,359 SNP markers were mapped to the 44 linkage groups spanning 4,243 cM. Paired BAC-end sequencing was conducted and 28,000 BAC end were obtained. Five BAC clones were sequenced and analyzed based on Sanger and Illumina platform to explore the genomic structure at the long DNA sequences level. PacBio single molecule sequencing was attempted and 2.6 GB data were generated for assisting assembling. The construction of a shrimp physical map is in progress. Seven transcriptomes ware sequenced and all reads were assembled and clustered into 66,815 unigenes, about 95% unigenes from transcriptomes were mapped into *L. vannamei* assembled genome. Using present shrimp genome data, we analyzed the genome-wide horizontal gene transfer (HGT) events in *L. vannamei*, among them 14 HGT genes were identified. The analysis of genome characterization indicated shrimp possess of a large and complex genome. A set of strategies have been utilized to facilitate assembly, a high quality, available whole genome sequence draft can be obtained in the near future.

P8012 Detection of large-scale variation among sheep, goat and cattle genomes. Yu Jiang (College of Animal Science and Technology, Northwest A&F University), Min Xie (BGI-Shenzhen), Yang Dong (State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences), John McEwan (AgResearch, Invermay Agricultural Centre), James Kijas and William Barendse (CSIRO Animal Food and Health Sciences), Yulin Chen and Xiaolong Wang (College of Animal Science and Technology, Northwest A&F University), Thibaut Hourlier (European Bioinformatics Institute, Wellcome Trust Genome Campus), Kim Worley (Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, 77030,USA), Alan Archibald (The Roslin Institute, University of Edinburgh), Noelle Noelle Cockett (Utah State University), Xu Xun (BGI-Shenzhen), Wen Wang (State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences) and Brian Dalrymple (CSIRO Animal Food and Health Sciences)

We assembled the reference genome sequence of the sheep (Oar v3.1) and goat (CHIR_1.0),

exhibiting ~97% sequence identity to each other. Sheep and cattle have ~90% DNA sequence identity and similar karyotypes. We confirmedthe four known Robertsonian translocations involving the autosomes, and the centromere loss and acquisition in chromosome X after the divergence of cattle and sheep.The centromere of Ovine chromosome X is now located at the boundary of pseudoautosome region (PAR). Thus the PAR region appears to represent the entire short arm of the X chromosome in sheep and possibly in goat. We identified 140 large breakpoints (>100 kb gaps, translocations or inversions) between the sheep Oar v3.1 and cattle UMD3.1, most of which containknown copy number variation regions (CNVRs). Two examples include the multidrug transporter *ABCC4* cluster and multiple clusters of olfactory receptor genes.To investigate in more detail the evolution of Bovidae family CNVRs, we used whole-genome shotgun sequence derived from 42 sheep breeds, 13 goat breeds and 24 cattle individuals. In sheep, 10,082 candidate duplicated regions with a total length of 28.4 Mb were identified, outnumbering the 1,524 candidate deletion regions with a total length of 3.9 Mb. Similar results were obtained for the goat and cattle populations. We detected 11.6 Mb of sheep lineage-specific duplicated regions and 2.0 Mb deletion regions. The work provides a genome-wide assessment of structure variation, segmental duplication and loss that covers much of ruminant evolutionary history.

P8013 The sheep genome illuminates biology of the rumen and lipid metabolism.. Brian Dalrymple on behalf of the ISGC (CSIRO Animal, Food and Health Sciences)

We have constructed a high quality reference sheep (*Ovis aries*) genome from two Texel individuals totaling ~150 fold sequence coverage using linkage and radiation hybrid maps to order and orientate the super-scaffolds. The final sheep genome assembly, Oar v3.1, has a contig N50 length of ~40 kb and a total assembled length of 2.61 Gb, with ~99% anchored onto the 26 autosomes and the X chromosome. RNA-Seq transcriptome data was generated from 94 tissue samples, including 83 from four additional Texel individuals. Our analysis of the genome and transcriptome identified a new mammalian gene, trichohyalin-like 2 (*TCHHL2*), and new members of the ruminant-specific *PRD-SPRRII* gene family highly expressed in the rumen and encoding probable keratin cross-linking proteins associated with the rumen epithelium. We identified a new mammalian gene (*LCE7A*) encoding a probable late cornified epithelium protein, expressed in sheep, goat and cattle skin, and in wool follicles. We also identified genes involved in lipid metabolism (*MOGAT2/3*) that had been amplified and had altered tissue expression patterns in the ruminants (high expression in the skin and not expressed in the liver) compared to non-ruminants. The presence of *MOGAT2/3* in sheep skin indicates that there may be an alternative pathway for di-acyl-glyceride synthesis, recycling mono-acyl-glyceride (MAG) generated from the mobilization of tri-acyl-glyceride stored within a cell to generate fatty acids for incorporation into other products. The MOGAT pathway bypasses glycerol cycling via the liver and phosphatidic acid (PA) synthesis. Mutations in LIPH (cleaves PA into 2-acyl LPA) in several mammalian species result in wool-like hair due to changes in follicle shape. These changes in the *MOGAT* genes may be in response to changes in the barrier lipids of the skin, an interaction between lipid metabolism and wool synthesis, and an increased role of volatile fatty acids in ruminants, compared to non-ruminants.

P8014 Genome-Wide Identification and Analysis the Repetitive DNA in Shrimp. Xiaojun Zhang, Jianbo Yuan, Chengzhang Liu, Cui Zhao, Yi Gao, Xiaoqing Sun, Fuhua Li and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

Shrimps are representative taxa in crustacean and the most economically important marine aquaculture species in the world and China. An important part of the Shrimp Genome Project is the analysis of genome content and specifically of the repeated regions, since they are believed to make up a very large fraction of the ~2.5 Gb genome. Using the massive amount of sequence data generated in Pacific white shrimp (*Litopenaeus vannamei*) from the Sanger, Illumina and PacBio platforms, we are able to perform an in-depth analysis of the repetitive DNA. The shrimp genome is known to be highly repetitive and our initial k-mer analyses confirms that repeats represent over 80%. Most part of these repetitive sequences were characterized as Simple Sequence Repeat (SSR), transposable elements (TEs) and low complexity sequences. SSRs accounted for over 8.23% of the whole genome, they widely distributed and occurred once in every 330 bp. In all types of SSR, the A/T, AT/TA, AAT/ATT and ATCT/AGAT were dominant. In all the major TEs classes, LTR retrotransposons were the most abundant (37.50% of all TEs), and the most abundant LTR was *gypsy*, which may contribute to the generation of the large genome size of *L. vannamei*. In addition, a significant portion (about 16.09%) of the sequences identified as repetitive still require further classification. To further characterize the repetitive component of the shrimp genome, we sequenced two additional species, Chinese shrimp (*Fenneropenaeus chinensis*) and ridgetail prawn (*Exopalaemon carinicauda*). Although the sequencing depth is not enough to obtain a complete genome assembly, it will be sufficient for the identification of the most abundant repetitive sequences. Based on these data, we are constructing a shrimp-specific repeat library. These work provided an important resource to study shrimp-specific repeats, and further understand the genome characterization of shrimp and crustacean.

P8015 Development of a High Density SNP-Based Linkage Map of Pacific White Shrimp (*Litopenaeus vannamei*) Using Next Generation Sequencing. Yang Yu, Xiaojun Zhang and Fuhua Li (Institute of Oceanology, Chinese Academy of Sciences), Xiaohan Chen and Yongzhen Zhao (Guangxi Institute of Fisheries), Long Huang and Hongkun Zheng (Biomarker Technologies Corporation) and Jianhai Xiang (Institute of Oceanology, Chinese Academy of Sciences)

Genetic linkage map is essential to Quantitative Trait Loci (QTL) detection and comparative genomics study. With the help of next generation sequencing (NGS) technology, Single Nucleotide Polymorphism (SNP) genotyping and high-density linkage map construction have become more and more efficient and cost-effective. Specific-locus amplified fragment sequencing (SLAF-seq) is an efficient method for large-scale SNP genotyping based on NGS technology and has proved to be effective for linkage map construction. In the present study, a high-density linkage map of Pacific white shrimp *Litopenaeus vannamei* was constructed using SLAF-seq method. Four sequencing libraries were constructed using genomic DNA from two parents and 205 corresponding offspring. A total of 456,620,260 reads were generated on Illunima HiSeq 2500 platform using paired-end sequencing technology. The average data coverage was 176X and 35X for parents and progenies, respectively. *De novo* SNP discovery generated 25,140 polymorphism markers. Considering the marker coverage among individuals and reads depth, a total of 6,359 markers with high quality were selected for linkage map construction based on double pseudo-test cross strategy using JoinMap 4.0 software. In the constructed linkage map, 4,396 markers were mapped to female map and 4,201 were mapped to male map. Both the female map and the male map contained 44 linkage groups,

which was in accordance with the number of chromosome pairs in *L. vannamei*. The integrated map was also constructed. A total of 6,146 markers spanning 4,271.43 cM were mapped to 44 sex-averaged linkage groups, with an average marker distance of 0.7 cM. The high density genetic linkage map will not only be useful in QTL detection, genetic improvement and marker-assisted breeding, but also play an important role in comparative genomics and genome assembly for *L. vannamei* and other penaeid shrimp.