ISAG
2012
33rd CONFERENCE
CAIRNS, AUSTRALIA
July 15 – 20, 2012

33rd Conference of the

International Society for Animal Genetics

July 15–20, 2012, Cairns, Australia

Programme and Abstract Book

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ISAG 2012 Conference Program

Time	Sunday, July 15	Monday, July 16	Tuesday, July 17	Wednesday, July 18	Thursday, July 19	Friday, July 20
8:30 – 9:00 am		*Opening ceremony*				
9:00 – 10:30 am		Plenary Session 1	Plenary Session 3		Workshop Session 3	Plenary Session 5
10:30 – 11:00 am		Morning tea	Morning tea		Morning tea	Morning tea
11:00 – 12:30 pm		Plenary Session 2	Plenary Session 4		Workshop Session 3 (continued)	Plenary Session 6
12:30 – 2:00 pm		Lunch + poster session (even numbers)	Lunch + poster session (odd numbers)		Lunch + poster session (all)	Lunch (12:30 – 1:30 pm)
2:00 – 3:30 pm		Workshop Session 1	Workshop Session 2	*Tours*	Workshop Session 4	Alan Wilton Memorial Plenary Session (1:30 – 2:15 pm);
3:30 – 4:00 pm	*Registration*	Afternoon tea	Afternoon tea		Afternoon tea	Award Ceremony (2:15 – 2:30 pm); Business Mtg + Closing (2:30 – 4:00 pm)
4:00 – 5:30 pm		Workshop Session 1 (continued)	Workshop Session 2 (continued)		Workshop Session 4 (continued)	Afternoon tea
5:30 – 7:30 pm	*Welcome reception*					
7:00 – 11:00 pm					*Conference dinner*	

Plenary Sessions

Monday, July 16

Session 1 (9:00 – 10:30 am): Quantitative Genetics Meets Molecular Genetics I

Prof. Mike Goddard, Professorial Fellow in Animal Genetics at the University of Melbourne and the Victorian Department of Primary Industries:
Genetic architecture of complex traits and prediction of genetic value

Prof. John Gibson, Director of the Centre for Genetic Analysis and Applications at the University of New England:
How genomic technologies can bring benefits to livestock farmers of the developing world

Session 2 (11:00 am – 12:30 pm): DNA Analysis

Prof. Alan Cooper, Australian Research Council Future Fellow and Director of the Australian Centre for Ancient DNA at the University of Adelaide:
Using ancient DNA to identify genetic diversity lost during the domestication process, past hybridization events, and cryptic species: From bovids to chickens

Assoc. Prof. Kathy Belov, Australian Research Council Future Fellow and Associate Professor in Animal Genetics at the University of Sydney:
Can genomics save the Tasmanian devil from extinction?

Tuesday, July 17

Session 3 (9:00 – 10:30 am): Quantitative Genetics Meets Molecular Genetics II

Prof. Morris Soller, Professor Emeritus at the Hebrew University of Jerusalem:
Sixty generations of broiler breeding: Secondary effects and the SIGV model for continued response to selection

Prof. Takashi Gojobori, Vice-Director of the National Institute of Genetics and Professor at the Center for Information Biology and DNA Data Bank of Japan:
Evolutionary origin and genetic differentiation of Japanese domesticated chickens

Session 4 (11:00 am – 12:30 pm): Quantitative Genetics Meets Molecular Genetics III

Mr. John McEwan, Senior Scientist at AgResearch in New Zealand:
Sheep genomics in New Zealand: Research progress and industry applications

Prof. John Quackenbush, Professor of Computational Biology and Bioinformatics at Harvard University and Director of the Center for Cancer Computational Biology at the Dana-Farber Cancer Institute:
Moving beyond the mean: The role of variation in determining phenotype

Friday, July 20

Session 5 (9:00 – 10:30 am): Epigenetics

Prof. Anne Ferguson-Smith, Professor of Developmental Genetics at Cambridge University:
Intergenerational epigenetic consequences of environmental compromise in a mouse model of under-nutrition

Prof. Emma Whitelaw, Senior Scientist and Department Coordinator of Cell & Molecular Biology at Queensland Institute of Medical Research:
Epigenetics in development

Session 6 (11:00 am – 12:30 pm): New Technologies

Prof. Ning Li, Professor of Animal Molecular Genetics at the China Agricultural University and Director of the State Key Laboratory of Agrobiotechnology:
Genomic editing of large farm animals comes of age

Prof. Claire Wade, Chair of Computational Biology and Animal Genetics at the University of Sydney:
Efficiency of light Illumina HiSeq 2000 whole-genome sequence for mutation detection

Alan Wilton Memorial Plenary Session (1:30 – 2:30 pm)

Dr. Barbara Zangerl, Research Assistant Professor of Medical Genetics at the University of Pennsylvania and Australian School for Advanced Medicine at Macquarie University.
When genetics goes to the dogs

Workshop Sessions

Monday, July 16

Session 1 (2:00 – 5:30 pm)

Horse Genetics and Genomics (Chair: Bianca Haase)

Domestic Animal Sequencing (Chairs: James Reecy, Dave Burt)

Animal Forensics Genetics (Chair: Sree Kanthaswamy)

Llama and Alpaca Working Group (Chairs: Cecilia Penedo, Eberhard Manz)

Genetics of Immune Response (Chairs: Claire Rogel-Gaillard, Sue Lamont)

Tuesday, July 17

Session 2 (2:00 – 5:30 pm)

ISAG-FAO Genetic Diversity (Chair: Hans Lenstra)

Avian Genetics and Genomics (Chair: Richard Crooijmans)

Ruminant Genetics and Genomics (Chair: Fiona Buchanan)

Equine Genetics and Thoroughbred Parentage Testing (Chair: Ann Trezise)

Comparative MHC Workshop/Genetics of Immune Response (Chairs: Keith Ballingall, Mike Stear)

Thursday, July 19

Session 3 (9:00 am – 12:30 pm)

Pig Genetics and Genomics (Chair: Barbara Harlizius)

Cattle Molecular Markers and Parentage Testing (Chairs: Romy Morrin-O'Donnell, Marie-Yvonne Boscher)

Dog and Cat Genetics and Genomics (Chairs: Kathryn Graves, Leslie Lyons)

Publishing in *Animal Genetics* (Chair: Chris Moran)

Domestic Animal Epigenetics (Chair: Ross Tellam)

Session 4 (2:00 – 5:30 pm)

Comparative and Functional Genomics (Chair: Klaus Wimmers)

Genetics and Genomics of Aquaculture Species

Livestock Genomics for Developing Countries (Chair: Wayne Pitchford)

Applied Genetics of Companion Animals (Chairs: Leslie Lyons, Cindy Harper)

Applied Sheep and Goat Genetics (Chairs: Gesine Luehken, Stephen White)

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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.

S0100–S0125

Invited Speakers

S0100 Can genomics save the Tasmanian devil from extinction? K. Belov*, *University of Sydney, Sydney, NSW, Australia.*

The Tasmanian devil, Australia's largest remaining marsupial carnivore, faces extinction in the wild due to the emergence of a new infectious disease. Devil Facial Tumour Disease (DFTD) is a contagious cancer that is spread as an allograft during biting. Devils have low genetic diversity at the major histocompatibility complex (MHC). We originally proposed that devils were essentially immunological clones, and that cancer cells were able to pass between unrelated animals without triggering an immune response due to this lack of MHC diversity. The discovery of MHC-disparate animals in northwestern Tasmania raised hopes that some of these animals may be able to mount an immune response against DFTD. Indeed, the frequency of disease in these populations remains low. However, recent experiments suggest that the tumor may be able to evade the immune response, as even skin grafts between MHC-similar devils are rejected. Furthermore, new strains of DFTD are emerging. I will discuss the use of genomics and transcriptomics to help us to understand the disease, its evolutionary trajectory and the role of genomics in the quest to save the species from extinction in the wild and in Australia′s largest captive insurance program.

Key Words: genomics, MHC, marsupial

S0101 Nutrigenetics of vitamin A supplementation and ADH1C genotype on intramuscular fat in feedlot cattle. F. C. Buchanan*, A. K. Ward, J. J. McKinnon, and S. Hendrick, *University of Saskatchewan, Saskatoon, SK, Canada.*

Vitamin A (VA) is an important supplement in feedlots, but in excess could hinder marbling. ADH1Cc.-64T>C, oxidizes retinol to retinaldehyde, could further affect marbling as the C allele removes a potential binding site for C/EPBα. We selected 130 steers (50TT, 50CT, 30CC), backgrounded them on a β-carotene deficient diet followed by a standard finishing diet with either no supplemental VA or 750,000 IU/month. Liver and serum samples were collected at the start and end-of-finishing (EOF). By the EOF, liver retinol was significantly ($P < 0.05$) lower in the unsupplemented steers, whereas serum retinol was higher in both treatments. Expression of ADH1C in the liver was additive with each T allele. Unsupplemented steers had significantly greater marbling scores than supplemented steers. There was a significant interaction between genotype and VA supplementation on intramuscular fat (IMF). TT steers had nearly 23% greater IMF than CC steers within the unsupplemented group. Within TT steers, unsupplemented had 24% greater IMF than supplemented. When VA is limiting it is likely that CC steers produce less retinaldehyde (and subsequently retinoic acid) leading to reduced IMF deposition. Reduced VA supplementation, in combination with ADH1Cc.-64T>C genotype could potentially be implemented in marker-assisted management to maximize marbling in finishing cattle.

S0102 Value of Beef CRC genomics research for developing countries. H. M. Burrow*, *CRC for Beef Genetic Technologies, Armidale, NSW, Australia.*

The Beef CRC undertakes genomics research to develop new products to improve productivity of beef herds and enhance animal welfare and the environment. The research uses sequence data from the bovine, cattle tick and methane-forming rumen microbes. Genetic (genomic predictions for economically important traits; DNA-based test for polledness; biological understanding of genes and gene pathways) and non-genetic (cattle tick vaccine; pro-biotic drench to reduce methane emissions; decision-support systems to improve compliance with market specifications) products are targeted. Much of Australia's beef production occurs in (sub)tropical environments and 56% of Australian cattle have *Bos indicus* content. Hence, the products have value for developed and developing countries, though commercialisation approaches must be modified for developing countries. Additionally, genome sequence data from developing country indigenous livestock breeds could be used to characterize breeds with unique attributes (e.g., assessing molecular diversity; identifying specific genomic regions associated with attributes of particular relevance to developing countries) for both production and conservation purposes. Ultimately outputs of genomics research could result in new applications for developing countries; e.g., creation of new domestic or international markets for elite germplasm from indigenous livestock.

Key Words: genomic applications, developing countries

S0103 Using ancient DNA to identify genetic diversity lost during the domestication process, past hybridization events, and cryptic species: From bovids to chickens. Alan Cooper*, *Australian Centre for Ancient DNA, Adelaide University, Adelaide, South Australia.*

Ancient DNA provides a novel viewpoint on the generation, maintenance and loss of genetic diversity through time. Furthermore, the recent timeframe of human domestication activities means that well-preserved specimens of many early domesticates are available, providing access to high-resolution genetic data. Next-generation sequencing and microarrays offer rapid and cost-efficient access to large amounts of variable genetic markers widely distributed across nuclear and organellular genomes, providing the ability to rapidly survey population-sized samples. Microarray SNP genotyping

chips may be applied across a broad taxonomic range, and recent studies demonstrate it is possible to obtain reliable nuclear SNP genotypes from ancient bovids, including the typing of methylated cytosines and other epigenetic modifications. We have used the Illumina BovineSNP50 assay and ancient mitochondrial sequences to explore the evolutionary history of living and extinct European and American bison species, and characterize potential biases due to degraded DNA. The research reveals a previously unknown species of bison, sister to the European bison (*Bison bonasus*), and records a previously unrecognised series of rapid population replacements between the new species, Steppe bison (*B. priscus*) and European bison over the past 60,000 years. Within North America, we characterize the genetic divergence between Plains and Wood populations of American bison (*Bison bison bison* and *Bison bison athabascae*, respectively), and propose a new scenario for their evolutionary origin from the Steppe bison. We have also applied ancient DNA analyses to early domestic chickens in the Pacific, and identified living descendants surviving on several isolated islands.

Key Words: ancient DNA, domestication, genetic diversity

S0104 Intergenerational epigenetic consequences of environmental compromise in a mouse model of undernutrition. A. Ferguson-Smith*[1], E. Radford[1], E. Iganaitis[2], and M. E. Patti[2], *[1]University of Cambridge, Cambridge, UK, [2]Joslin Diabetes Institute, Harvard Medical School, Boston, MA, USA.*

Environmental factors during early life are critical for the later metabolic health of the individual and of future progeny. Imprinted genes are generally dosage-sensitive, exquisitely epigenetically controlled and are critical for early growth and metabolic axis development. They have therefore been proposed to be uniquely susceptible to environmental compromise in utero and contribute to later adult disease. Using a mouse model of maternal caloric restriction during pregnancy affecting the metabolic health of two generations including paternal transmission to the second generation, we will explore two questions: (1) Are imprinted genes more or less susceptible to environmental compromise and hence may be 'developmental programming genes' and (2) is the paternal experience of in utero undernutrition, resulting in metabolic defects in his offspring, an epigenetically inherited memory transmitted via his sperm methylome?

Key Words: epigenetic inheritance, methylation, imprinting

S0105 How genomic technologies can bring benefits to livestock farmers of the developing world. J. P. Gibson*, *Centre for Genetic Analysis and Applications, University of New England, Armidale, NSW, Australia.*

Genetic improvement in the low-input systems of the developing world has often failed because of (a) failure to simultaneously improve other aspects of the livestock system (e.g., market access, nutrition, health), and/or (b) implementation of technologies and processes in systems without the capacity (expertise, infrastructure and finance) for sustainable application after external expertise and support are withdrawn. Application of genomic technology will be most valuable where it overcomes or avoids capacity constraints, such as application for a short period or in a restricted setting (e.g., an AI bull stud). An example of use for a short period that is currently being tested is to use dense marker assays to determine the breed composition of mixed-breed animals without pedigree in existing populations and to combine that with measures of performance in the field to determine optimum breed composition in situ. Application of genomic selection may appear attractive because there will rarely be an existing phenotype-based improvement program against which it has to compete or add value. But the rapid breakdown of accuracy of gEBV across generations coupled with an ongoing requirement for technical and financial capacity is a major obstacle. The breakdown of accuracy across generations may also limit the direct use of results obtained in developed countries, for example to select young Bos taurus dairy bulls in a developing world AI stud where opportunities for selection are currently minimal. Approaches such as detection and analysis of signatures of selection might identify breed-specific genome regions useful for introgression or in synthetic breed formation.

Key Words: genomic applications, developing world, GWAS

S0106 Comparison of the effectiveness between STR and SNP panels for forensic DNA analysis in bovine breeds. Guillermo Giovambattista*, *Instituto de Genética Veterinaria (IGEVET), CCT La Plata, CONICET, Fac Cs Veterinarias, UNLP, La Plata, Argentina.*

STRs have been successfully used in animal genetic identification in forensic investigations, however in the last years, SNPs have gained traction. An efficient SNP identification system requires a marker set with enough power to identify individuals. In this study, information obtained from SNPs and STRs in Taurine and Cebuine breeds was compared. Samples (n = 378) from 15 breeds and 7 forensic caseworks were genotyped using18 STRs and 32 SNPs. The effects of quality, amount, and tissue sources (blood, hair, bone, beef) of DNA on genotype performance were evaluated. The SNP results showed a performance of 81.1% of call rate and a percent concordance between both replicates of 98.7%. These results were mainly explained by 2 factors: that assay design,

and DNA samples quality rather than DNA quantity were important. Cumulative SNPs exclusion power values for sample matching, using double exclusion criteria, within each breed showed that in Taurine breeds information from the analyzed SNPs are equivalent to the 12 STRs ISAG recommended set ($\sim10^{-11}$). In Cebuine breeds, this set exhibited only a match probability between $\sim10^{-7}$ and $\sim10^{-9}$. The test also showed a range of 61 to 99% of correct assignment within each breed. These results could provide a valuable population data that support the consensus SNP panel for bovine genetic identification.

Key Words: animal forensic, SNP, forensic index

S0107 Genetic architecture of complex traits and prediction of genetic value. M. Goddard*[1,2], *[1]University of Melbourne, Melbourne, Victoria, Australia, [2]Department of Primary Industries, Victoria, Bundoora, Victoria, Australia.*

Complex traits are those controlled by many genes and by environmental factors. They are important in human health (e.g., obesity), in agriculture (e.g., yield of wheat) and in natural evolution (e.g., clutch size in birds). Despite their importance, few of the genes and mutations causing variation in these traits have been identified. However, recently, the use of genome-wide genetic markers has greatly increased our knowledge of these traits. It now appears that thousands of genes cause the normal variation in most complex traits. Nearly all these genes have very small effects which explains why they have been difficult to identify. There are many situations in which it would be useful to be able to predict the genetic value of individuals for complex traits. In agriculture, we use prediction of genetic value to select parents of future generations. In human medicine, we use genetic information to predict an individual's risk of a given disease. It is now possible to predict individual's genetic value from a complex trait from genome-wide genetic markers. The most accurate method for predicting genetic value from markers is a Bayesian method that uses knowledge of the genetic architecture of the trait. The accuracy of the prediction is limited by the proportion of the genetic variance that is tracked by the markers and the accuracy with which marker effects are estimated. In cattle, the markers track about 80% of the genetic variance but in humans only about 50%.

Key Words: quantitative genetics, genomic selection

S0108 Evolutionary origin and genetic differentiation of Japanese domesticated chickens. Takjashi Gojobori*[1], Kazuho Ikeo[1], and Tomoyoshi Komiyama[2], *[1]Center for Information Biology, National Institute of Genetics, Mishima, Japan, [2]Tokai University School of Medicine, Shimokasuya, Isehara, Kanagawa 259-1193, Japan.*

Domesticated chickens have played a valuable role in the culture development and food supply of humans: The chicken has spread worldwide and has differentiated into many varieties, bred for specific purposes, from food production to entertainment. Conducting gene sequencing and comparative analysis, we investigated the evolutionary origin and process of 3 major Japanese domesticated varieties of chickens: fighting cocks, long-crowing chickens, and ornamental chickens, all of which have been typical manifestation of domestic chicken as part of Japanese culture for over a thousand years. In particular, authentic fighting cocks are typical examples of chickens that have been bred for purposes other than food. The results obtained have shown that long-crowing and ornamental chickens have originated from fighting cocks, implying that chickens may have been domesticated and bred first for the for cultural purposes, but not food supply.

S0109 GWAS of economically important traits in NZ sheep using data with significant genetic substructure. M. A. Lee*, B. Auvray, K. Dodds, S. A. Newman, S. Phua, P. Johnson, J. Everitt-Hincks, G. Shackell, R. Anderson, H. Mathias-Davis, M. Bixley, G. Greer, E. Young, W. Bain, J. C. McEwan, *AgResearch, Mosgiel, Otago, New Zealand.*

In many agriculturally important species, genome-wide selection (GWS) has provided the impetus for generating large amounts of genotype data. Individuals that have been genotyped typically have estimated breeding values (eBVs) already available or in some cases just their own phenotypes. The New Zealand sheep industry, through the Ovita Consortium, has implemented GWS and accrued thousands of such individuals mainly from progeny tested rams encompassing more than 20 traits of economic importance. The analysis of this data, which contains significant genetic sub-structure consisting of both breed and pedigree relationships, is discussed in the context of identifying quantitative trait loci (QTL) that explain a reasonable level of the total genetic variance (i.e., genome wide association study, GWAS).

Key Words: GWAS, sheep, breeding

S0110 Genomic editing of large farm animals comes of age. Xiangqing Li[1], Zhiyuan Song[1], Fei Chang[1], Junjie Luo[1], Wenping Hu[1], Li Li[1], Xiaorong Gu[2], Rui Fang[2], Lei Zhang[2], Qiuyan Li[1,2], Janwu Wang[2], and Ning Li*[1], *[1]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, China, [2]Beijing GeFuCare Biotechnology Company, Beijing 100193, China.*

Precisely genomic modification of large farm animals (LFAs) would not only create new animal models for deep investigation of fundamental mechanisms of

life, but also provide novel genetic resources for animal selection breeding. Due to the absence of embryonic stem cells, genomic editing of LFAs is completely impracticable. The successful birth of Dolly sheep encourages many scientists to employ somatic cells followed up by nuclear transfer to generate live genomic modified animals. So far, 3 kinds of animal genomic editing were developed: (1) Gene addition, with manner of randomly insertion or Rosa locus-specific insertion or loxP/Frt site-specific insertion, is to add one or multiple genes from different species into the genome. In recent study, the transposons are used as cargo to efficiently deliver targeted gene. (2) Gene deletion, previously homologous recombination (HR) with positive and negative selection is the only tool to approach this target, but newly developed zinc finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs) technology has been invented and the efficiency are greatly enhanced. ZFNs and TALENs create double-strand breaks in DNA sequence at user-specified locations to stimulate the cell's natural DNA-repair processes like HR and non-homologous end-joining. Double knockout may occur with ZFNs or TALENs, which is time saving to breed homozygote animals. (3) Single nucleotide substitution, could also be reached through HR with moderate efficiency. For each of these genomic editing, some examples will be given, such as β lactoglobulin gene knockout in dairy cattle and serum albumin gene replacement in pig, which are currently carried out in our laboratory. Obviously the latest genomic editing allows us to modify any single nucleotide in the genome of LFAs, which imply a new era of genetic engineering for livestock breeding comes.

Key Words: genomic editing, large farm animals

S0111 Making your process, evidence, and reports bulletproof. C. Lindquist*, *University of California at Davis, Veterinary Genetics Laboratory Forensic Unit, Davis, CA, USA.*

While accreditation provides a solid framework for ensuring that DNA test results will stand up in court, it is a process that takes a dedicated effort and a large commitment of time and resources. That level of oversight may be unrealistic for those laboratories that perform forensic testing infrequently. This presentation will cover practical steps that laboratories can take to strengthen their procedures and assure that their reports are accepted by the courts. The points covered will include documentation, evidence handling, security and reviews. In addition to providing sensible approaches to addressing these vulnerable points, resources that are becoming available through the work of the US-based SWGWILD (Scientific Working Group for Wildlife Forensic Science) will be presented.

Key Words: forensic, accreditation, quality assurance

S0112 Sheep genomics in New Zealand: Research progress and industry applications. J. C. McEwan*, K. G. Dodds, B. Auvray, M. Lee, T. J. Johnson, J. Everitt-Hincks, S. Phua, N. Pickering, and S. Clarke, *AgResearch Ltd., Invermay Agricultural Centre, Mosgiel, Otago, New Zealand.*

An update will be provided on results of using the Ovine 50K chip for genomic selection in New Zealand sheep breeds. This currently includes weaning weight, carcass weight, meat yield, parasite resistance, number of lambs born, facial eczema and wool weight. Work to include lamb survival, dagginess, adult ewe weight, meat quality, and a proxy for ewe longevity will also be described. In addition, results from the development and use of a low density 5K SNP chip for 2-stage selection in ram lambs and an approximately 100 SNP parentage plus product will be outlined.

Key Words: SNP chip, sheep, genomic selection

S0113 Moving beyond the mean: The role of variation in determining phenotype. J. Quackenbush*[1,2], *[1]Dana-Farber Cancer Institute, Boston, MA, USA, [2]University of Queensland, Brisbane, QLD, Australia.*

Two trends are driving innovation and discovery in biological sciences: technologies allowing holistic surveys of genes, proteins, and metabolites and a realization that biological processes are driven by complex networks of interacting molecules. However, there is a gap between the gene lists emerging from genome sequencing projects and the networks that link between genotype and phenotype. 'Omic technologies were once heralded as providing a window into those networks, but so far their success has been limited, in large part because the high-dimensional they produce cannot be fully constrained by the limited number of measurements and in part because the data themselves represent only a small part of the complete story. To circumvent these limitations, we have developed methods that combine 'omic data with other sources of information in an effort to leverage the compendium of information that we have been able to amass. We will present several approaches with an emphasis on the how those methods have provided into the role that particular cellular pathways play in driving differentiation, and the role that variation in gene expression patterns influences the development of disease states. In particular, we will challenge the basic analytical framework used in biomedical research and argue that one should move beyond a simple comparison of the means relative to variance (the *t*-test) but also consider how variance itself changes between phenotypes. Looking forward, we will examine more abstract state-space models that may have potential to lead us to a more general predictive, theoretical biology.

Key Words: genomics, systems biology, computational

biology

S0114 Genome-wide association and transcriptome studies for immunity traits in French Large White pigs. Claire Rogel-Gaillard*, Nuria Mach, and Jordi Estellé, *INRA, UMR1313, Laboratory of Animal Genetics and Integrative Biology, Jouy-en-Josas, France.*

Global robustness and disease resistance have emerged as key concepts for a sustainable resilience in animal production systems. Including health criteria in selection schemes is one of the challenging objectives of the current decade. We have launched a project that combines genetics, genomics and functional studies in a cohort of French Large White pigs with the aim to characterize immune response levels as a measure of individual's immunocompetence. A wide range of both innate and adaptive immunity traits are heritable, confirming that many parameters are under genetic control and susceptible of being included in selection protocols. Genome-wide association studies allowed us to detect significant associations for traits that quantify white blood cell subpopulations, specific antibody levels after vaccination, phagocytosis, and cytokines produced by blood cells after in vitro stimulation. Transcriptome analyses have revealed differentially expressed genes in peripheral blood of animals with contrasted levels of various parameters (white blood or CD4-CD8+ cell counts, phagocytosis, in vitro production of IL2 and IL10). Genetics and genomics data will be integrated to identify biologically relevant candidate genes that underlie the phenotypic variability of the heritable studied immune traits. A next challenge will be to investigate the value of immunocompetence traits to predict resistance of animals facing various pathogens.

Key Words: swine, genetics, immunity

S0115 Consideration of genomic solutions to food security in the developing world. Max F. Rothschild*[1,2], *[1]Bureau of Food Security, US Agency for International Development (USAID), Washington DC, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, 50011 USA.*

Feed the Future is the US government's global hunger and food security program and it supports country-driven approaches to address the root causes of hunger and poverty and forge long-term solutions to chronic food insecurity and under nutrition. As part of this program long-term research efforts are being employed to use genomics to improve and sustain food production and food quality. Such solutions are aimed at intensifying production and making animals more resilient to disease and the effects of climate change. Under the Norman Borlaug Commemorative Research Initiative funded by USAID, researchers from the USDA have received funding to participate in an international goat genome project involving other US researchers and researchers from around the world. Following a workshop at ILRI in Nairobi, efforts are underway to sequence the goat genome using goats from Africa, the Middle East and South America. Efforts to search for signatures of selection related to climatic differences and disease tolerance are but the first step toward identifying genomic solutions to food security. Genomic solutions to finding useful animal vaccines and eventually developing cropping systems more favorable to animal production are also being considered. Discussion of these approaches and expected outcomes and possible pitfalls are presented.

Key Words: developing countries, food security, genomics

S0116 Sixty generations of broiler breeding: Secondary effects and the SIGV model for continued response to selection. Y. Eitan and M. Soller*, *Dept. Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel.*

The past 60 years have seen major genetic gains in production of the main agricultural animals. This is often attributed to the transformation of selection beginning in the 1940s, from an "art" to a science based on population genetics and advanced statistics. More important, however, was the transformation of an animal husbandry based on limited food sources inedible by man (pasture, farm and household wastes, forest mast) to one based on unlimited grain feeding. Consideration of the secondary effects that accompanied the selection process in broiler chickens shows that these appeared in a "punctuated" manner (i.e., at a specific point in time from onset of selection; rather than appearing simultaneously and gradually increasing in severity), and in a "coordinated" manner (i.e., at about the same point in time and severity in the stocks of all major breeders). Two alternative hypotheses: endo-environmental effects and selection induced genetic variation (SIGV) are proposed, and recent literature results supporting the SIGV hypothesis are presented. The SIGV hypothesis provides an explanation for the continued response to long-term directional selection, and includes the genetic endowment of the animal as a third essential source of the said genetic gains. (This research supported by the EC-funded FP7 Project 'Quantomics.')

Key Words: broilers, selection, endo-environment

S0117 Imputation of microsatellite alleles from dense SNP genotypes for parentage verification. M. McClure, C. P. Van Tassell, and T. S. Sonstegard*, *USDA, ARS, Bovine Functional Genomics Laboratory, Beltsville, MD USA.*

Microsatellite markers (MS) have traditionally been

used for parental verification and are still the international standard in spite of their higher cost, error rate, and turnaround time compared with single nucleotide polymorphisms (SNP)-based assays. Despite domestic and international demands from the livestock and research communities, no viable means currently exist to verify parentage for any individual unless all familial connections were analyzed using the same DNA marker type (MS or SNP). We have devised an effective and inexpensive method to impute MS alleles from SNP haplotypes within and potentially across breeds. This method was >98% accurate in imputing the MS alleles for 12 markers of ISAG recommended parentage panel using SNP genotypes from 479 dairy animals across 4 dairy cattle breeds (Brown Swiss, Guernsey, Holstein, and Jersey). Implementation of this method will allow producers to parentally verify an individual when separate genotyping platforms have been used across the generations. While MS are currently the international standard for parentage verification of exported semen, this work represents a tool to quickly migrate toward SNP based verification in 1 generation. Finally, these imputation methods can be integrated into any livestock species that desires to move from MS- to SNP-based parental verification.

Key Words: imputation, parentage, SNP

S0118 Lethal haplotypes in dairy cattle: What lies beneath? T. S. Sonstegard*[1], P. M. VanRaden[2], C. P. Van Tassell[1,2], H. A. Adams[3], and H. A. Lewin[4,3], *[1]USDA, ARS, Bovine Functional Genomics Laboratory, Beltsville, MD USA, [2]USDA, ARS, Animal Improvement Programs Laboratory, Beltsville, MD USA, [3]University of Illinois, Champaign, IL USA, [4]University of California Davis, Davis, CA USA.*

SNP genotyping tools for cattle continue to have major impact on research and the dairy AI industry. The breadth of sampling within breed has allowed for discovery of genomic regions containing recessive mutations affecting embryonic survival and fertility. A search for common haplotypes that do not become homozygous led to the discovery of 5 new recessive lethal alleles. These 5 Mbp haplotypes had confirmed effects on fertility with no observed homozygotes. Continuous genotyping by industry identified crossover events that narrowed 2 of these haplotypes to about 1 Mbp. Whole genome sequencing and SNP discovery analysis of select affected bulls revealed stop-gain mutations within Jersey Haplotype 1 (JH1) and Holstein Haplotype 1 (HH1). The stop-gain mutation underlying HH1 was found in APAF1, a gene critical for neural tube closure during development. The stop-gain mutation for JH1 was found in CWC15, a gene involved in the Prp19/CDC5L spliceosome complex. We assume this knockout results in abnormal splicing that affects early development, because embryo loss occurs between d 30 and 60 post-conception. Diagnostic SNP tests for these mutations run on a larger sampling of the breed validated these findings. These results demonstrate how the rare lethal variants can be amplified in a livestock population under intense selection pressure for specific production traits.

Key Words: fertility haplotypes, recessive mutation, SNP

S0119 Re-analysis of the *CW-1* QTL revealed the *PLAG1-CHCHD7* QTN for stature in Japanese Black cattle. A. Takasuga*[1], S. Nishimura[1], T. Watanabe[1], K. Mizoshita[2], K. Tatsuda[3], and Y. Sugimoto[1], *[1]Shirakawa Institute of Animal Genetics, Nishigo, Fukushima, Japan, [2]Cattle Breeding Development Institute of Kagoshima Prefecture, So, Kagoshima, Japan, [3]Hyogo Prefectural Institute of Agriculture, Forestry & Fisheries, Kasai, Hyogo, Japan.*

We previously mapped a carcass weight QTL on bovine chromosome 14, using a Japanese Black paternal half-sib family. The QTL was named *CW-1*, and narrowed down to a 1.1-Mb region by identical-by-descent and linkage disequilibrium (LD) mapping. However, the strongest association (P = 1.03E-12) was obtained at 2.3-Mb centromeric from the critical region in a genome-wide association study for carcass weight using 1156 Japanese Black steers that were selected preferentially from the tails of the distribution of more than 27,500 collected DNA samples. Re-analysis of haplotype and LD mapping revised the *CW-1* region within a 0.9-Mb interval containing the SNP with the strongest association. Candidate causative variations were searched for by targeted re-sequencing of 3 *Q*-homozygous, 3 *Q/q*, and a *q*-homozygous animals. Of 213 variations obtained, one non-synonymous SNP and 8 variations within highly conserved elements were examined for an association with carcass weight. The strongest association was obtained for the variations in the *PLAG1-CHCHD7* intergenic region that were recently identified by Karim et al. as regulatory QTNs influencing bovine stature. The *Q* haplotype of Japanese Black had a mosaic structure of the *Q* and *q* haplotypes of the F1 (Holstein × Jersey) sires, and shared the same *Q* alleles only at the causative variations for stature.

Key Words: stature, cattle, QTL

S0120 Efficiency of light Illumina HiSeq 2000 whole-genome sequence for mutation detection. C. E. Willet and C. M. Wade*, *Veterinary Science, University of Sydney, NSW, 2006, Australia.*

Our laboratory has been employing low budget Illumina HiSeq2000 sequencing as an alternative to sequence capture for mutation detection in mapped genomic intervals. We have employed paired-end sequencing

at approximately 5- to 7-fold cover in both canine and equine samples. With four animals sequenced, we have been able to gain approximately 4,000,000 polymorphic loci with moderate to high quality base calls on all individuals by applying calling parameters tailored for low-cover data. In this presentation, our experiences with identification of insertion-deletion events, duplications and pseudogenes will be discussed. We will also discuss our experiences with low quality DNA from formalin fixed tissues.

Key Words: whole-genome, sequencing, variation

S0121 A pilot study of the genetic basis of separation-related stress disorder in dogs. D. VanRooy, P. D. McGreevy, P. C. Thomson, and C. M. Wade*, *Veterinary Science, University of Sydney, NSW Australia.*

An understanding of the genetic underpinnings of behavior has been elusive because until recently we lacked the necessary repertoire of tools to begin to understand why we do what we do and feel what we feel. Dogs are an exceptional cohort to study behavior as they are highly observed and have an exceptionally good population structure for gene mapping.
Dogs with separation-related distress show obvious signs of distress that negatively affect their quality of life and welfare. Separation-related distress can disrupt the human-animal bond and create problems in the community. Separation-related distress is a common reason for relinquishment of pet dogs to shelters (where symptoms often become far worse) or euthanasia. The disorder presents a compelling case for us to resolve while genomics provides us with a feasible way of providing tools and therapeutic targets that can potentially improve the lives of living dogs and dogs yet to be born. More than 300 questionnaires have been received and analyzed for phenotypic associations among traits. DNA from >160 dogs from targeted breeds are available to date. Thirty cases have been identified which represent an incidence of 12.4%. A pilot cohort of 24 cases and 24 controls has been genotyped with Illumina CanineHD array. Further results are expected soon and the outcomes of the work will be discussed.

Key Words: canine, anxiety

S0122 Epigenetics in development. E. Whitelaw*, *Queensland Institute of Medical Research, Royal Brisbane Hospital, Qld, Australia.*

It is well recognized that there is a surprising degree of phenotypic variation among genetically identical individuals even when the environmental influences, in the strict sense of the word, are controlled. Genetic textbooks acknowledge this fact and refer to it as "intangible variation" or "developmental noise." We have carried out a "sensitized" ENU mutagenesis screen in the mouse to identify genes that modify epigenetic state. In most cases they are homozygous lethal, indicating the obligate requirement for the genes that have been hit (Blewitt et al., 2005). We have now screened 5000 F1s and identified 50 MommeDs. So far, we have identified the underlying mutations in 30; some of these are novel. Mice haploinsufficient for such proteins show a range of subtle phenotypes, including obesity and behavioral abnormalities. In some instances, the range of phenotypes seen in a MommeD colony is greater than that seen in the wildtype colony, supporting the idea that epigenetics plays a role in intangible variation. These mutant lines will be a valuable resource to study the role of epigenetics in gene / environment interactions. Funding from the National Health and Medical Research Council of Australia and the Australian Research Council.

Key Words: metastable epialleles, intangible variation, phenotypic variation

S0123 Feline osteochondrodysplasia of Scottish Fold cats. C. Willet*[1], B. Gandolfi[2], R. Malik[3], L. Lyons[2], C. Wade[1], and B. Haase[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [2]Population Health & Reproduction, School of Veterinary Medicine, University of Davis, CA, USA, [3]Centre for Veterinary Education, University of Sydney, Sydney, NSW, Australia.*

The distinctive and defining physical trait of the Scottish Fold cat breed is the characteristic ear phenotype. Scottish Fold cats have ears which fold forward, this presumably reflecting lack of resilience of the pinna and auricular cartilages. There is convincing clinical, radiologic, histologic and genetic evidence that Scottish Fold cats have an underlying congenital defect which affects the structure and function of cartilage, resulting in progressive bone, joint and cartilage abnormalities that subsequently lead to progressive dysfunction. Cats develop a variable osteochondrodystrophy causing abnormal bone development through defective endochondral ossification, progressive osteoarthritis and lameness. From pedigree analyses and breeding experiments the phenotype has been shown to be inherited as a monogenic autosomal dominant trait with incomplete penetrance. Thus, cats with 2 copies of the abnormal gene invariably have severe disease from an early age, whereas cats with one copy of the defective gene have disease which may vary from mild to moderate severity (in terms of extent of involvement and clinical progression). We applied a whole-genome SNP association mapping approach using a total of 78 cats (comprising 53 Scottish Fold cats and 25 Scottish shorthairs). DNA samples were genotyped with the feline Illumina 60kSNP genotyping microarray. We identified a region on chromosome D3 associated with this phenotype. However, the associated region is close to a large gap

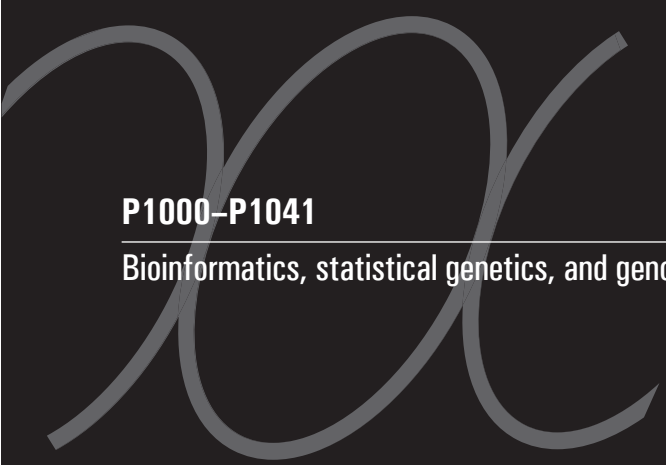
in the feline genome assembly, which complicates the identification of a positional candidate gene. Validating the associated region will be possible upon release of the new high coverage feline genome assembly.

Key Words: osteoarthritis, endochondral ossification, SNP association mapping

S0124 When genetics goes to the dogs. B. Zangerl*[1,2], *[1]Clinical Studies, University of Pennsylvania, Philadelphia, PA, USA, [2]Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia.*

The last decade has seen the completion of the canine genome sequence, development of genomic and expression arrays, and an explosion in the discovery and analysis of genetic variation within and between breeds. The advantages of these techniques are well documented in the rapid identification of causative sequence alterations for numerous traits, including the loci responsible for cutaneous lupus erythematosus in the German shorthaired pointer and primary open angle glaucoma in beagles, circumventing the often tedious and time-consuming discovery phase. However, multifactorial inheritance or unfavorable population history are still unchallenged disadvantages of modern genetics that we are just beginning to tackle by dissecting the unique structure of ancient and modern dog breeds. More importantly, as our focus shifts from cataloguing sequence changes to understanding and altering their impact on the organism, the canine model still stands out as one of the leaders in the understanding of disease mechanisms and development of treatments. As demonstrated by the latest developments of therapeutic intervention for canine bestrophinopathies, the dog days are far from being over!

Key Words: canine model, mutation discovery, gene therapy



P1000–P1041

Bioinformatics, statistical genetics, and genomic technologies

P1000 Meta-analysis of gene expression studies to identify genes involved with *Haemonchus contortus* resistance in sheep. M. Al-kalaldeh* and C. Gondro, *University of New England, Armidale, NSW, Australia.*

Gastrointestinal nematodes cost the Australian sheep industry over $370 million annually or 8.7% of its total value. Several gene expression studies have been performed to identify potential gene markers that contribute to resistance to infection in sheep. The resulting lists of differentially expressed genes from such studies are often highly inconsistent which makes interpretation and downstream adoption difficult. To get a handle on more robust markers we performed a meta-analysis using 5 separate global gene expression studies that measured transcriptional profiles under different models of infection to *H. contortus*. Additionally, functional gene-set enrichment approaches were performed. A total of 121 candidate resistance genes were identified, out of which, 99 were more highly expressed in resistant sheep and 22 had reduced expression. The biological functions associated with the upregulated genes evidenced that most of them were involved in inflammation and immune response processes. Eight overexpressed genes (C3, C7, C1QA, C4A, BRE, ANXA2, CORO1A, and SBDS) were highly enriched in immune-related GO terms and KEGG pathways. Further analysis of these overexpressed genes might provide insights into the biological mechanisms associated with *H. contortus* resistance in sheep and assist in selection for increased resistance.

Key Words: meta-analysis, gene expression

P1001 Sequencing of Brahman cattle for single nucleotide polymorphisms. R. Bunch[1,2], S. McWilliam[1,2], B. Harrison[1,2], and W. Barendse*[1,2], *[1]CRC for Beef Genetic Technologies, Armidale, NSW, Australia, [2]CSIRO, St. Lucia, QLD, Australia.*

Recent progress in genome sequencing has allowed rapid progress in the sequencing of cattle since 2009. Most of the additional sequencing has been of taurine cattle. Indicine and African cattle have lagged behind this, with the first 2 publications of genome sequences of Indicine and African bulls (Barris et al. 2012 Anim. Prod. Sci. 52:133–142; Canavez et al. 2012 J. Hered. doi:10.1093/jhered/esr153) published recently, representing the Africander, Brahman, Nelore, and Tuli breeds. In these studies, indicine cattle in particular have been shown to have higher levels of polymorphism than taurine cattle. To gather additional DNA variants for indicine cattle, we genotyped a collection of 83 Brahman cattle using the Illumina Bovine HD SNP array and calculated a genome relationship matrix between the individuals. The mean relatedness of pairs of the 83 animals was g = −0.013 (s.d. = 0.078), and the mean level of inbreeding was 7.0% (s.d. = 0.13). Sorting through the group we identified the 32 animals with the lowest degree of relatedness between each another, discarding animals with strong evidence of cross-breeding. The most related 2 individuals had g = 0.062, which is equivalent to sharing a grandparent. Illumina next generation sequencing of these individuals has commenced, more than 200 Gb of data have already been collected, and further sequencing is ongoing.

Key Words: Brahman, genome, sequencing

P1002 Estimation of genetic parameters for body weight in Moghani sheep breed (Jafar Abab station) by using random regression model. Mehdi Bayeriyar,* *Faculty of Agriculture, Moghan, Ardebil Province, Iran.*

In this study, weight records of Moghani sheep, from birth to365d of age, that was recorded every 3monthly analyzed by using random regression model. After basic edits, there were 6,758 sheep weights recorded between 2000 and 2011. The analysis was done by different assumption about residual variance, including the assumption of constant (homogenous) residual variance and different assumption about variable (heterogeneous) residual variances during growth. Maximum residual variance was estimated in ages 270 to 365 d. Amounts of direct heritability of birth weight, 90 d, 180 d, 270 d, and 360 d were estimated 0.36, 0.28,0.36, 0.39, and 0.44, respectively. The result showed that direct heritability estimates decreased after birth until animals were about 90 d old, increased slowly until 180 d of age. Maternal heritability decrease fast from birth to about 90d of age and decreased slowly after that. Correlations tended to decrease with increasing number of days between records. Additives genetic direct correlation estimates between weights at standard ages (birth, 90 d, 180 d, 270 d, and 360 d) were moderate to high and maternal genetic and environmental correlation were consistently high.

Key Words: body weight, genetic parameters, random regression

P1003 Investigation of correlations between lean meat yield measurements. M. J. Bixley,* S. N. Newman, K. G. Dodds, and P. L. Johnson, *AgResearch Ltd., Mosgiel, Otago, New Zealand.*

Methods used to estimate lean meat yield vary between breeder and processing companies. With producers generally having relationships with a specific processor it has been difficult to quantify the relationships between the different measurement systems. Many of the animals used in breeding programs have measurements obtained from ultrasound scanning of the loin around 8 mo of age. However, this is only a predictor of total carcass lean meat yield. CT scanning of the whole animal can provide accurate total lean meat yield, but

is both costly and access is limited. At the progeny test level, several different methods have been used to estimate lean meat yield. ViaSCAN and X-Ray are imaging systems used by 2 different New Zealand meat processors and other methods include weighing either the primal cuts or the total cuts from a whole carcass. Using data recorded in the SIL (Sheep Improvement Ltd.) database provided by Beef and Lamb NZ, we calculated the phenotypic and genetic correlations between these different meat trait measurements with a view to developing new lean meat yield breeding values, and compared them to existing values.

Key Words: lean meat yield, breeding value, SIL

P1004 Sire error rates and the impact on genetic progress in the New Zealand dairy cattle population. F. E. Bowley*[1], P. R. Amer[1], and S. Meier[2], *[1]AbacusBio Limited, Dunedin, Otago, New Zealand, [2]DairyNZ Limited, Hamilton, Waikato, New Zealand.*

Incorrect pedigree information has been shown to reduce estimates of heritability and correlations between true and estimated breeding values (EBVs), leading to loses in genetic gain. Rates of sire misidentification in dairy cattle have been investigated in several countries, but no studies using New Zealand data have been published. Analysis of sire test results from 97 herds participating in the DairyNZ InCalf program revealed a mean sire error rate of 23.3%, where 16.9% were incorrectly assigned and 6.4% were missing. To evaluate the effect of sire errors on EBV accuracy, 20 replicates of a simulation were performed, involving 360 progeny test sires each with 80 daughters in a progeny test herd, 40 widespread sires each with 80 daughters in a progeny test herd and 1000 daughters in a commercial herd, and 160 natural service sires each with 15 daughters in a commercial herd, using either a correct pedigree or one where a 23% sire error rate existed in the commercial herd. EBV accuracies for milk volume ($h^2 = 0.36$) and fertility ($h^2 = 0.03$) calculated via BLUP were lower when the erroneous pedigree was used (97% vs. 96% and 55% vs. 53%, respectively). The accuracy of estimated Breeding Worth also decreased from 91% to 89%. Hence, reduction of pedigree errors would facilitate more rapid genetic progress in the dairy industry.

Key Words: dairy, breeding value, pedigree error

P1005 Association of blood serum insulin-like growth factor I (IGF-I) concentration with reproductive, growth, and body composition traits of female Angus beef cattle. X. Zhang, M. E. Davis,* S. J. Moeller, and J. S. Ottobre, *The Ohio State University, Columbus, OH, USA.*

Reproductive performance of animals affects lifetime productivity. However improvement of reproductive traits via direct selection is generally slow due to low heritability. Therefore, identification of indicator traits for reproductive performance may enhance genetic response. Previous studies showed that serum insulin-like growth factor I (IGF-I) concentration is a candidate indicator for growth and reproductive traits. The objectives of our study were to estimate the (co) variances for IGF-I concentration and reproductive, body composition, and growth traits. Data were collected from a divergent selection experiment for serum IGF-I concentration at the Eastern Agricultural Research Station owned by The Ohio State University. The study included a total of 2,662 calves in the 1989 to 2005 calf crops. (Co)variance components were estimated for additive genetic effects using an animal model in multiple-trait, derivative-free, restricted maximum likelihood and ASReml computer programs. The results of direct additive genetic correlation suggest that selection for greater IGF-I concentration (heritability = 0.50 ± 0.07) could lead to increased conception (heritability = 0.11 ± 0.06, r = 0.32, $P < 0.001$) and calving rate (heritability = 0.13 ± 0.06, r = 0.43, $P < 0.001$), increased d 140 postweaning ribeye area (heritability = 0.63 ± 0.08, r = 0.38, $P < 0.001$) and weight (heritability = 0.62 ± 0.08, r = 0.15, $P < 0.001$), and decreased age at first calving in heifers (heritability = 0.35 ± 0.20, r = –0.40, $P < 0.001$).

Key Words: genetic correlation, insulin-like growth factor-I, reproduction

P1006 The turkey genome sequence: An update on the assembly and latest build. R. A. Dalloul*[1], A. V. Zimin[2], R. E. Settlage[3], and K. M. Reed[4], *[1]Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, USA, [2]Institute for Physical Science and Technology, University of Maryland, College Park, MD, USA, [3]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA, [4]Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA.*

Since the release of the turkey genome sequence in 2010, efforts to improve its assembly, gene annotation, and genomic analyses continue. The initial assembly build (2.01) represented about 89% of the genome sequence with 17X coverage depth (931 Mb). Sequence contigs were assigned to 30 of the 40 chromosomes with approximately 10% of the assembled sequence corresponding to unassigned chromosomes (Chr_Un). The turkey genome sequence is being refined through both genome-wide and area focused sequencing, including additional shotgun and paired-end sequencing, and targeted sequencing of chromosomal regions with low or incomplete coverage. These subsequent sequencing efforts have improved the sequence assembly resulting in 2 subsequent genome builds mostly using additional 3Kb and 8Kb libraries with sequencing on the Roche/454 Titanium platform resulting in higher

genome coverage (25X/Build3.0 and 30X/Build4.0) with a current sequence amount of 1,01Mb. These builds (3.0 and 4.0) were created to assess the value of the newly added sequences and did not undergo extensive annotation as additional sequences are being added. As such, neither build was released as efforts continue to enhance coverage of the smaller microchromosomes and the sex chromosomes, detailed gene annotation, and further genomic analysis of the latest build (5.0) expected to be completed in 2012. For example, BACs with end sequences assigned to the Z/W chromosomes, Chr_Un, or not placed in the current build have been isolated, deeply sequenced (Illumina), and are being incorporated into the latest build (5.0), which completion and release are anticipated within the coming year.

Key Words: turkey genome, sequencing, assembly

P1007 Detecting positive selection on X chromosome in pig through high density SNPs. Yunlong Ma, Qin Zhang, and Xiangdong Ding,* *China Agricultural University, Beijing, China.*

Many important traits were suffered strong selection pressure in pig. With the availability of high density SNPs in farm animals, the selection occurring in those traits could be traced by detecting selection signatures on genome, and genes experiencing selection can also be further mined based on selection signatures. Due to the special characteristic of X chromosome, many approaches of genetic analysis fitted for autosomes are not plausible for X chromosome, detecting selection signature provide one effective tools to settle such situation. In this study, XP-EHH was implemented to identify selection signatures on chromosome X across 3 pig breeds of Landrace, Chinese Songliao and Yorkshire using 1307 SNPs, afterward, genes located within selection signature regions were revealed in bioinformatics analysis. In total, 29, 13 and 15 regions with selection signatures were identified in Landrace, Songliao and Yorkshire, respectively. Some selection signatures regions in Songliao overlapped with those in Landrace, the similar situation was also found between Landrace and Yorkshire. However, there are no overlap regions between Yorkshire and Songliao. After gene GO analysis, many genes related with reproduction and immune traits were identified in selection signature regions, some of them were not reported in pig by far, which might be as important candidate genes in future study.

Key Words: positive selection, pig

P1008 A reference genome of the domestic goat (*Capra hircus*) generated by Illumina sequencing and whole genome mapping. Yang Dong[1], Xiaoyong Du*[3,1], Shuhong Zhao[3], Jun Wang[2], and Wen Wang[1], *[1]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences (CAS), Kunming, Yunnan, People's Republic of China, [2]BGI-Shenzhen, Shenzhen, People's Republic of China, [3]Huazhong Agricultural University, Wuhan, Hubei, People's Republic of China, [4]CSIRO Livestock Industries, St Lucia, QLD, Australia, [5]Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, People's Republic of China.*

The domestic goat, *Capra hircus* (2n = 60), is one of the most important domestic livestock species in the world. Here we report its high quality reference genome generated by combining Illumina short reads sequencing and a new automated and high throughput whole genome mapping system based on the optical mapping technology which was used to generate extremely long super-scaffolds. The assembly is strongly supported by the RH map of goat chromosome 1. We annotated 22,175 protein-coding genes, most of which are recovered by RNA-seq data of 10 tissues. Rapidly evolving genes and gene families are enriched in metabolism and immune systems, consistent with the fact that the goat is one of the most adaptable and geographically widespread livestock species. Comparative transcriptomic analysis of the primary and secondary follicles of a cashmere goat revealed 51 genes that were significantly differentially expressed between the 2 types of hair follicles. This study not only provides a high quality reference genome for an important livestock species, but also shows that the new automated optical mapping technology can be used in a de novo assembly of large genomes.

Key Words: *Capra hircus*, reference genome, whole genome mapping

P1009 MiRNA targeting in host-virus interaction: Inter-strain comparison in Pseudorabies Virus (PrV). M.-L. Endale Ahanda,* T. Letellier, and E. Giuffra, *UMR de Génétique Animale et Biologie Intégrative, Equipe GIS, INRA, Jouy-en-Josas, France.*

During a viral infection an intricate interaction takes place between a virus and its host. MiRNAs, a class of small regulatory molecules, have been shown to be major actors in this process. Several host miRNAs are essential to immune response, with few of them proposed to directly target viral genes. Viruses are able to use the miRNA-induced gene-silencing pathway to mediate immune evasion by modulating host and viral transcripts expression, and many herpesviruses including PrV are known to encode miRNAs. Here we compared three recently sequenced strains of PrV to predict the influence of DNA variations on the miRNA-mediated cross-talk between PrV and its natural host, the pig. High miRNA-binding prediction specificity was obtained by focusing on 3′UTRs target sites and by combining site accessibility and evolutionary conservation criteria. Most PrV miRNAs/PrV target sites interactions were highly

conserved (42 in all strains, 8 in two strains). Pig miRNAs/PrV target sites interactions (from a catalogue of ~350 porcine miRNAs) included UL24, a gene widely conserved among herpesviruses. Remarkably, striking differences in miRNA-binding sites were predicted between vaccine and virulent PrV strains. This approach is being extended to other viruses infecting livestock, including fast evolving RNA viruses.

Key Words: microRNAs, host-pathogen interaction, herpesvirus

P1010 A whole-genome association study for pigmentation traits in Chinese Holstein. Yipeng Fan,* Weixuan Fu, Xiaogang Cui, Cong Li, Weihui Gao, Peng Wang, Dongxiao Sun, Yi Zhang, Qin Zhang, and Yuan Zhang, *College of Animal Science and Technology, China Agricultural University.*

Pigmentation traits are essential characteristics of livestock. Although the pathway of melanogenesis, which is the genetic basis of pigmentation, has been well known, few genes influencing pigmentation traits were reported in big domestic animals. Base on the Illumina BovineSNP50 BeadChip, we herein conducted a genome-wide association (GWAS) study for 2 traits including proportion of black coat color and teat color in 707 Chinese Holstein cows from 14 sire families. With the case-control design, total 6 genome-wide significant SNPs were detected to be associated with pigmentation traits ($P < 10^{-6}$). Of them, a great significant locus within KIT gene in BTA6 accounts for the variation of proportion of black. As for teat color, one locus close to WNT16 gene and another one near PTPRZ1 gene might be candidate key genes which together affect the phenotype of such trait in Chinese Holstein. Our findings implied that complex pigmentation traits could be controlled by a few genes.

Key Words: GWAS, pigmentation traits, Chinese Holstein

P1011 Total computational performance comparison of two methods of genetic simulation. Michel M. Farah*[1], Aldrin V. Pires[2], Ricardo da Fonseca[3,1], Camila T. Meira[1], Adam T. H. Utsunomiya[1], and Luis Orlando D. Carreño[1], *[1]São Paulo State University/Genetics and Breeding Program, Jaboticabal, São Paulo, Brazil, [2]Federal University of the Jequitinhonha and Mucuri Valleys, Diamantina, Minas Gerais, Brazil, [3]São Paulo State University, Dracena, São Paulo, Brazil.*

The genetic simulation is an important tool for the animal breeding. And their performance on the efficiency of memory consumption and processing speed of simulation methodologies are affected by many factors, such as the amount of animals founders and the number of loci that affect feature. Although important to the research, was not found in the literature comparisons between the methods of individual-level simulation (ILS) and gene-level (GLS) with respect to their properties, parameters and computational performance. The objective of this study is to compare the factors affecting on memory demands (MDT) and processing (PDT) totals of 2 simulation methodologies. For a base population of 1000 individuals and a trait controlled by 500 loci the MDT of ILS is lower than the GLS until the formation of 3000 new individuals, this is because the process of generating new individuals in the ILS demand more memory than to form the base population, while in GLS the MDT is dependent only on the number of new individuals and loci generated. The PDT to ILS was superior to GLS, mainly due to the algorithm used in ILS utilize a relationship matrix to consider the effects of Mendelian sampling and in the GLS these effects are simulated during the gametogenesis process. Thus, ILS is growing exponentially and SNG is a linear growth.

Key Words: additive genetic breeding, algorithms, animal breading

P1012 A collaborative European network on rabbit genome biology: RGB-Net. H. Garreau*[1], Z. Bosze[2], I. Curik[3], M. Piles[4], C. Rogel-Gaillard[5], C.G. Thulin[6], and L. Fontanesi[7], *[1]INRA UR631, Castanet-Tolosan, France, [2]Agricultural Biotechnology Center, Gödöllő, Hungary, [3]University of Zagreb, Zagreb, Croatia, [4]IRTA, Caldes de Montbuí, Spain, [5]INRA UMR1313, Jouy en Josas Cedex, France, [6]Swedish University of Agricultural Sciences, Umeå, Sweden, [7]University of Bologna, Bologna, Italy.*

COST Action TD1101 "Rabbit Genome Biology-Net" is an action granted by COST in the domain of Biomedicine and Molecular Biosciences. Chaired by the University of Bologna and INRA, it will last for 4 years from November 2011, and gather 94 experts from 20 European countries and from USA, China, Japan, Taiwan and South Africa. Rabbit Genome Biology-Net (RGB-Net) aims at building an open international network of research organizations, associations and companies in all rabbit research areas (breeders, geneticists, bioinformaticians, physiologists, evolutionists, embryologists, immunologists, industry experts, etc.) to facilitate the transition of rabbit genomic information from experimental data into usable benefits and applications. Four Working Groups are focused on i) the refinement of the European rabbit genome resources and the development of genome-based platforms, ii) genetic aspects in meat, fur and pet rabbits and biodiversity resources, iii) the rabbit as a model in basic biology and human diseases and as a tool for biotechnology applications and iv) genetic and comparative genomic aspects for the study, exploitation and management of wild lagomorphs. The outcome is a coordination of rabbit

research activities and a transfer of knowledge, that will produce a strong European added value across a broad spectrum of biology research fields.

Key Words: European rabbit, genome biology, networking expertise

P1013 Understanding the microbial diversity of the bovine rumen in relation to feed efficiency. Bibaswan Ghoshal,* Mi Zhou, Emma Hernandez-Sanabria, Leluo Guan, and Paul Stothard, *University of Alberta, Edmonton, Alberta, Canada.*

The bovine rumen is rich in microorganisms among which bacteria play a key role-their fermentation providing the host animal with requisite energy to perform daily functions. Feed efficiency, often measured as residual feed intake (RFI), is considered one of the most important traits determining the productivity and profitability of cattle. To gain a better understanding of the relationship between bacterial diversity and feed efficiency we applied 454 pyrosequencing to generate partial (~400 bp) 16S rRNA gene sequences from rumen contents of beef cattle from different RFI classes (high and low). The sequencing data was analyzed using the QIIME software package for comparing bacterial diversity and species abundance. Thus far, in total, 296,492 reads were obtained. A total of 12568 OTUs were identified, which correspond to 11 distinct bacterial phyla. Species richness and phylogenetic diversity measures were found to be higher in high-RFI animals than in low-RFI animals. Furthermore, principle-coordinate-axes and hierarchical clustering of samples based on phylogenetic composition produced clusters corresponding to low-RFI group distinct from the high-RFI group. Although it is not yet clear whether these microbial differences contribute to feed efficiency differences, these preliminary results could lead to the development of microbial markers for predicting host RFI.

Key Words: RFI, bovine rumen, QIIME

P1014 DNA sequence capture for targeted resequencing of the major histocompatibility complex across wild pig and peccary species. J. Gongora*[1], M. Moroldo[2], N. Mach[2], P. Wahlberg[2], S. Marthey[2], J. Lecardonnel[2], M.-T. Bihoreau[3], C. Rogel-Gaillard[2], and J. Estelle[2], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [2]INRA, UMR GABI, CRB-GADIE, Domaine de Vilvert, Jouy-en-Josas, France, [3]CEA/IG/CNG, 2 rue Gaston Crémieux, Evry, France.*

Sequence capture is a widespread and cost-effective approach to resequence genomic regions of interest in large sets of individuals mainly within the same species. Our aim was to test the efficiency of this method across species of the family Suidae and distantly related family Tayassuidae for capturing and re-sequencing their MHC genome regions. A solid phase array encompassing the whole porcine MHC region (2.8Mb) was used in 9 species of Suidae (n = 69) and 2 species of Tayassuidae (n = 19). In Suidae, the average values of coverage and proportion of reads on target sequences were 86X and 32%, respectively, while in Tayassuidae these parameters were significantly lower. Given the segmented nature of the data aresued from capture, it was not possible to reconstruct a continuous sequence for each species. However, we could recover and update the sequence of 85% of the bases in the original reference haplotype using feral pig and wild boar data sets, which represents 95% of the sequence included in the array. The efficiency was similar in other Sus species and reasonably good in other Suidae. On the contrary, it was possible to recover ~70% of the targeted sequence in Tayassuidae. We conclude that capture across species appears to be efficient in Suidae while further optimization will be required in order that it be implemented in Tayassuidae.

Key Words: MHC, suids, peccaries

P1015 H3K27me3 regulation in lymphocytes and the association with bovine subclinical mastitis. Yanghua He*, Ying Yu, and Yuan Zhang, *China Agriculture University, Beijing, China.*

Bovine subclinical mastitis (SM) is the most expensive disease in dairy cattle. Since it is a complex disease and influenced by pathogen, environment and genetics, epigenetic regulation among their crosstalk is particularly important. In the present study, 6 Chinese Holstein cows were selected based on pathogenic bacteria identification and divided into 2 groups (SM and controls). Peripheral blood lymphocytes used in the study were isolated from each cow. The distribution and regulation pattern of histone H3 trimethylation on lysine 27 (H3K27me3) were investigated by high-throughput ChIP-Seq and digital gene expression techniques. The enrichment of H3K27me3 and the expression level of the target genes were validated by ChIP-qPCR and qRT-PCR approach. Our results discovered that bovine lymphocytes H3K27me3 marks tended to repress expressions of target genes via acting on 5′ and 3′ regulatory regions on genome-wide, and genes promoter is the crucial region. A set of target genes of H3K27me3 were detected differentially expressed in healthy and SM cows. These genes are involved in immune responses and inflammatory diseases, such as CD4, IL10 and IFNB3. Our data suggest that H3K27me3 was associated with bovine SM and it can be considered as potential epigenetic marks in SM for further pathogenesis studies.

Key Words: bovine mastitis, H3K27me3, ChIP-seq

P1016 A high-throughput DNA extraction method for sheep ear tissue. H. Henry,* R. Anderson, J. McEwan, and S. Clarke, *AgResearch, Mosgiel, New Zealand.*

High-throughput SNP genotyping has revolutionized our ability to obtain both high and low density genotypes. It is therefore essential to develop a high-throughput DNA sampling and extraction method that yields good quality DNA of sufficient quantity for application across the various genotyping platforms. The extraction method needs to be robust, high-throughput and reproducible. Further to this, selecting a sample type that could be collected in a high-throughput and user-friendly way would be an asset in a commercial environment. We have developed a simple, cost effective, 96-well plate-based high-throughput DNA extraction method, using sheep ear tissue. Several techniques were investigated focusing on robotics and scaling-down, to minimise the cost of consumables and reagents. The chosen method is a proteinases K digest followed by salt/ethanol precipitation which delivers consistently greater than 10ug of high quality DNA. Validation of this DNA extraction method will be presented where-by DNA samples have been tested on both the Sequenom and Illumina genotyping platforms with excellent results. In addition to genotyping platforms, this method also produces good quality DNA for next generation sequencing.

P1017 Databases for animal genome research: The needs, the trends, and the potentials—A case study on development of the pig genome database. Zhi-Liang Hu* and James M. Reecy, *Iowa State University, Ames, IA, USA.*

A large number of databases have been constructed in the past decade for animal genome research. Many of these databases were developed to serve a specific purpose; however, not all of them are well maintained over time. The increase in the number of databases has resulted in several negative consequences: one, there are "too many" databases for users to look through when one needs to find something; 2, many of these databases overlap in terms of resources and contents; 3, the methods to develop these databases are similar, which has often resulted in reinventing the wheel. In construction of a pig genome database, we have explored possibilities to avoid these problems with a "federated database" idea. The main feature of this approach is to utilize all publicly available resources/tools by establishing dynamic links between the remote databases for users to easily traverse through the related information without physically bringing them to a database on local hardware. The advantages of this implementation are several fold: maximize the productivity with limited resources, share the resources and duties maintaining the data, and minimize the workload for continued database updates. Toward this end, we propose a "database network" for the community.

Key Words: federated database, database network, data sharing

P1018 Extensive variant detection in the Fleckvieh population by low-coverage re-sequencing. Sandra Jansen,* Bernhard Aigner, Hubert Pausch, Michal Wysocki, and Ruedi Fries, *Chair of Animal Breeding, Technische Universitaet Muenchen, 85354 Freising, Bavaria, Germany.*

Although sequencing costs have dropped dramatically in the last few years, re-sequencing of a large number of animals for exhaustive variant detection is still not feasible. However, re-sequencing of a small number of key ancestors allows the capture of a major proportion of the population-wide variation. We have chosen 39 key ancestors and 4 contemporary animals of the Fleckvieh population for re-sequencing. These animals explain 68% of the population's gene pool. Next-generation sequencing with Illumina instruments provided an average effective coverage of 7.4 x. Multi-sample variant detection yielded 17.3 million SNPs and short insertion / deletions polymorphisms (InDels). Combining a read-pair with a read-depth copy number variant detection method allowed to uncover 1782 deletion variants with high reliability. After BEAGLE imputation, 97% of SNP and InDel genotypes had phred-scaled quality scores of at least 10. Sensitivity amounted to 98% and specificity to 3% after comparing the sequence-derived genotypes with high-density array genotypes. Annotation of high-quality variants revealed 89,533 variants in the coding region of 16,460 genes. Of the coding variants, 44.37% are non-synonymous and 689 lead to stop codons. The re-sequencing derived genotypes of key ancestors will be the basis for imputation in high-density genotyped animals and will facilitate QTL fine-mapping by genome-wide association studies at a very high resolution.

Key Words: population-wide sequencing, variant detection, functional annotation

P1019 The domestic sheep reference genome assembly. Yu Jiang*[1], Min Xie[2], Brian Dalrymple[1], James Kijas[1], Richard Talbot[3], Alan Archibald[3], Jillian Maddox[4], Thomas Faraut[5], Wen Wang[6], and Noelle Cockett[7], *[1]CSIRO Livestock Industries, St Lucia, QLD, Australia, [2]Beijing Genomics Institute, Shenzhen, China, [3]The Roslin Institute, Easter Bush, Scotland UK, [4]University of Melbourne, Melbourne, VIC, Australia, [5]INRA, Toulouse, France, [6]Kunming Institute of Zoology, Kunming, Yunnan, China, [7]Utah State University, Utah, USA.*

Oarv3.0 of the ISGC coordinated reference genome of the domestic sheep was released in April 2012. This

is a rebuild of the Oarv2.0 assembly incorporating a range of improvements. Additional sequencing was undertaken at the Roslin Institute to increase the coverage of high-GC rich regions that were underrepresented in previous Illumina sequencing. Sanger sequence from the BAC-ends and 454-derived sequence from the Oarv1.0 assembly (6 different animals) was also used to fill gaps. A large number of the short gaps present in Oarv2.0 have been filled and the N50 of contigs and scaffolds has significantly increased. Appropriate revisions of the sheep genome assembly were also made where supported by the mapping of the paired-ends of long insert sheep clones. Localized reassembly of a small number of regions of that were difficult to assembly in Oarv2.0 was undertaken. The assembly has been deposited in GenBank and GigaDB. A revised list of Ovine SNP50 BeadChip SNP coordinates and an assessment of the major remaining gaps in the assembly is available from the authors. The result is a high quality assembly essential for underpinning experiments across the community. Key among these will be its application to generate reference guided assemblies of influential sires and sheep with traits of interest as they are sequenced.

Key Words: Oarv3.0, fill gap, high-GC

P1020 The imprintome of a domestic sheep. Yu Jiang*[1], Brian Dalrymple[1], James Kijas[1], and Wen Wang[2], *[1]CSIRO Livestock Industries, St Lucia QLD, Australia, [2]Kunming Institute of Zoology, Kunming, Yunnan, China.*

Imprinted genes are mono-allelically expressed in a parent-of-origin dependent manner because of epigenetic modification. By combining sequencing of the transcriptome and the genome itself allelically imbalanced genes can be accurately identified. Using Illumina sequencing we identified 5.1 million SNPs in a female Texel sheep and from 7 tissues of the same individual we acquired 15 Gb of RNA-Seq data. Using > 90% expression in one allele of a SNP and with a sequence coverage of at least 20-fold as a stringent cut off, 8.9% of the 41K expressed SNPs showed allelic imbalance. Of the 636 genes with allelically imbalanced expression, 332 were supported by one SNP and 304 by multiple SNPs. Several loci containing more than one adjacent gene with allelically imbalanced gene expression were identified. The largest such region (198 adjacent mono-allelically expressed SNPs) included DLK1 and is involved in the polar over-dominant callipyge phenotype. Highly expressed genes, and genes containing SNPs generating non-conservative amino acid changes, were more likely to have allelically imbalanced expression. The sheep data set was compared with a similar data set from a female Yunlin black goat. Just over 50% of the genes showing allelic-imbalance in the sheep also showed allelic imbalance in the goat. Allelic gene expression and hence imprinting appears to be moderately conserved between species of ruminants.

Key Words: allelically imbalance, imprinting, RNA-seq

P1021 Introducing mouse, canine, and zebrafish exomes using the Agilent Technologies SureSelect Target Enrichment System. Moraima Guadalupe[1], Chee Yang Lee*[4], Angelica Giuffre[1], Carlos Pabón-Peña[2], Harini Ravi[1], Barbara Novak[2], Owen Hardy[3], Marc Visitacion[2], Joseph Ong[1], Swati Joshi[1], Eric Lin[2], Kyeong-Soo Jeong[2], Julia Barboza[1], Albert Lee[2], Margherita Corioni[2], Micah Hamady[2], Francisco Useche[2], Douglas Roberts[2], Scott Happe[1], and Emily Leproust[2], *[1]Agilent Technologies, Genomics Division, Cedar Creek, TX, USA, [2]Agilent Technologies, Genomics Division, Santa Clara, CA, USA, [3]Agilent Technologies, Genomics Division, La Jolla, CA, USA, [4]Agilent Technologies, Genomics Division, Petaling Jaya, Selangor, Malaysia.*

The dramatic increase in throughput of sequencing data from next-generation sequencing platforms has enabled scientists to study the genome with unprecedented depth and accuracy. It has revolutionized the discovery of rare polymorphisms, structural variants, and novel transcripts. The SureSelect Target Enrichment System for human exomes has been shown to accurately detect common and rare SNPs, indels, CNVs, and splicing variants. To enable exome studies in common model organisms, 3 new catalog kits have been introduced targeting mouse, canine, and zebrafish exomes. Each exome kit demonstrates high performance as measured by specificity of capture, coverage depth, uniformity of coverage, and duplicate read counts. In addition to the 3 model organism exome kits, Agilent's eArray web-based software supports the design of custom libraries for 17 unique species to facilitate analysis of smaller, focused regions. We demonstrate that the SureSelect portfolio is a flexible system that is rapidly expanding to meet the needs of next-generation sequencing users. This provides a cost-effective approach to analyzing specific genomic regions with unprecedented depth and accuracy.

Key Words: SureSelect Target Enrichment, model organisms, exome

P1022 Potential bull traits for prediction of percent normal sperm at 24 months of age. Y. Li*[1,2], N. Corbet[1,3], B. Burns[1,4], D. Corbet[1,5], J. Crisp[1,6], B. Venus[1,4], M. McGowan[1,6], and R. Holroyd[1,5], *[1]CRC for Beef Genetic Technologies, [2]CSIRO Livestock Industries, St Lucia QLD, Australia, [3]CSIRO Livestock Industries, Rockhampton QLD, Australia, [4]Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, Rockhampton QLD, Australia, [5]Department of Employment, Economic Development and Innovation, Rockhampton QLD, Australia, [6]School*

of Veterinary Science, University of Queensland, Gatton QLD, Australia.

Reproductive performance is one of the most important traits which directly affect the profitability and productivity in the northern Australian beef cattle industry. Percent normal sperm at 24 mo (PNS24) has been regarded as a key indicator of male fertility. Seventeen bull traits were examined for their phenotypic and genetic associations with PNS24 to ascertain if any could be used as early predictors of PNS24. These traits included growth and carcass, adaptation, conformation, circulating blood hormones, scrotal circumference and semen quality traits measured at various developmental stages. Three statistical models, namely linear (LM), generalized linear (GLM) and non-linear statistical models (NLS), were applied to evaluate the relationships of traits measured at weaning, 12, 18 or 24 mo of age with PNS24 in Brahman and Tropical Composite populations. PNS24 was analyzed either as a continuous response variable or a binary variable using 70% as a threshold value. The results from different models clearly demonstrate non-linear curvature of the phenotypic relationships between PNS24 and the indicator traits. The relationships vary between genotypes, analytical methods and developmental stages. None of the phenotypic measures could accurately predict PNS24 with high statistical power (R-squared value).

Key Words: percent normal sperm, bull traits, statistical models

P1023 RNA sequencing to identify specific expressed genes and microRNAs in wool follicles during anagen, catagen and telogen phases. G. Liu,* S. Zhao, X. Li, J. Cao, M. Zhu, and M. Yu, *Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, PR China.*

The wool quality is one of the most important economic traits in the sheep. The wool is the fiber derived from the specialized skin cells called follicles. Therefore, it would be of importance to understand the mechanisms controlling the follicle development and fiber formation in sheep. By Solexa RNA-sequencing, we characterized genes from follicles and 4 pooled tissues from the Tibetan sheep. The results showed that a total of 1341 genes were highly expressed in follicles. Further experiments in 16 different tissues confirmed that 17 of them are specifically expressed in follicle cells. We also characterized microRNAs from wool follicles during anagen, catagen and telogen phases. A total of 914 microRNAs were detected to be expressed in wool follicles. The level of the 39 most highly expressed microRNAs were found to be significantly changed among the phases. Additionally, the integrative analysis of gene and microRNA profiles showed that 2894 candidate genes could be targeted by the 39 microRNAs. 167 of the predicted targets are involved in 15 different KEGG pathways, including Wnt, TGF β and Hedgehog pathways which play important roles in cell proliferation and differentiation. The results revealed important genes and microRNAs that might be involved in regulation of wool fiber formation and growth in the sheep.

Key Words: RNA-sequencing, wool follicle, microRNA

P1024 Accuracy of imputation using 6K and 50K SNP chips in multi-breed and crossbred beef cattle populations. R. V. Ventura[1,2], F. S. Schenkel[1], M. Sargolzaei[1,4], Z. Wang[3], C. Li[5], and S. P. Miller*[1], *[1]CGIL, Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, [2]Beef Improvement Opportunities, Guelph, ON, Canada, [3]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [4]L'Alliance Boviteq, Saint-Hyacinthe, Quebec, QC, Canada, [5]Agriculture and Agri-Food Canada, Edmonton, AB, Canada.*

Genotyping with lower density, but lower cost panels enables more genotypes for genomic selection, which should increase accuracy of genomic EBV. Imputation enables the determination of missing SNP genotypes in animals genotyped with a low density panel by using information from a reference population genotyped with a higher density panel. In this study, population imputation, utilizing linkage disequilibrium among markers, was implemented using the software BEAGLE and FImpute 2.0 in a multi-breed, crossbred taurine beef cattle population genotyped with the Illumina 50K SNP panel. Different combinations of reference populations and imputed animals were defined based on breed composition. Number of animals (n = 1500, 2000, 2500, 3000 or 4732) and the presence of parents in the reference population (only for n = 4732) were investigated. The overall imputation accuracy ranged from 90.99% to 98.28%. Higher imputation accuracies were obtained when both parents of imputed animals were in the reference population and the breed composition of imputed animals was well represented in the reference population. FImpute yielded an average overall increase of 2.21% in imputation accuracy over all scenarios and reduced the run-time by 10x (81 vs. 816 min) compared with BEAGLE.

Key Words: imputation, beef cattle, crossbred

P1025 Genomic prediction in Merino sheep for varying reference population size and marker density. N. Moghaddar*[1,2] and J. H. J. Van der Werf[1,2], *[1]School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia, [2]Sheep Cooperative Research Center, Armidale, NSW 2351, Australia.*

Incorporation of genomic information in genetic evaluation of farm animals can considerably enhance the rate of genetic improvement by increasing the accuracy of selection and decreasing the generation interval. The aim of this study was to determine the accuracy of genomic prediction for yearling greasy fleece weight (YGFW), early breech wrinkle (EBRWR) and post weaning weight (PWW) in Australian Merino sheep, depending on the size of the reference population (Nref) and the number of SNP markers genome wide (Nmark = 5k, 10k and 50k SNPs). The marker subsets were chosen at random, and always evenly distributed across the genome. Genomic prediction of breeding value (GEBV) was obtained via the GBLUP method, using genomic relationships between a reference population of merino sheep and 150 to 184 merino industry sires with progeny and the GEBV accuracy was calculated from its correlation with the Australian Sheep Breeding Value (ASBVs) on those sires. For Nref = 4000 and Nmark = 50k, the GEBV accuracy was 0.64, 0.31 and 0.56 for YGFW, EBRWR and PWW, respectively. With Nref = 1000, the accuracy decreased between 11% and 13.8%. When Nmark decreased to 10k, the accuracies decreased between 9 and 10.5% and for Nmark = 5k accuracy decreased between 11.1% and 13.2%, on average. The decline in accuracy was higher in low heritable traits. We suspect that a fair part of the prediction accuracy is due to the degree of genetic relationships and population substructure.

Key Words: genomic prediction, Merino sheep

P1026 The complete swine olfactory subgenome: Expansion of olfactory receptor genes in the pig genome. Dinhtruong Nguyen[1], Kyooyeol Lee[1], Hojun Choi[1], Minkyeung Choi[1], Minhthong Le[1], Kyungtae Lee[2], Taehun Kim[2], and Chankyu Park*[1], *[1]Department of Animal Biotechnology, Konkuk University, Gwangjin-gu, Seoul, South Korea, [2]Animal Genomics and Bioinformatics Division, National Institute of Rural Development Administration, Suwon, South Korea.*

Olfaction is a complicated process of specific binding of volatile odorant molecules to dedicated olfactory receptors (ORs). OR proteins are encoded by the largest gene superfamily in the mammalian genome. We report here the whole genome analysis of the olfactory receptor genes of *Sus scrofa* using conserved OR gene specific motifs and known OR protein sequences from diverse species. We identified 1,638 OR related sequences from the *S. scrofa* genome assembly, Sscrofa 10.2 including 1,113 functional OR genes, 188 pseudogenes, and 337 partial genes. OR genes were located in 46 different loci on 16 pig chromosomes. We classified the ORs into 17 families, 3 class I and 14 class II families, and further grouped them into 349 subfamilies. We also identified inter- and intra-chromosomal duplications of OR genes residing on 11 chromosomes. A significant number of pig OR genes (n = 212) showed less than 60% amino acid sequence similarity to known OR genes of other species. As the genome assembly Sscrofa 10.2 covers 99.9% of the pig genome, our analysis represents almost complete OR gene repertoire from an individual pig genome. We show that *S. scrofa* has one of the largest OR repertoires, suggesting an expansion of OR genes in the swine genome. A significant number of unique OR genes in the pig genome may suggest the presence of swine specific olfactory stimulation.

Key Words: olfactory receptor, pigs, OR genes

P1027 Imputation of whole-genome sequence information for QTL fine-mapping in the Fleckvieh population. Hubert Pausch,* Christine Wurmser, Sandra Jansen, Bernhard Aigner, and Ruedi Fries, *Chair of Animal Breeding, Technische Universitaet Muenchen, 85354 Freising, Germany.*

Next generation sequencing of 43 key and contemporary animals, explaining 68% of the gene pool of the German FV population, and subsequent multi-sample variant calling yielded genotypes at 17.3 million sites. Pre-phasing both the sequence and the array data with Beagle and subsequent population-wide imputation with MiniMac facilitated to extrapolate genotypes at 12 million SNPs and 1.5 million InDels for 3668 FV animals via high- and medium-density genotypes. The accuracy of the imputed genotypes exceeded 95%. Thus, imputed 13.5 million genotypes were used in genome-wide association studies (GWAS) with progeny-derived phenotypes for milk-fat content at different lactation stages. The sequence-based GWAS identified 9 QTL, among them a highly significantly associated QTL for milk-fat content in early lactation on chromosome 27. The QTL contains GPAT4 which encodes a rate-limiting enzyme in the triacylglycerol biosynthesis pathway and plays a key role in milk fat biosynthesis. The association was more significant than that obtained from using array-based markers only (5.79×10^{-20} vs. 3.63×10^{-11}). The most significantly associated SNP is located in the 3′-UTR of GPAT4 and affects a putative microRNA binding site. The SNP reached high significance (4.01×10^{-17}) in an independent validation study with 2327 animals of the German Holstein-Friesian population and is an excellent candidate to be the underlying QTN for the milk-fat content QTL on chromosome 27.

Key Words: sequence imputation, genome-wide association, QTL fine mapping

P1028 Automated phenotyping of livestock. I. Purvis*[1], P. Hunt[1], P. Valencia[2], and L. Overs[2], *[1]CSIRO Livestock Industries, Armidale, NSW, [2]CSIRO ICT Centre, Brisbane, QLD.*

Genomic evaluation systems in the dairy and beef industries have entered a phase of implementation and refinement, but their full potential will not be realized until all economically important traits in relevant production systems are incorporated. Many of the traits not yet incorporated, are either difficult or highly technical to measure. One example is individual animal feed intake for pasture-based production systems. Currently evaluation uses data from feedlot measurement using grain-based diets. The relevance of grain based feed intake data for predicting the merit of pasture fed animals must be questioned. The development of miniaturised wireless sensors and data capture systems now offers us the capability to study animals in their (various) production environment(s), and in a way that does not constrain them from expressing their full range of genetic drivers for the traits of interest. We describe an automated high throughput system for deep phenotyping of livestock maintained in environments that reflect commercial conditions, and present an early assessment of the system for more precise and relevant genetic/genomic evaluation.

Key Words: phenotyping, livestock, automation

P1029 A composite test to detect positive selection in cattle and sheep. Imtiaz A. S. Randhawa,* Mehar S. Khatkar, Peter C. Thomson, and Herman W. Raadsma, *REPROGEN Animal Bioscience group, Faculty of Veterinary Science, University of Sydney, Camden, NSW 2570, Australia.*

Rapidly emerging research in quantitative and qualitative genetics demands robust analytical tools to identify genetic loci involved in phenotypic appearance and underlying molecular mechanisms. Here we present a robust method for genome-wide selection scans (GWSS) using dense polymorphism data to map high-resolution signals by combining evidence from multiple selection tests. We investigated multi-breed data sets from 375 cattle and 2803 sheep for the presence or absence of either polledness or double muscling using 3 independent tests for selection signatures and a novel composite test. All cattle and sheep samples were genotyped with Illumina's BovineSNP50 and OvineSNP50 chip assays, respectively. Total of 38,610 SNPs for cattle and 47,502 SNPs for sheep were retained. For each trait, single (FST, ΔDAF or ΔAFD) and multiple (XP-EHH) marker based estimates for evidence of selection were computed. In addition, a novel composite signal was obtained by converting the fractional ranks of test statistics of each method into Z-statistics and combined in a composite score at each locus to detect a common selection signature. High-resolution peak scores were detected by GWSS in each analysis in the known candidate regions of cattle's autosome 1 (gene: SYNJ1) and 2 (MSTN), and sheep's autosome 10 (RXFP2) and 2 (GDF8), for the functional mutations which underpin polledness and double muscling, respectively. The strong association of the single and the combined signal at these loci confirms the robustness of composite signals for detecting selection signatures. This method can be used to identify the candidate regions harbouring functional SNPs in genes of complex networks, e.g., domestication, adaptation and production traits.

Key Words: genome-wide selection scans, composite signals, cattle and sheep

P1030 Multitrait composite interval mapping reveals pleiotropic QTL on chicken chromosome 1. Millor Rosario*[1,5], Rodrigo Gazaffi[2], Ana Moura[3], Monica Ledur[4], Katia Nones[1], Antonio Garcia[2], and Luiz Coutinho[1], *[1]Departamento de Zootecnia, USP/ESALQ, Piracicaba, 13418-900, Brazil, [2]Departamento de Genétcia, USP/ESALQ, Piracicaba, 13418-900, Brazil, [3]Universidade Estadual Paulista, UNESP/FMVZ, Botucatu, 18618-000, Brazil, [4]Embrapa Suínos e Aves, Concórdia, 89700-000, Brazil, [5]Departamento de Genética e Evolução, UFSCar/CCBS, São Carlos, 13565-905, Brazil.*

Genetic correlations between traits are caused by linkage or pleiotropy. Multivariate approaches are useful to help clarify this point. We implemented multitrait composite interval mapping to map pleiotropic QTL associated with performance, carcass, organs and physiological traits on chromosome 1 of a Brazilian chicken F2 population (broiler x layer). Genotypes from 453 F2 chickens were obtained using 26 microsatellite markers. Phenotypes for 24 traits were adjusted using family (5) and sex (2) as fixed effects and hatch (17) as a random effect in PROC MIXED. Conditional probabilities of QTL genotypes were obtained from GridQTL and cofactors were selected only if located outside of the chromosome 1, avoiding overparametrization. QTL analysis was carried out in R software and a significant threshold was defined as LOD > 17.0, considering Bonferroni correction. We mapped 4 pleiotropic QTL: at 173 cM (LOD = 20.5) associated with body weight 41 d (BW41), weights of heart and abdominal fat, and ash content in dry matter; at 203 cM (LOD = 20.4) associated with BW41, weights of wings, head, heart, gizzard and abdominal fat, and crude protein, ether extract and ash contents in dry matter; at 267 cM (LOD = 18.7) associated with BW41, weights of breast, shank, head, gizzard and crude protein content in dry matter; and at 436 cM (LOD = 22.9) associated with BW41, weights of breast, wings, shank, gizzard, intestinal length and cholesterol and triglycerides plus cholesterol levels. These 4 genomic regions will be useful to mine for SNP using 60k DNA chip to identify associations between SNP and poultry economical traits simultaneously.

Key Words: poultry, pleiotropy, multivariate analysis

P1031 A selective genotyping approach identifies copy number variants associated with backfat thickness in Italian Large White pigs. G. Schiavo*[1,3], M. Dolezal[2], P. L. Martelli[5,3], D. G. Calò[4], G. Galimberti[4], E. Scotti[1], R. Casadio[5,3], L. Buttazzoni[6], A. Bagnato[2], V. Russo[1], and L. Fontanesi[1,3], *[1]Department of Agro-Food Science and Technology, University of Bologna, Bologna, Italy, [2]Department VSA, University of Milan, Milan, Italy, [3]Centre of Genome Biology, University of Bologna, Bologna, Italy, [4]Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy, [5]Biocomputing Group, Department of Experimental Evolutionary Biology, University of Bologna, Bologna, Italy, [6]CRA, Centro di Ricerca per la Produzione delle Carni e il Miglioramento Genetico, Roma, Italy.*

Copy number variants (CNVs) are a major source of genetic variability in mammalian genomes. CNVs are involved in many human disorders, including obesity. For several biological reasons pig could be a biomedical model for human obesity and associated diseases. Fat deposition is a key process with practical and economical implications in pig breeding. This trait determines carcass value and consumers' acceptance of pork. In this study we applied a selective genotyping approach to identify CNVs associated with backfat thickness (BFT) in Italian Large White pigs. Pigs with extreme and divergent estimated breeding values (EBVs) for BFT were selected among a performance-tested population of ≈12,000 animals and genotyped with the Illumina PorcineSNP60k Beadchip. CNVs were called using pennCNV using strict criteria. Fifteen copy number variation regions (CNVRs) (in at least 4 pigs) were present only in the positive BFT-EBV group whereas 12 CNVRs were reported only in the negative BFT-EBV tail. Other CNVRs differed in frequency ($P < 0.05$) between the tails. Identified CNVRs include genes involved in fat metabolism, growth regulation, immune system, and neuronal regulation of eating behavior. These results provide additional insights into mechanisms affecting fat deposition in pigs useful for understanding aspects of human obesity.

Key Words: CNV, backfat, pig

P1032 Constrained GERP elements and the annotated bovine genome. M. Dolezal[1], D. Kedra[2], E. Lipkin[3], C. Notredame[2], A. Bagnato[1], H. Tafer[4], M. Soller*[3], and J. Herrero[5], *[1]Dept. VSA, University of Milan, Milan, Italy, [2]Centre de Regulacio Genomica (CRG), Barcelona, Spain, [3]Dept. of Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel, [4]Institut für Informatik, Universität Leipzig, Leipzig, Germany, [5]European Bioinformatics Institute (EBI), Hinxton, UK.*

Top-down identification of genomic functional elements (GFEs) involves projecting onto the genome identified features such as protein-coding mRNA. A bottom-up approach to GFE identification can be based on the assumption that across evolutionary time scales, GFEs accumulate fewer mutational changes than neutral regions. Genomic regions of 35 mammalian species were aligned, yielding a neutral substitution rate of 4.95 nucleotides per site. The GERP (Genomic Evolutionary Rate Profiling) score was estimated for each nucleotide site as the expected number of substitutions under neutrality minus the observed number. A positive GERP score indicates constraint and function. By permutation test, 1,356,030 bovine GERP-GFEs were identified of average size 110 bp, comprising about 6% of the bovine genome (149M nucleotides). Working top-down, a total of 26,740 GFEs were annotated to the bovine genome, including 22,118 protein coding genes, but not (as yet) lncRNAs and sncRNAs uncovered by RNaseq. Of total bovine GERP-GFEs, 52.7% do not overlap annotation-GFEs, suggesting incompleteness of the bovine "top-down" annotation. Of total bovine annotation GFEs, 40% are not overlapped by GERP-GFEs. This is unexpected considering the high proportion of large protein coding elements among the annotation-GFEs. (This research supported by the EC-funded FP7 Project 'Quantomics'.)

Key Words: GERP score, genome annotation, genomic functional elements

P1033 Missing homozygous genotypes in SNP chip analysis—Artefacts or clues to lethal traits? I. Tammen*[1,3], M. Khatkar[1,3], M. Hobbs[1,3], E. Jonas[1], B. Hayes[2,3], P. C. Thomson[1,3], and H. W. Raadsma[1,3], *[1]Reprogen - Animal Bioscience, Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia, [2]Biosciences Research Division, Department of Primary Industries, VIC Australia, [3]Dairy Futures Cooperative Research Centre (CRC), Australia.*

Data from SNP arrays provides dense genotype information for the mapping of genetic conditions. We proposed previously that the analysis of extreme segregation distortion clusters (ESDC) can be used to identify regions that are likely to carry embryonic lethal genes or genes with a strong negative selection response. Raw genotypic and signal intensity data on 845 cows genotyped with the Illumina 800k SNP chip were analysed, and SNP with extreme segregation distortion (ESD-SNP) were defined as those having a minor allele frequency (MAF) > 0.01 and a missing homozygous genotype. The MAF of the ESD-SNP were plotted against their chromosomal position on the Bos taurus UMD3.1 assembly and several distinct ESDC were noticed. Further analysis involving different scoring methods, candidate gene screening and haplotype analysis were conducted. A high number of haplotypes was identified leading to the assumption that genotypes for ESD-SNP might be incorrect. SNP signal intensity data

was plotted to visually assess the accuracy of genotype assignment. Many ESD-SNP presented with atypical clustering and more than 2 clusters, and were excluded from the analysis. Only one strong ESDC remained, which was located in a region subsequently identified to harbor a copy number variation region. It was concluded that ESDC were not a useful approach to identify lethal traits.

Key Words: SNP array, segregation distortion

P1034 Application of non-linear modeled heritability estimates for GWAS. Tozaki Tozaki*[1], Takeshi Miyake[2], Hironaga Kakoi[1], Hitoshi Gawahara[1], Kei-ichi Hirota[1], and Masahiko Kurosawa[1], *[1]Laboratory of Racing Chemistry, Utsunomiya, Japan, [2]Comparative Agricultural Sciences, Kyoto University, Kyoto, Japan.*

A categorical trait analysis for non-linear models is known to be useful for estimating the heritability of non-normally distributed traits. This study estimated heritability by performing linear and non-linear model analyses concerning lifetime earnings or ranking of Japan Racing Association to investigate the variation in heritability within the various categorizations of the phenotypes. The heritability (0.25) obtained from a non-linear model concerning formal ranking was much higher than that obtained from a linear model (0.11). In particular, the heritability (0.34) for binary categorizations with non-winning and winning horses was much higher than those for the other categorizations. These results may reflect possible presences of genetic discriminations between the binary categorizations. The binary categorizations were consistent with the case/control classification in the GWAS that only identified the MSTN gene variant (g.66493737C/T, OR = 2.31). It was also demonstrated by a large-scale cohort study that the allele frequency distribution of the non-winning category (C: 39%) was particularly different from those of the winning categories (C: 44–49%). Those findings suggested that the non-linear modeled heritability estimates from several categorizations of phenotypes are useful for the determination of thresholds for QTL phenotypes and the selection of case/control populations in GWAS.

Key Words: heritability, horse

P1035 Statistical associations between questionnaire-derived canine behaviour phenotypes. D. Van Rooy,* P. Thomson, P. McGreevy, and C. Wade, *Faculty of Veterinary Science, University of Sydney, Sydney NSW Australia.*

Questionnaires are an efficient and effective way of phenotyping behavior for genetic studies. As part of a study into the genetic basis of canine separation-related distress disorder, common canine behaviors were phenotyped using a questionnaire based on the Canine Behavior Assessment & Research Questionniare (CBARQ). To date, 354 questionnaires have been completed by owners/carers of Golden retrievers and Labrador retrievers. Data obtained have been analysed for relationships among behavior traits. We have identified positive correlations that are statistically significant ($P < 0.001$) among several traits: excitability with energy levels; among fear-response phenotypes; and among different categories of aggression. For instance, aggression towards strangers was significantly positively associated with aggression towards dogs, and aggression towards owners was positively associated with aggression between household dogs. Additionally, associations were found between fear and aggression phenotypes, namely fear of strangers was associated with aggression towards strangers. Attention-seeking behaviour was associated with separation-related problems and excitability. Sensitivity to touch was associated with separation-related problems and fear of places, objects and noises. These associations form an important resource for the genetic study of canine behaviour.

Key Words: phenotype, behaviour

P1036 Identification of miRNA expression profile in pig thyroid at different growth stages by miRNA microarray technology. Ying Wang,* Zhaozheng Yin, and Ningying Xu, *College of Animal Science, Hangzhou, Zhejiang, China.*

In this study, the 10 small RNA libraries were constructed by pig thyroid with 10 developmental stages: 3 prenatal stages (60, 80, and 105 d after insemination, referred to as E60d, E80d, E105d respectively) and 7 postnatal stages (1, 25, 60, 90, 120, 150 and 180 d after birth). The expression profile of miRNAs in thyroid at different development stages was detected by microarray technology, then the quantitative real-time PCR (RT-PCR) was carried out to verify the accuracy of the microarray assay, and the gene structure and target genes of the target miRNAs were predicted by bioinformatics. Our results showed that totally 248 miRNAs were significant difference among developmental stages ($P < 0.01$), and 1930 target genes in accordance with top 10 abundance of miRNAs were also revealed. Furthermore, KEGG pathway analysis suggested that highly changed miRNAs were involved in tissue development and regeneration, signal transduction, cell-cell and cell-extracellular matrix communication and neural development and function. Our results reexplore the molecular mechanism of thyroid development from the epigenetics, provide comparative medical material of pathogenesis, prophylaxis and treatment for some human thyroid disease.

Key Words: thyroid, miRNA, microarray

P1037 Comparison of predictability of genomic selection methods. Z. Hu[1,2], S. Xu[2], G. Plastow[1], and Z. Wang*[1], *[1]University of Alberta, Edmonton, Alberta, Canada, [2]University of California, Riverside, Riverside, California, USA.*

The MCMC implemented Bayesian methods are commonly used for genomic selection (GS). However, they are time consuming and often not reliable when the marker density is too high. We used 3 fast and well-known methods: the empirical Bayes (EB), the least absolute shrinkage and selection operator (Lasso), the genomic best linear unbiased prediction (GBLUP); and a new method (Bin-model), to perform genomic value prediction for 5 beef carcass and 3 ultrasound traits. Data of 922 beef steers genotyped using the Bovine50kSNP chip were used to study the predictability of the SNP markers for the 8 quantitative traits. The correlations between the observed and predicted trait values obtained from 10-fold cross validation were used as the criteria to evaluate the 4 methods. Among the 8 traits, 3 of them (carcass weight, carcass ribeye area and ultrasonic backfat) had reasonable predictability for all 4 methods and they were ranked as Bin-model > GBLUP = EB > Lasso (r = 0.57, 0.28, 0.28, and 0.19) respectively. For the remaining 5 traits, Lasso failed to detect any markers with nonzero effects, EB gave poor prediction and GBLUP provided reasonable prediction. The Bin-model produced the best accuracies of prediction for all traits considered in this study. Our conclusion was the Bin-model method is the preferred method for GS in this beef cattle study.

Key Words: quantitative traits, genomic value prediction

P1038 Maternal and paternal genetics differentially affect myofibre characteristics and muscle weights of bovine fetuses at midgestation. Ruidong Xiang*[1], Bill Johns[2], Tanja Eindorf[1], David Rutley[1], Dana Thomsen[1], Zbigniew Kruk[1], Carolyn Fitzsimmons[1,3], Claire T. Roberts[4], Brian Burns[5], Gail Anderson[1], Paul Greenwood[2], and Stefan Hiendleder[1], *[1]JS Davies Epigenetics and Genetics Group, School of Animal and Veterinary Sciences, Roseworthy Campus, and Robinson Institute, The University of Adelaide, Roseworthy, South Australia, Australia, [2]Department of Primary Industries, Beef Industry Centre, Trevenna Rd, University of New England, Armidale, New South Wales, Australia, [3]Agriculture and Agri-Food Canada/ University of Alberta, Edmonton,Alberta, Canada, [4]School of Paediatrics and Reproductive Health, University of Adelaide, South Australia, Australia, Adelaide, South Australia, Australia, [5]The University of Queensland, Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, Rockhampton, Queensland, Australia, Rockhampton, Queensland, Australia.*

Skeletal muscle contributes up to half of mammalian body mass. It is composed of type I and II myofibers, which originate from mesenchymal stem cells during embryonic and fetal myogenesis. Thus, postnatal muscle development is to a considerable extent determined prenatally. Postnatal myofiber characteristics and skeletal muscle mass in mammals may be significantly affected by genetic and epigenetic factors, including parent-of-origin effects. However,maternal and paternal genetic effects on prenatal muscle development have not been investigated. We investigated these effects on myofiber characteristics and selected muscle parameters in 73 Day-153 purebred and reciprocal hybrid fetuses with *Bos taurus* (Angus) and *Bos indicus* (Brahman) genetics. Myofiber characteristics were determined immunohistochemically in M. semitendinosus; muscle parameters including absolute and relative weights were determined for M. supraspinatus, M. longissimus dorsi, M. quadriceps femoris and M. semimembranosus. Using general linear models with R2-values ranging from 0.07 to 0.68, we show that parental genetics accounted for significant proportions (0.56 to 1.00) of explained variation in myofiber characteristics and muscle parameters. Maternal genetics explained most or all (0.60 to 1.00) of the genetic variation in absolute muscle weights. Paternal genetics, in contrast, explained most or all genetic variation (0.54 to 1.00) in relative muscle weights except M. semimembranosus, where fetal gender was the only source of explained variation. Comparison of these results with postnatal data suggests that midgestation is an important time point for bovine muscle development, driven by non-equivalent parental genetic effects. In conclusion, our findings demonstrate differentiated roles of maternal and paternal genetics in mammalian myogenesis.

Key Words: bovine fetus, muscle, parental genetic effects

P1039 Expressional analysis of immunoglobulin D in cattle (*Bos taurus*), a large domesticated ungulate. Beilei Xu*[1], Jing Wang[1], Min Zhang[1], Ping Wang[1], Zhiguo Wei[2], Yi Sun[1], Qiqing Tao[1], Liming Ren[1], Xiaoxiang Hu[1], Ying Guo[1], Jing Fei[1], Lei Zhang[1], Ning Li[1], and Yaofeng Zhao[1], *[1]China Agricultural University, Beijing, China, [2]Henan University of Science and Technology, Henan, China.*

For decades, it has remained unknown whether artiodactyls, such as cattle, pigs and sheep, express immunoglobulin D, although the delta gene was identified in these species nearly 10 years ago. With the development of a mouse anti-bovine IgD heavy chain McAb, we were able to show that secreted bovine IgD is present mainly as a monomer in serum and is heavily glycosylated by N-linked saccharides. Nonetheless, IgD was detectable in some but not all Hostein cattle examined. Flow cytometry analysis demonstrated that

IgD-positive B cells constituted a much lower percentage of B cells in the bovine spleen, jejunal Peyer's patches and peripheral blood leukocytes than in humans and mice. Furthermore, IgD-positive B cells were almost undetectable in bovine bone marrow and ileal Peyer's patches. We also demonstrated that the bovine delta gene can be expressed via class switch recombination. Accordingly, bovine delta germline transcription, which involves an Idelta exon and is highly homologous to Imu, was confirmed. However, we could not identify an Idelta promoter, despite bovine Emu demonstrating both enhancer and promoter activity, and being able to drive gene transcription. This study has answered a long-standing question in cattle B cell biology and significantly contributes to our understanding of B cell development in this species.

Key Words: bovine, immunoglobulin D, class switch recombination

P1040 Identification of melatonin and photoperiodic regulated genes in sheep pituitary using ISGC sheep genome assembly. L. Yu*[1], S. Dupré[2], B. Paton[1], B. Dalrymple[3], S. McWilliam[3], A. West[2], K. Miedzinska[4], R. Talbot[1], S. Smith[1], M. Fell[1], J. Davis[2], A. McNeilly[4], A. Loudon[2], and D. Burt[1], *[1]Roslin Institute, University of Edinburgh, Edinburgh, Lothian, UK, [2]University of Manchester, Manchester, Great Manchester, UK, [3]MRC Centre for Reproductive Health, Edinburgh, Lothian, UK, [4]CSIRO Livestock Industries (LI) livestock genomics, Clayton South, Australia.*

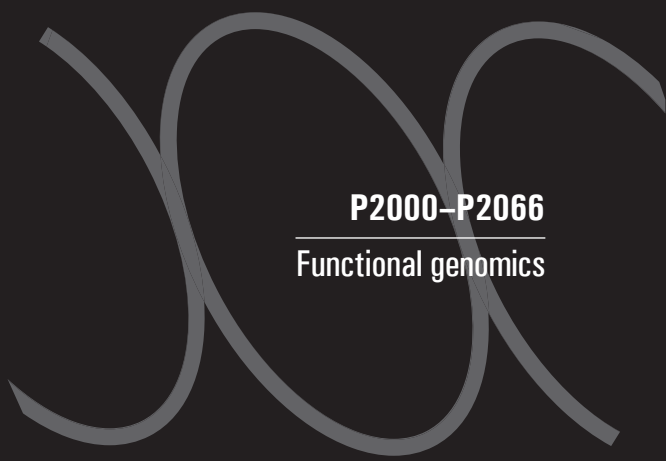
Seasonally breeding mammals use photoperiod, encoded by rhythmical production of the nocturnal pineal hormone melatonin. Photoperiod via melatonin acts directly on the pars tuberalis (PT) of the pituitary, regulating expression of thyrotropin. To understand the molecular control in the PT we undertook detailed gene expression analysis on this target organ using RNASeq. RNAs were analyzed in sheep PTs after both melatonin implant and photoperiodic treatments. Millions of 36bps single-ended RNASeq tags were generated by Illumina GAII. We used ISGC sheep reference genome assembly (OARv2.0) for alignments. Gene annotation was integrated from 5 different annotation resources. We successfully identified 22146 genes, of which 17423 had human gene symbols. RNA-Seq tags were then mapped to the sheep reference genome and cross referenced to our integrated annotations. Next we calculated counts of tags for each gene and statistical analysis was adopted to identify genes that were regulated by melatonin and photoperiod. On the basis of this, 90% tags were mapped to OARv2.0, 80% of which were successfully annotated to known genes. Compared with our previous analysis using the Bos taurus genome, our integrated annotations based on the sheep OARv2.0 assembly allowed us to identify more melatonin and photoperiod regulated genes. Project funded by BBSRC (UK).

Key Words: RNASeq, sheep genome

P1041 Extensive diversification of IgH subclass encoding genes and IgM to IgM class switching in Crocodylia. Gang Cheng, Tao Wang, YI Sun, Xiaoxiang Hu, Ning Li, and Yaofeng Zhao,* *State Key Laboratory of Agrobiotechnology, Beijing, China.*

Herein, we report a distinct immunoglobulin heavy chain gene locus that is organized as Vn-Dn-Jn-μ1-δ-α1-μ2-α3-μ3-ψα-ψμ-α2-υ3-υ2-υ1 in 2 crocodilian species, the Siamese crocodile (*Crocodylus siamensis*) and the Chinese alligator (*Alligator sinensis*). This locus appears to have experienced intensive gene duplications and rearrangements leading to diversification of the μ, α and υ genes and inversion of the α genes. Both the μ2 and μ3 genes are expressed through class switch recombination involving all of the essential elements, such as the switch region, germline transcription and an intronic promoter. These 2 μ genes also accumulate more mutations in their variable regions than does the μ1 gene. Both IgM1 and IgM2 are present in the serum as polymers, suggesting that the crocodilians may use class switch recombination to produce IgM with both high avidity and affinity for antigens.

Key Words: Crocodylia, immunoglobulin, class switch



P2000–P2066

Functional genomics

P2000 Effects of hormonal growth implant on feed efficiency and expression of RFI-associated genes in beef cattle. W. Al-Husseini*[1,2], C. Gondro[1,2], K. Quinn[3], L. M. Cafe[1,3], R. M. Herd[1,3], J. P. Gibson[1,2], P. L. Greenwood[1,3], and Y. Chen[1,4], *[1]Australian Cooperative Research Centre for Beef Genetic Technologies, Armidale, NSW, Australia, [2]Centre for Genetics Analysis and Applications, University of New England, Armidale, NSW, Australia, [3]NSW Department of Primary Industries, Beef Industry Centre, Armidale, NSW, Australia, [4]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia.*

Hormonal growth promotants (HGPs) have been used to improve feed conversion ratio (FCR) and growth rates of cattle by modifying protein turnover rates in the body.Residual feed intake (RFI) has been adopted in Australia as a measure of feed efficiency in cattle for the purpose of genetic improvement. Eight genes (AHSG, GHR, GSTM1, INHBA, PCDH19, S100A10, SERPINI2 and SOD3) have been previously reported to be highly associated with RFI and can be used to predict RFI in bulls and steers. In this study gene expression levels of these genes were measured by quantitative real-time PCR in liver tissue of 46 cattle. These cattle were part of a larger Beef CRC tenderness gene marker experiment consisting of 2 breeds (Angus and Brahman); 2 sexes and HGP treatment (implanted vs. control). Cattle were measured for daily growth rate, RFI and FCR and carcass traits. Our results showed that HGP treatment affected neither RFI nor expression levels of the RFI-associated genes. HGP treatment increased average daily gain by 20% ($P < 0.05$), improved FCR by 18% ($P < 0.1$), and increased rib eye-muscle area of 7.5% ($P < 0.05$). HGP treatment was effective in improving growth rate, presumably by its known action in reducing rates of protein degradation. This mechanism is among those hypothesized as regulators of RFI. Lack of effect of HGP treatment on RFI or the RFI-associated genes does not support this hypothesis.

P2001 Characterization of micro-RNAs in Atlantic salmon (*Salmo salar*). R. Andreassen*[1] and B. Hoyheim[2], *[1]Oslo and Akershus University College of Applied Sciences, Oslo and Akershus University College of Applied Sciences, Oslo, Norway, [2]Norwegian School of Veterinary Science, Norwegian School of Veterinary Science, Oslo, Norway.*

MicroRNAs (miRNAs) are an abundant class of endogenous small RNA molecules. Some miRNAs are highly conserved from species to species while other miRNAs seems to be species specific. MicroRNAs are often expressed in a tissue-specific manner and contribute to establish and maintain the characteristic tissue specific gene expressions by affecting the gene expression of target genes. The target genes regulated by miRNAs control multiple biological processes like developmental timing, growth, stem cell division and apoptosis. Despite the position Atlantic salmon (*Salmo salar*) has as an important domesticated animal, and despite the focus on functional genomics in aquaculture, there has been little research on miRNAs in *Salmo salar*. Thus, the objective of this study has been to identify and characterize miRNAs in Atlantic salmon. Small RNAs from ten different tissues as well as from fertilized eggs sampled at different stages from newly fertilized to eyed eggs was extracted and subjected to next generation sequencing (Illumina). Using high quality sequence data, a homology based approach and miRBase any micro-RNAs conserved across species may be identified. A number of such conserved miRNAs were identified in all tissues and developmental stages of Atlantic salmon. Alignments of deep sequencing data to the first version of the *Salmo salar* genome identified putative *Salmo salar* miRNA genes.

Key Words: micro-RNA, *Salmo salar*

P2002 Effect of feeding linseed and algae on lipogenic enzyme gene expression of lamb subcutaneous and intramuscular adipose tissues. O. Urrutia, B. Soret, K. Insausti, J. A. Mendizabal, and A. Arana,* *Universidad Publica de Navarra, Pamplona, Navarra, Spain.*

Health concern is contributing to reduced consumption of lamb meat due to its content in saturated fatty acids, so increasing PUFA content would be desirable. Nevertheless, this approach may have unwanted effects by inhibiting endogenous fatty acids synthesis. The effects of ω3 PUFA on key lipogenic enzymes expression in Longissimus (IM) and subcutaneous (SC) tissues were investigated. Three groups of 11 lambs were studied: C group fed on barley and soya concentrate, L and LA received the same feed as C including 10% linseed or 5% linseed and 3.75% algae. Gene expression of ACC, LPL, and SCD was measured by real time RT-PCR and relative expression (Ct method) was calculated by normalizing against β-actin using the C group as a calibrator. In both tissues, L lambs had higher content of linolenic acid than C ($P < 0.001$) and LA lambs higher content of EPA and DHA ($P < 0.001$) than C and L. IM tissue showed a higher increase of EPA, DHA and ω3 PUFA than SC both in L and LA. Linseed and algae inclusion caused a decrease in gene expression of ACC in both tissues ($P < 0.001$) and an increase in LPL expression on IM ($P < 0.01$), reflecting higher incorporation of exogenous fatty acids. L and LA SCD expression decreased in IM ($P < 0.01$), suggesting that exogenous ω3 PUFAs inhibit endogenous synthesis through down regulation of lipogenic enzimes.

Key Words: PUFA, gene expression, lambs

P2003 Enabling the reading of genome sequences for farmed and companion animals—A proposal for ENCODE consortia. A. L. Archibald*[1], P. Flicek[2], and E. Birney[2], *[1]The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK, [2]European Bioinformatics Institute, Hinxton, Cambridge, UK.*

A high quality annotated reference genome sequence is critical to contemporary biological research. Draft reference genome sequences have been established for the major farmed and companion animal species and important aquatic species. Establishing its sequence is only the first step in characterizing a genome. Identifying the functional elements within the genome sequence is essential for understanding the phenotypic consequences encoded in the genome. The annotation of these genome sequences is currently limited to gene models deduced from alignments with expressed sequences (cDNA, ESTs, RNaseq) and some sequence variation (SNPs, CNVs). The aim of the ENCODE project is to identify the functional elements in the human genome to facilitate understanding of human biology. A similar effort is required for farmed and companion animals. Given the resources available for animal research it might appear naive to try to replicate the ENCODE project. However, next-generation sequencing technologies have transformed the ease with which functional DNA elements can be identified on a genome-wide scale and dramatically reduced the costs of such experiments. By focusing on a subset of assays - RNAseq, Transcription Start Sites (CAGE), histone marks and methylation states - and by coordinating efforts to minimise redundant activity it should be possible to make significant progress.

P2004 Porcine follicle-stimulating hormone improves fecundity in female transgenic mice. Mingjun Bi*[1], Jia Tong[1], Fei Chang[1], Jing Wang[1], Yunping Dai[2], Yaofeng Zhao[1], and Ning Li[1], *[1]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China, [2]GenProtein Biotech Ltd., Beijing, China.*

FSH is a pituitary glycoprotein that, together with LH, plays a crucial role in ovarian folliculogenesis and female fertility. Here we report the generation of a novel gain-of-function mouse model using a bacterial artificial chromosome (BAC) system to jointly introduce 92 kb and 165 kb genomic fragments comprising the porcine FSH (pFSH) α- and β- subunit genes. These directed the physiological expression of pFSH with the same temporal and spatial pattern as endogenous FSH in female transgenic (TG) mice. Serum levels of biologically active pFSH heterodimers in independent TG lines ranged from 6.36 to 19.83 IU/L. High basal pFSH activity led to a significant reduction of serum LH and estradiol levels in TG females compared with wild-type (WT) littermates. Endogenous FSH levels were significantly increased but testosterone levels remained normal. Ovarian histology showed that the number of corpora lutea was significantly higher at 14 and 28 weeks of age in TG females and breeding curves revealed that mean litter sizes of TG females were obviously larger than for WT littermates, suggesting that elevated FSH enhances ovulation and finally results in increased litter sizes. Our TG mouse model is useful for exploring the biological effects of pituitary targeted expression of pFSH in gonadal development and function.

Key Words: porcine FSH, folliculogenesis, reproductive ability

P2005 Is RNA involved in transgenerational epigenetic inheritance? Martin H. Braunschweig,* *Institute of Genetics, Vetsuisse Faculty, University of Bern, Berne, Switzerland.*

Recently, we accomplished a 3 generation feeding experiment to investigate transgenerational epigenetic inheritance down the male line in Large White pigs. In mammals, there are a few studies demonstrating that environmentally induced epigenetic effects were transmitted to the next generations. However, it still needs to be elaborated on the molecular mechanisms including the molecules that are involved in the transmission of epigenetic information from one generation to the next. DNA methylation, histone modifications, RNA and trans effects similar to paramutation were proposed to transport epigenetic information. In our 3 generation pig feeding experience we fed F0 boars a diet enriched with methyl donors or a control diet to study heritable epigenetic effects in F2 offspring. We found differences in carcass traits, gene expression and DNA methylation between the 2 groups of descendants from the experimental F0 boars and the control F0 boars, respectively. To investigate the mechanism of this form of inheritance we performed RNA-Seq to quantify sperm RNA of boars that received either a methyl enriched diet or a control diet. Results of this RNA analysis from sperm cells, their interpretation and implication will be presented.

Key Words: transgenerational epigenetic inheritance, RNA-Seq, pig

P2006 Contribution of mammary epithelial cells to the immune response during early stages of a bacterial intramammary infection. P. Brenaut*[1], C. Bevilacqua[1,2], L. Lefèvre[1,2], C. Morgenthaler[1], S. Dauchy[2], D. Laloë[1], F. Jaffrezic[1], and P. Martin[1,2], *[1]INRA, UMR 1313 GABI, Jouy en Josas, France, [2]Plateforme de microgénomique expressionnelle, Jouy en Josas, France.*

We examined in goats the innate immune response of mammary epithelial cells (MEC) at the early steps

of an experimental intra mammary infection (IMI) with *Staphylococcus aureus*, using milk fat globules (MFG) as a source of RNA representative of MEC. Experiments were performed using sheep microarrays to compare gene expression patterns before infection, at 12h, 18h and 24h post-infection. Our results were confirmed using microdissected MEC which can be considered as the gold reference to analyze the MEC transcriptome in their pathophysiological context. We showed that at 18h post infection MEC secrete large amounts of cytokines and chemokines to stimulate recruitment and activation of inflammatory cells. They also express factors contributing directly to fight infection, including acute-phase proteins such as SAA3. Taken together, our results underlined the coordinated induction of inflammatory response by MEC following infection. Furthermore, we have demonstrated unambiguously that SAA3 is rapidly and specifically expressed by MEC. In summary, we first demonstrated in vivo how MEC orchestrate innate immune response during an IMI to *S. aureus* in the goat species. Besides, the production of SAA3 by MEC, in the early stages of IMI, provides a sensitive indicator for early detection and therefore, treatment of mastitis.

Key Words: mastitis, transcriptome, non invasive sampling

P2007 Efficient gene modification in livestock using custom designed TALENs. D. F. Carlson*[1,3], W. Tan[1,2], S. G. Lillico[4], D. Stverakova[4], C. Proudfoot[4], M. Christian[1,5], D. F. Voytas[1,5], and C. B. A. Whitelaw[4], *[1]Center for Genome Engineering, University of Minnesota, Minneapolis, MN, USA, [2]Department of Animal Science, University of Minnesota, Saint Paul, MN, USA, [3]Recombinetics, Inc., Saint Paul, MN, USA, [4]The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom, [5]Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, USA, [6]Veterinary Physiology & Pharmacology, College of Veterinary Medicine, College Station, TX, USA.*

TALENs are programmable nucleases that join the modular DNA binding domain of transcription activator-like (TAL) effectors with FokI endonuclease. Though zinc-finger nucleases (ZFN) enable a variety of genome modifications, their application to livestock engineering has been slowed by technical limitations of embryo injection, primary cell culture and difficulty in producing reliable reagents with a limited budget. In contrast, we found that TALENs could be easily manufactured and that over half (23/36; 64%) demonstrate activity in primary cells ranging from 1.5 to 40%. TALEN mRNA injected into the cytoplasm of livestock zygotes was capable of inducing gene knockout (KO) in up to 29% of embryos analyzed, nearly half of which harbored bi-allelic modification. We also developed a simple transposon co-selection strategy for TALEN-mediated gene modification in primary fibroblasts that enabled both enrichment for modified cells and efficient isolation of modified colonies. Treatment with a single TALEN pair enabled isolation of clones with mono- and bi-allelic modification in up to 54% and 17% of colonies screened. We also used co-selection to isolate clones harboring large chromosomal deletions and inversions (10% and 4% of colonies respectively) mediated by 2 TALEN pairs targeting the same chromosome. Further, as a model for allelic introgression, we precisely transferred the 11 bp Myostatin deletion from Belgium Blue cattle into the Wagyu genome using TALEN-stimulated homologous recombination, without the introduction of a selection gene at the targeted locus. This study demonstrates the flexibility and reliability of TALEN-mediated genome engineering and novel methods for application to livestock and beyond.

Key Words: TALENs, introgression, genome engineering

P2008 Solexa sequencing identification of microRNAs in backfat of Large White and Chinese Meishan pigs. Chen Chen*[1], Bing Deng[1], Jin Chai[1], Jian Peng[2], and Siwen Jiang[1], *[1]Key Laboratory of Swine Genetics and Breeding of Agricultural Ministry and Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China.*

The domestic pig, an important species in animal production industry, is a right model for studying fat deposition. To expand the repertoire of porcine miRNAs and explore potential regulatory miRNAs influence on adipogenesis, Solexa sequencing approach was adopted to identify miRNAs in backfat of Large White and Meishan pigs (Chinese indigenous fatty pig). We identified 215 unique miRNAs comprising 75 known pre-miRNAs, of which 49 miRNAs were first identified in our study, and 140 were newly predicted miRNAs. We analyzed the sequence variations, seed edits and phylogenetic development of the miRNAs. Seventeen miRNAs widely conserved from vertebrates to invertebrates, suggesting that they may serve as potential evolutional biomarkers. Nine conserved miRNAs with significantly differential expressions were determined. The expression of miR-215, miR-135, miR-224 and miR-146b was higher in Large White pigs, opposite to the patterns shown by miR-1a, miR-133a, miR-122, miR-204 and miR-183. Almost all novel miRNAs could be considered pig-specific except ssc-miR-1343, miR-2320, miR-2326, miR-2411 and miR-2483 which had homologs in Bos taurus, and these miRNAs were

validated in backfat tissue by stem-loop qPCR. Our results displayed a high level of concordance between the qPCR and Solexa sequencing method in 9 of 10 miRNAs comparisons except for miR-1a.

Key Words: miRNA, Large White pig, Meishan pig

P2009 Hereditary sensorineural hearing loss in Chinese Rongchang pigs result from promoter mutations in Mitf. Lei Chen*[1,3], Weiwei Guo[2], Jiyong Wang[3], Shiming Yang[2], Xiaoxiang Hu[1], and Ning Li[1], *[1]China Agricultural University, Haidian, Beijing, P.R. China, [2]Chinese PLA General Hospital, Haidian, Beijing, P.R. China, [3]ChongQing Academy of Animal Science, Rongchang, Chongqing, P.R. China.*

Chinese native pigs harbour rich spontaneous mutants with similar disease phenotype as human. Here we present a hereditary hearing loss family in Chinese Rongchang pig. ABR assessment on mutants shows no signs of hearing indicating a profound congenital hearing impairment. SEM and Immunohistochemistry analysis indicated a progressive outer and inner hair cell degeneration in mutant cochlear, TEM analysis revealed the deficiency of melanocyte in cochlear stria vascularis. A phenotype segregation family with 3 generations were constructed, and genome wide linkage and association analysis were used to mapping the mutant gene. A 24 cM linkage disease interval on SSC.13 were detected, and the strongest association signal were observed for 2 SNP markers in this interval (Log p = −8.77). A melanogenesis and hearing relate gene MITF were located between the 2 SNP markers, fine mapping revealed 7 mutations in the M-promoter of Mitf, promoter mutations specifically downregulates the expression of Mitf-M transcripts. Our study suggesting that Mitf is a critical factor of hearing development, and hereditary hearing loss Rongchang pig is a model for studying the mechanism of Waardenburg syndrome type II and other hereditary sensorineural hearing loss.

Key Words: hereditary sensorineural hearing loss, animal model, pathogenetic gene

P2010 Profiling bovine liver microRNA by deep sequencing. Yizhou Chen*[1,3], Wijdan Al-Husseini[1,2], Cedric Gondro[1,2], Robert Herd[1,4], John Gibson[1,2], and Paul Arthur[1,3], *[1]Australian Cooperative Research Centre for Beef Genetic Technologies, Armidale, NSW 2351, Australia, [2]School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia, [3]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568, Australia, [4]NSW Department of Primary Industries, Beef Industry Centre, Armidale, NSW 2351, Australia.*

MicroRNAs (miRNAs) are short non-coding RNAs that regulate the stability and translation of mRNAs. Profiling experiments in bovine by deep sequencing technology have been reported in testicular and ovarian tissues previously. Here we report comprehensive liver miRNA profiles by deep sequencing using cattle from genetically divergent lines for residual feed intake (RFI; a measure of feed efficiency), and identify candidate miRNAs related to feed efficiency in cattle. Two pools of liver RNA were made from low and high RFI selection-line cattle and were sequenced by Illumina Genome Analyzer. In total 24 million sequence reads were obtained and 60% of the sequence reads were mapped to bovine genome (Btau4.2). We identified 352 known miRNAs in the liver samples. bta-miR-143, bta-miR-30a-5p, bta-miR-122, bta-miR-378, bta-let-7f were the top 5 most represented miRNA families. We also identified 73 putative miRNAs using a MirAnalyzer based on precursor sequence and the secondary structure. We compared the miRNA profile between high and low RFI animals and ranked the most differentially expressed known bovine miRNA and putative miRNA. The most differentially expressed known bovine miRNA family was bta-mir-455, which was up-regulated in high RFI animals. We also identified 5 putative bovine miRNA which were expressed differentially between high and low RFI animals. Candidate-82 was highly expressed in low RFI (high efficiency) animals. The results of this miRNA profiling provide a list of known and putative bovine miRNAs potentially regulating feed efficiency in beef cattle.

Key Words: microRNA, cattle, sequencing

P2011 A gene-expression phenotype for cattle skeletal muscle energy source preferences. N. De Jager[1,2], N. J. Hudson[2,4], A. Reverter[2,4], R. Barnard[3], L. M. Cafe[1,5], P. Greenwood[1,5], and B. P. Dalrymple*[2,4], *[1]CRC Beef Genetic Technologies, Armidale, NSW, Australia, [2]CSIRO Livestock Industries, St Lucia, QLD, Australia, [3]SCMB University of Queensland, St Lucia, QLD, Australia, [4]CSIRO Food Futures Flagship, St Lucia, QLD, Australia, [5]NSW DPI, Armidale, NSW, Australia.*

This work investigated the hypothesis that differences in diet lead to different interactions between hormone growth promotant treatment (HGP), intramuscular fat (IMF)% and triacylglyceride (TAG) module gene expression in the longissimus muscle in Brahman cattle at 2 experimental sites (NSW and WA),. The overlap between the top 100 genes differentially expressed between WA and NSW animals and genes differentially expressed between feedlot and grass fed *Bos taurus* cattle in both high and low marbling cattle was highly significant (P = 3.4E-26). Several the genes in the overlap (PDK4, ANGPTL4, CPT1A, FOXO1 and MLYCD) encode proteins which play roles in mediating the Randle effect, where high levels of circulating

long chain fatty acids (LCFAs) induce expression of the above genes (among others) leading to increased usage of LCFAs and reduced usage of glucose by the muscle. This set of genes (and SERPINE1, whose expression also responds to LCFA availability) was used as a gene expression phenotype to explore the interactions. The WA cattle had much higher levels of expression of the set of genes than the NSW cattle. The difference in expression between animals at the 2 sites was reduced in HGP treated animals, primarily due to the reduced expression of these genes in the WA animals. However, although these expression changes were consistent with differences in IMF%, unlike the TAG module genes. the expression of this set of genes was not correlated with IMF%.

Key Words: PDK4, Randle effect, intramuscular fat

P2012 Gene-expression phenotype for lipid metabolism and intra-muscular fat in cattle skeletal muscle. N. De Jager[1,2], N. J. Hudson[2,4], A. Reverter[2,4], R. Barnard[3], L. Cafe[5], P. Greenwood[5], and B. P. Dalrymple*[2,4], *[1]CRC for Beef Genetic Technologies, Armidale, NSW, Australia, [2]CSIRO Livestock industries, St Lucia, QLD, Australia, [3]SCMB University of Queensland, St Lucia, QLD, Australia, [4]CSIRO Food Futures Flagship, St Lucia, QLD, Australia, [5]NSW DPI, St Lucia, QLD, Australia.*

This work aimed to identify and evaluate gene-expression phenotypes for intramuscular fat % (IMF%) in skeletal muscle as an alternative to traditional approaches. Gene expression data from a time-course of longisimus muscle development in high and low marbling *Bos taurus* cattle crosses were compared with identify modules of genes involved in lipid metabolism. In a separate analysis in the LM of 48 *Bos indicus* cattle the genes in the triacylglyceride (TAG) synthesis and storage modules were enriched in the top 100 genes most correlated with IMF% (P = 1.2E-24). We investigated the effect of a steroid growth promotant treatment, 2 experimental sites (NSW and WA) and 2 tenderness genotypes on the expression levels of genes in the TAG module. Cattle possessing the favored tenderness alleles of calpain 1 and 3 and calpastatin exhibited a significant (P = 0.008) reduction in expression in NSW (1.8-fold reduction, P = 0.0002) compared with WA (1.2-fold reduction, P = 0.03). In general the interactions between genotype, treatment and location, and TAG module gene expression were consistent with the interactions between the same factors and IMF% detected using conventional approaches with around 300 animals of which the 48 in this study were a subset. Thus the TAG module constitutes a gene-expression phenotype able to predict the effects of different genotypes and treatments on IMF% using a much smaller number of individuals than current approaches, even in animals with very low IMF%.

Key Words: lipid metabolism, triacylglyceride synthesis, intra-muscular fat

P2013 Co-expression analysis of fetal weight-related genes in ovine skeletal muscle during medium and late fetal development. Lingyang Xu, Fuping Zhao, Caihong Wei, Li Zhang, and Lixin Du,* *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China.*

In this research, we investigated the gene expression profiles of developing fetal longissimus muscles from Ujumqin and Texel sheep using systems genetic methods. Longissimus muscle tissues were collected at 70, 85, 100, 120, and 135 d, with 3 biological replicates for each sample. In total, 1472 differentially expressed genes were identified using the maSigPro method in the SEA web tool. Further systems genetic analysis was performed using weighted gene co-expression network analysis (WGCNA) methods. A total of 5, 11, 7 and 6 distinct gene modules were identified at the Texel sheep medium fetal development stage (d 75 and 80), Ujumqin medium fetal development stage, Texel late fetal development stage (d 120 and 135), and Ujumqin late fetal development stage, respectively. We also identified gene modules that were significantly correlated with fetus weight. The genes with high gene significant values were selected for further network visualization and pathway analysis. And hub genes in trait-related modules identified by WGCNA suggested that it may be possible to develop muscle biomarkers based on LD muscle gene expression. The results from co-expression network analyses based on systems-oriented genetic approaches may shed new light on pathways underlying economic traits in other animals.

Key Words: co-expression analysis, fetal development, longissimus muscle tissue

P2014 Transcript characteristic of myostatin in sheep fibroblasts. Jian Lu, Fuping Zhao, Caihong Wei, Li Zhang, and Lixin Du,* *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, Beijing, 100193, China.*

Myostatin, a secreted growth factor highly expressed in skeletal muscle, negatively regulates skeletal muscle growth and differentiation. Recently, myostatin has emerged as a potential target for anti-atrophy and anti-fibrotic therapies. To investigate the regulation mechanism of myostatin in sheep adult fibroblasts, we used the RNA interference mediated by lentiviral vector to gene silence myostatin and constructed the sheep myostatin overexpression vector. The results showed that the lentiviral vector could significantly reduce myostatin gene expression both at the mRNA and protein level by 71% and 67%, respectively (P < 0.01). In addition, inhibition of myostatin led to a remarkable increase of activin receptor 2B (ACV2B), p21, PPARγ, leptin, C/EBPβ and

MEF2A expressions, and a decrease of Akt1, CDK2, MEF2C, and Myf5. On the contrary, overexpression of myostatin contributed to an increase of Akt1, CDK2, Myf5, and PPARγ, and a decrease of p21, C/EBPα and leptin expressions at the transcript level. These results suggested that myostatin positively regulated Akt1, CDK2, Myf5, leptin and C/EBPα, but negatively regulated p21 mRNA expression in adult fibroblasts. This research would provide a reference for utilizing the lentiviral system inactivated myostatin gene in fibroblasts to generate transgenic sheep and ameliorate muscle fibrosis and atrophy by gene therapy in the future.

Key Words: myostatin, sheep, lentiviral vector

P2015 Resources of pig expressed genes: Full-length-enriched cDNA libraries and large-scale sequencing based on the libraries. Hirohide Uenishi[1], Takeya Morozumi[2], Daisuke Toki[2], Tomoko Eguchi-Ogawa*[1], Lauretta A. Rund[3], and Lawrence B. Schook[3], *[1]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan, [2]Institute of Japan Association for Techno-innovation of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-0854, Japan, [3]University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.*

Collection of the nucleotide sequences of genes expressed in various porcine tissues and determination of entire cDNA sequences are necessary for investigations of gene function in pigs. The cDNA sequences are also valuable for annotation of the pig genome, the draft sequencing of which was completed by the international consortium. Here we present a summary of sequencing analysis by using 32 full-length-enriched cDNA libraries derived from 28 kinds of porcine tissues and cells, including tissues derived from pigs that were cloned from a sow subjected to the genome sequencing. We conducted a large-scale expressed sequence tag (EST) analysis in pigs with the libraries and obtained more than 330,000 EST reads from the 5′-ends of the cDNA clones, corresponding to more than 15,000 genes. In parallel with the EST analysis we conducted sequencing of the entire inserts of the representative cDNA clones in the libraries. We have finished sequencing more than 31,000 clones corresponding to at least 12,000 genes. Mapping of the sequences of these cDNA clones on the latest draft sequence of the pig genome indicated that the clones originate from about 15,000 independent loci on the pig genome. The porcine ESTs and cDNA sequences presented here are not only useful for the genome annotation, but also valuable for molecular biology-based analyses in pigs.

P2016 Genetic effects on expression of *IGF2R* and *AIRN* in bovine fetal tissues. M. Ghanipoor*[1], A. Javadmanesh[1], D. Thomsen[1], G. Nattrass[2], K. Kind[1], and S. Hiendleder[1], *[1]JS Davies Epigenetics and Genetics group, School of Animal and Veterinary Sciences, and Robinson Institute, University of Adelaide, Roseworthy, SA, Australia, [2]Livestock & Farming Systems, SARDI, Roseworthy, SA, Australia.*

The insulin-like growth factor 2 receptor gene (*IGF2R*) encodes a transmembrane receptor that binds and regulates bioavailability of insulin-like growth factor 2, a potent growth promoting hormone. Mouse gene knockout experiments have demonstrated the essential role of *IGF2R* in prenatal growth and development. The gene is subject to genomic imprinting and expressed from the maternal allele in mouse and bovine, but is not imprinted in human. Imprinted *IGF2R* expression is regulated by expression of a partially overlapping, reciprocally imprinted noncoding antisense RNA, *AIRN*. We analyzed expression of *IGF2R* and *AIRN* in brain, cotyledon, heart kidney, liver, lung and skeletal muscle of bovine Day153 fetuses with *Bos taurus* (Angus), *Bos indicus* (Brahman) and *B. taurus* × *B. indicus* reciprocal cross genetics (n = 74) by real time quantitative PCR to determine effects of fetal genetics and sex. Statistical analysis of qPCR data in general linear models (PASW Statistics 19) showed that *AIRN* expression in brain, and *IGF2R* expression in cotyledon, was affected by fetal genetics (ANOVA, both $P < 0.001$). Expression of both genes was significantly higher in Brahman (sire) × Angus (dam) fetuses compared with all 3 other groups of fetuses (*t*-tests $P < 0.01$). In addition, expression of *IGF2R* in skeletal muscle was affected by an interaction between fetal sex and genetics ($P < 0.05$) where female fetuses with Brahman maternal genetics showed significantly higher transcript levels than all other groups. These findings suggest a complex tissue-specific pattern of expression for *IGF2R* and *AIRN* which is controlled by the interplay between genetics, epigenetics and fetal sex.

Key Words: bovine, *IGF2R*, *AIRN*

P2017 MiRNAs in host-virus interaction: The Pseudorabies Virus (PrV) model. Nada Mahjoub[1], Barbara Klupp[2], Walter Fuchs[2], Marie-Laure Endale Ahanda[1], Sophie Dhorne-Pollet[1], Francois Lefevre[3], Thomas C. Mettenleiter[2], and Elisabetta Giuffra*[1], *[1]National Institute for Agronomical Research (INRA), Animal Genetics and Integrative Biology Unit, GIS Team, Jouy-en-Josas, France, [2]Friedrich-Loeffler-Institut (FLI), Institute of Molecular Biology, Greifswald-Insel Riems, Germany, [3]National Institute for Agronomical Research (INRA), Virology and Molecular Immunology Unit, Jouy-en-Josas, France.*

MicroRNAs (miRNAs) are micromanagers of gene expression. Due to their non immunogenic nature, viral miRNAs represent an efficient tool to control the cellular environment. Most herpesviruses encode miRNAs to manipulate the post-transcriptional regulation of their

own genomes and that of their host. The alphaherpesvirus PrV establishes latent infections primarily in the trigeminal ganglia of its natural host, the pig. Deep sequencing has identified five miRNAs expressed from the Large Latency Transcript region which are highly conserved among different PRV strains and possibly involved in regulation of latency. To unravel the biological role of these miRNAs we use a combination of *in silico*, *in vitro* and *in vivo* approaches. PrV strains Ka and NIA-3 were deleted of the 2.5kb cluster of five miRNAs. All mutants displayed growth kinetics and plaque sizes in various cell lines similar to parental virus. *In silico* predictions and comparative analyses of the PrV genome vs. other herpesviruses identified potential viral and host target genes. Profiling of viral targets (e.g. IE180, EP0) in porcine PK15 cells confirmed slight down-regulation correlated with increasing levels of miRNAs up to 12h post infection. An infection experiment in pigs will be conducted to assess how deletion of the miRNAs affects the establishment of latency in PrV's natural host.

Key Words: herpesvirus, mutant, miRNA

P2018 Different strategies of coping with thermal stress in two rainbow trout strains identified by transcriptome profiling in gill tissue. Alexander Rebl[1], Marieke Verleih[1], Judith M. Köbis[1], Tomas Korytar[2], Carsten Kühn[3], Bernd Köllner[2], Klaus Wimmers[1], and Tom Goldammer*[1], *[1]Leibniz-Institut für Nutztierbiologie (FBN), Fachbereich Molekularbiologie, Dummerstorf, Germany, [2]Friedrich-Loeffler-Institut (FLI), Institut für Infektionsmedizin, Greifswald, Germany, [3]Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern (LFA-MV), Institut für Fischerei, Born, Germany.*

Local selection and breeding of robust rainbow trout strains characterized by low stress susceptibility is a sustainable alternative to growing trout from globally marketed strains. We compared the local selection strain BORN with an import strain and found the local strain better adapted to regional farming conditions as indicated by faster growth, higher weight gain, and higher survival rate after pathogen-induced stress. Since feed intake and growth correlated partially with higher water temperatures in summer, we performed a holistic transcriptome analysis after moderate thermal stress (8 to 23°C) to screen for genetically determined variations in the acclimation response of gills, the organ responsible for gas and electrolyte exchange as well as excretion. Besides well characterized mediators of thermoregulation such as genes encoding cold-inducible RNA-binding protein (CIRBP) and heat shock proteins (HSP), the present microarray study suggests several new candidate genes commonly regulated in gills of the 2 trout strains. The comparison of transcriptome profiles provides evidence for strain-specific employed expression patterns. Our data provide temperature change regulated genes and suggest links between different temperature-dependent pathways and gene networks. EFF pilot project VI56073084.

Key Words: rainbow trout, thermal stress, gill transcriptome

P2019 Evaluation of globin depletion methods for reduction of alpha- and beta-globin transcripts from porcine and bovine blood RNA. A. Hosseini[1], X. Sun[1], M. Yan[1], P. Stothard[1], C. K. Tuggle[2], G. S. Plastow[1], and L. L. Guan*[1], *[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada, [2]Department of Animal Science, Iowa State University, Ames, IA, 50011, USA.*

Alpha- and β-globin mRNA transcripts are the most abundant transcripts (~52–76%) in whole blood, and impede the ability to detect less abundant transcripts. Our objective was to evaluate the effect of different α- and β-globin depletion approaches on RNA transcriptome analysis in bovine and porcine blood. Total RNA was extracted from whole blood collected in PAXgene tubes. Alpha- and β-globin mRNAs were depleted using Ambion's GLOBINclear kit (GCK) and peptide nucleic acid (PNA) oligos in bovine and porcine. Additionally Affymetrix globin reduction (AGR) using custom designed oligos targeting globin variants with different length, was used with porcine blood samples. Remaining globin mRNAs were quantified using qPCR. The results indicated that α- and β-globin mRNAs were efficiently depleted 96% and 93%, respectively using GCK in bovine; they were 98% and 90% depleted in porcine using AGR. Depletion using PNA was less efficient than GCK in bovine, whereas PNA did not deplete α- and β-globin transcripts in swine. Substantial depletion of α- and β-globin transcripts was achieved in both species, which may provide improvements in accuracy and reproducibility of transcriptome analysis and allow the detection of higher numbers of lowly expressed genes.

Key Words: blood transcriptome, globin, qRT-PCR

P2020 Molecular cloning and expression analysis of an avian specific genes highly expressed in the ovary. Zhenhe Zhang, Long Liu, Junyin Li, Guiyun Xu, Ning Yang, and Zhuocheng Hou,* *National Engineering Laboratory for Animal Breeding and MOA Key Laboratory of Animal Genetics and Breeding, China Agricultural University, Beijing 100193, China.*

This study focuses on the investigation of one bird specific gene which specifically expressed in ovary and its functions in the process of ovary development and egg formation. All the bird specific genes were generated based on the Ensemble comparative genomics.

Our RNA-Seq data combined with published EST data showed that 16 genes were highly expressed in the chicken mature ovary. Among these 16 genes, a novel gene (LOC772391) which belongs to CYP2J super gene family was indentified. RT-PCR and qRT-PCR results demonstrated that LOC772391 was abundant in the mature ovary, while presented at very low expression levels in liver, ileum, magnum, isthmus, shell gland, and breast muscle. Full-length cDNA of LOC772391 was cloned from ovary mRNA by RACE. Similarity of LOC772391 with chicken and human CYP2J2 is only 8.67%, 8.59%, respectively. Dynamic expression analysis during the ovary development and egg laying period showed that LOC772391 was strongly expressed in the ovary of 25 weeks and 40 weeks (laying period), but weakly expressed in 16 weeks and 20 weeks (before laying). Our data showed that this gene might play an important role in the formation of egg yolk/oocyte mature. This study supported species-specific genes are related with species-specific traits. Our study also provide a new method to study specifies specific traits.

Key Words: comparative genomics, species-specific traits, ovary

P2021 Myostatin deficient cattle have delayed muscle growth and increased lipid gene expression in early prenatal development. B. Dalrymple[1,2], N. Hudson*[1,2], A. Reverter[1,2], P. Greenwood[3,4], Y. Wang[1,3], and S. Lehnert[1,2], *[1]CSIRO Livestock Industries, St.Lucia, QLD4067 Australia, [2]CSIRO Food Futures Flagship, St.Lucia, QLD4067 Australia, [3]CRC for Beef Genetic Technologies, [4]NSW DPI University of New England, Armidale NSW2351 Australia.*

The impact of myostatin deficiency on the expression of genes in the longissimus muscle (LM) of cattle during development was investigated. Microarray data from samples of LM across a time course from 60 d post-conception to 30 mo postnatal were compared from Piedmontese × Hereford (PxH) and Wagyu × Hereford (WxH) cattle. The PxH animals were heterozygous for the myostatin C313Y mutation. Increased expression of genes involved in innervation and early development, and reduced expression of genes encoding muscle structural proteins, at d 60 in PxH relative to WxH LM suggested delayed development of the PxH LM. By d 135 post-conception (near the start of adipogenesis and during secondary myogenesis) the PxH individuals had even more reduced muscle subunit gene expression compared with WxH animals than at d 60, but increased expression of genes encoding products involved in triacylglyceride (TAG) and fatty acid (FA) synthesis. Thus the additional space within developing muscle associated with reduced muscle cells appeared to be occupied by developing adipocytes. However, by d 195 post-conception (near the end of secondary myogenesis) the between genotype differences in TAG and FA metabolism genes and expression of genes encoding fast twitch muscle structural proteins had been reversed. Thus, the PxH animals appear to have an extended secondary myogenesis, compensating for their reduced primary myogenesis. Expression of fiber type differences based on slow twitch myofiber structural protein genes (reduced expression in PxH relative to WxH) was significant at d 60 and 135 post-conception, primarily as a consequence of the general delay in muscle development, and again became highly significant between the genotypes postnatally from 20 mo of age.

Key Words: myostatin, prenatal development, muscle development

P2022 Tissue-specific regulatory network of the porcine transcriptome. D. Pérez-Montarelo[1,2], N. Hudson*[1], A. Fernández[2], B. Dalrymple[1], and A. Reverter[1], *[1]Computational and Systems Biology, CSIRO Animal, Food and Health Sciences, Queensland Bioscience Precinct, 306 Carmody Road, St.Lucia, Brisbane, Queensland 4067, Australia, [2]Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. Coruña Km 7.5, 28040 Madrid, Spain.*

Cell and tissue differentiation proceeds from tightly orchestrated patterns of gene expression conducted by transcription factors (TF) and other regulatory elements. The processes, networks and TFs that drive tissue differentiation remain unclear for most tissues, especially non model organisms like the pig. Further, a gene atlas for the pig does not exist. To better understand the regulation of genes responsible for tissue identity, we inferred regulatory networks from a meta-analysis of 20 gene expression studies spanning 480 Porcine Affymetrix chips for 134 experimental conditions on 27 tissues. Despite disparity in the origin of the individual studies, a hierarchical cluster analysis revealed an anatomically sensible arrangement of tissues. Using this resource, we constructed a network based on the co-expression patterns of 1,072 TFs and 1,232 tissue specific genes. The resulting network is biologically consistent. That is, genes clustered by tissue and tissues clustered by site of embryonic origin. The normalization of the meta-analysis and inference of the gene co-expression network, operated synergistically toward a successful search for tissue-specific regulators. Together, our results recapitulate the known biology behind tissue specificity and provide new valuable insights in a model species.

Key Words: network, transcription factor, tissue

P2023 A gene expression atlas of the domestic pig. D.A. Hume*[1], T. C. Freeman[1], D. Beraldi[1], K. M. Summers[1], C. K. Tuggle[2], and A. L. Archibald[1], *[1]The Roslin Institute, University of Edinburgh, Midlothian, Scotland, UK, [2]Iowa State University, Ames, Iowa, USA.*

We have developed a new Affymetrix expression array that has comprehensive coverage of the known pig transcriptome. Expressed sequences (DNA) were collated from public repositories to generate a non-overlapping collection of 52,355 expressed sequences. The final array represents 47,845 expressed transcripts, with a mean probe coverage of 22. Annotation of the array was performed by iterative homology searches across multiple mammalian genomes to assign putative orthology to annotated genes in other species. The new array was used to generate a gene expression atlas of pig tissues. In total, 104 arrays were run on samples derived from 62 tissue/cell types. Following normalization, the data were subjected to correlation network analysis and clustering using the tool BioLayout Express3D. These analyses provide a detailed functional clustering of the pig transcriptome. Based upon this clustering, one can infer the function of a gene of unknown function from the transcriptional company it keeps. For example, we identified a comprehensive set of genes associated with oxidative phosphorylation and with endocytosis. The new array and the gene expression atlas provide a resource for many aspects of future omics research in the pig.

Key Words: microarray, annotation, pig

P2024 Genetic and sex effects on insulin-like growth factor system components in brain of bovine purebred and hybrid fetuses. A. Javadmanesh*[1], K. Kind[1], C. Fitzsimmons[2], D. Thomsen[1], and S. Hiendleder[1], *[1]JS Davies Epigenetics and Genetics group, School of Animal and Veterinary Sciences, and Robinson Institute, University of Adelaide, Roseworthy Campus, Roseworthy SA 5371 Australia, Adelaide, South Australia, Australia, [2]Agriculture and Agri-Food Canada/University of Alberta, Edmonton, Alberta, Canada, Edmonton, Alberta, Canada.*

Insulin-like growth factor (IGF) system components are crucial for pre- and postnatal development as they stimulate cell proliferation and mitogenesis. The IGF system consists of 2 ligands: IGF1 and IGF2; type 1 and 2 receptors: IGF1R and IGF2R; and the 6 binding proteins: IGFBP1–6. IGF system regulation is tightly connected with insulin (INS) and growth hormone (GH), and their receptors, GHR and INSR. IGF1 is highly expressed in brain and essential for normal brain development in human and rodents. Partial inactivation of *IGF1R* in the embryonic mouse brain selectively inhibits GH and IGF1 pathways postnatally which leads to a longer average lifespan. We used quantitative real time-PCR to measure transcript abundance for *IGF1*, *IGF1R*, *IGFBP5* and *GHR* in brain tissue of Day153 fetuses of pure-bred Brahman and Angus cattle and their reciprocal hybrids (n = 74). Expression levels of other investigated transcripts including *INSR*, *IGFBP1*, *IGFBP2*, *IGFBP3*, *IGFBP4* and *IGFBP6* were too low to measure consistently. Statistical analysis of genetic and sex effects on relative gene expression in hybrids versus pure-breds were performed with a linear model (JMP 4.0, SAS Institute, Inc.). *IGF1R* and *GHR* expression levels were lower in hybrids than in pure-breds ($P < 0.01$). *GHR* transcript was also affected by sex ($P < 0.05$) and by a genetics × sex interaction ($P < 0.01$). Other transcripts measured were not significantly different. As *IGF* receptors in brain strongly promote the development of somatotropic function in the mouse model, this may explain observed developmental differences in hybrid cattle.

Key Words: bovine brain, gene expression, IGFs

P2025 Comparison of the muscle transcriptome between samples with divergent intramuscular fat content in Berkshire by RNA-Seq. T. H. Kim*[1], Y. G. Lee[2], S. C. Kim[2], H. J. Jeon[1], S. W. Lee[1], K. T. Lee[1], E. S. Cho[1], and N. Kim[2], *[1]National Institute of Animal Science, Suwon, Republic of Korea, [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea.*

RNA-Seq has been widely used to understand complex transcriptome landscape of various tissues in mammals. Intramuscular fat content is one of the most crucial variables determining meat quality. A muscle transcriptome analysis was carried out to compare gene expression profiles between loin eye muscles with high and low fat content. We produced an average of 3 Gb from 12 samples by Solexa platform (101bp paired-end sequencing). Tophat and SAMseq were used to identify expression levels of each sample. An expression analysis revealed 55 differentially expressed genes between muscles with high and low intramuscular fat content. Genes within pig QTL database have been compared with differentially expressed genes. Gene Ontology (GO) enrichment analysis and functional analysis also has been performed. This study is a first step toward the development of DNA markers associated with intramuscular fat content. In addition, transcriptome assembly will provide a deep insight into 2 divergent fat muscle tissues such as alternative splicing, polyadenylation, novel genes and transcripts.

Key Words: pig, transcriptome, intramuscular fat content

P2026 Differential expression profiling by deep RNAseq in pigmented and non-pigmented bovine skin. C. Kuehn,* F. Hadlich, and R. Weikard, *Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

Next generation sequencing enables a comprehensive picture of the transcriptome of a specific cell, tissue or developmental stage. Piebald spotting is

characterized by patches of pigmented skin interrupted by white areas. Our studies targeted on elucidating mechanisms responsible for divergent pigmentation in mammals. Pigmented and non-pigmented samples from immediately adjacent skin areas of 2 bulls with piebald pattern were collected. Isolated RNA was used for TruSeq mRNA library preparation. The libraries were sequenced by a paired-end multiplex 2x 61 cycle run on an Illumina GAIIx sequencer. After demultiplexing and quality control, reads were mapped to the bovine genome using Bowtie/Tophat mapping tools. After filtering for mapped reads that uniquely mapped to the bovine reference sequence with less than 3 mismatches, $35.6 – 70.0 \times 10^6$ fragments per sample were available for transcript assembly. Expression profiling analysis showed that 310 transcripts were significantly ($q < 0.05$) differentially expressed in pigmented vs. non-pigmented skin. Whereas many of those transcripts belong to the known melanin synthesis pathway, also genes relevant for intra- and intercellular trafficking were affected. Our data highlight novel functional candidate genes that may affect the modulation of mammalian pigmentation.

Key Words: RNAseq, pigmentation, cattle

P2027 Study on microRNAome associated with goat hair cycle. Zhihong Liu, Jinquan Li,* Suangying Lai, Yanhong Zhao, Hongmei Xiao, Xinlei Yu, and Ting Cai, *Inner Mongolia Agricultural University, Hohhot, Inner Mongolia,China.*

Research on microRNA in skin and hair follicle started late. Taking advantage of gene chip, q- PCR, in situ hybridization, and the expression profiles of miRNAs have been reported in skin development processes in human and mouse skin, which suggesting that MicroRNAs (miRNAs) play significant roles in regulating the expression of the post-transcriptional skin and hair follicle gene. In this study, the skin samples were collected from the body of Inner Mongolian cashmere goats during the growth (anagen), involution (catagen), and rest (telogen) phases of hair growth. Total RNA was extracted and sequencing was performed. By comparing with miRbase, We found that 68 goat sequences matched known miRNAs; 248 sequences were assumed to be novel goat-specific miRNAs which are conserved in other species and not been detected in goat before. The analysis on differently expressed miRNA families in anagen, catagen and telogen indicates that there are 11 miRNAs differently expressed from anagen to catagen, 20 ones from catagen to telogen and 17 ones form telogen to anagen.This study showed the specifically periodically expressed miRNA, providing a basis for further reaearch on hair growth cycle regulation and constructed complete microRNAome profiles of goat skin cycle development, which is an important step toward artificially regulating hair growth.

Key Words: microRNAome, goat, hair cycle

P2028 Heritability and gene SNP associations with fatty acid composition in beef cattle. C. Ekine[1], N. Aldai[2], M. Vinsky[3], L. Chen[1], P. Stothard[1], M. Dugan[3], T. McAllister[4], and C. Li*[1,3], *[1]Department of AFNS, University of Alberta, Edmonton, Edmonton, Alberta, Canada, [2]Food Science and Technology, Faculty of Pharmacy, University of the Basque Country, Vitoria-Gasteiz, Spain, [3]Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Alberta, Canada, [4]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada.*

A DNA marker set of 1536 SNPs in 456 growth and fat metabolism related genes was genotyped on 223 crossbred beef steers with fatty acids measured in brisket adipose tissue. Heritability and SNP marker effects on 22 major fatty acids and heath index were estimated using an animal model incorporating animal′s relationship matrix that was defined based on 961 gene SNP markers. Estimates of heritability ranged from 0.03 for branched-chain fatty acids to 0.51 for 9c-14:1. Single SNP marker association analyses identified a panel of 25 to 46 gene SNPs that had significant associations with each of the fatty acids ($P < 0.05$). The results suggest that fatty acids are low to moderate heritable depending on the type of fatty acid and are influenced by multiple bovine genes. Further validation of the SNP marker associations will enhance our understanding of the genetic control of fatty acids in beef, which may lead to the genetic improvement of fatty acid composition in beef through marker assisted or genome selection.

Key Words: beef cattle, fatty acids, SNP

P2029 Discovery of potential piRNAs from next generation sequences of the sexually mature porcine testes. G. Liu[1], B. Lei[1], Y. Li[1], K. Tong[1], Y. Ding[1], L. Luo[1], X. Xia[2], S. Jiang[1], C. Deng[1], Y. Xiong[1], and F. Li*[1], *[1]Key Laboratory of Pig Genetics and Breeding of Ministry of Agriculture & Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan 430070, PR China, [2]College of Science, Huazhong Agricultural University, Wuhan 430070, PR China.*

piRNAs, a new class of small RNAs discovered from mammalian testes, are involved in transcriptional silencing of retrotransposons and other genetic elements in germ line cells. To identify a full transcriptome set of piRNAs expressed in the sexually mature porcine testes, small RNA fractions were extracted and were subjected to a Solexa deep sequencing. We cloned 6,913,561 clean reads of small RNAs (18–30 nt) and performed functional characterization. *Sus Scrofa* small RNAs showed a bimodal length distribution with two peaks at 21 nt and 29 nt. Then from 938,328 deep-sequenced small RNAs (26–30 nt), 375,195 piRNAs were identified by a k-mer scheme and 326 piRNAs were identified

by homology searches. All piRNAs predicted by the k-mer scheme were then mapped to swine genome by SOAP, and 81.61% of all 197,673 uniquely mapping piRNAs were located to 1124 defined genomic regions (5.85 Mb). Within these regions, 536, 501, 48 and 39 piRNA clusters were designated as minus, plus, divergent, and mixed type, respectively. Furthermore, expression pattern of 7 piRNAs identified by homology searches showed 5 piRNAs displayed a ubiquitous expression pattern, although 2 piRNAs were specifically expressed in the testes.

Key Words: piRNA, Solexa deep sequencing, testis

P2030 Clenbuterol upregulates histone demethylase JHDM2a via the cAMP/PKA/CREB pathway. Y. L. Li,* J. H. He, Y. Z. Zhao, X. H. Hu, and N. L. Li, *China Agricultural University, Beijing, China.*

The importance of beta2-adrenoceptor signaling stimulated by clenbuterol in the metabolism of muscle and adipose tissue has been acknowledged. Meanwhile, the significant role of demethylase JHDM2a in regulating metabolic gene expression was also confirmed in JHDM2a−/− mice. To determine the molecular mechanism involved in adipose tissue reduction from an epigenetic aspect, our research focused on CREB to determine whether JHDM2a is regulated by the beta2-AR/cAMP/PKA pathway. In porcine tissues treated with clenbuterol, JHDM2a was found to be upregulated at both the mRNA and protein level. Exogenous CREB expression increased JHDM2a as shown by transient transfections and luciferase reporter assays. In addition, the changes in JHDM2a expression were coincident with a variation in endogenous CREB phosphorylation and p-CREB/CBP interaction capability in various drug-stimulated porcine cells. Finally, we found that CREB regulates JHDM2a by directly binding to the CRE site of its promoter near to the transcription start site. Our results uncovered a signaling pathway upstream of JHDM2a by means of CREB as an intermediate link which is regulated by cAMP-PKA and promotes activity of the JHDM2a promoter. These findings suggest that clenbuterol decreases adipose cells in porcine tissues requiring JHDM2a demethylation to regulate metabolic genes.

Key Words: clenbuterol, JHDM2a, CREB

P2031 Systems biology of piglet maturity. L. Liaubet*[1], Y. Billon[2], G. Boudry[5], L. Canario[1], F. Gondret[4], I. Le Huerou-Luron[5], L. Lefaucheur[4], I. Louveau[4], P. Mormede[1], A. Paris[6], M-C. Pere[4], J. Riquet[1], M. SanCristobal[1], E. Terenina[1], H. Quesnel[4], *[1]INRA, UMR444 Cellular Genetics, F-31326 Castanet Tolosan, France, [2]INRA, U367 Experimental Genetics and Animal Production, F- 17700 Surgères, France, [3]INRA, UMR1331 ToxAlim, F-31027 Toulouse, France, [4]INRA, UMR1348 PEGASE, F-35590 Saint-Gilles, France and AgroCampus-Ouest, F-35042 Rennes, France, [5]INRA, UR1341 Nutrition & Digestive, Nervous and Behavioural Adaptations, F-35590 Saint-Gilles, France, [6]INRA, UR1204 Food Risk Analysis Methodologies, Mét@risk, F-75231 Paris, France.*

In recent decades, there has been improvement of prolificacy and body composition (toward more muscle) but the genetic progress has been accompanied by a substantial increase in the mortality of piglets before weaning. The most critical period is the perinatal period, mostly during the first 24–48 h following birth. The maturity of piglets, defined as the state of full development for survival at birth, is an important determinant of early mortality. However, evaluation of maturity at birth is difficult because of the lack of markers of this complex trait. The objective of our project is to take advantage of current knowledge about 2 pig breeds, Large White (LW) pigs selected for prolificacy and body composition and Meishan (MS) pigs being more robust piglets, to identify new markers of this trait. Maturity of several tissues and metabolite profiles of various fluids were analyzed in fetuses (LW, MS and reciprocal F1) at d 90 or 110 of gestation. The 1H-NMR metabolic profiles of fetal plasma show 1) the 4 genotypes were effectively different and 2) the litter heterogeneity was set up at 110 d of gestation. Physiological parameters (e.g., cortisol, fructose and albumin) differed significantly between genotypes and developmental stages. These data combined with transcriptomic and proteomic data should enrich our understanding of piglet maturity determinism. Funds: ANR-09GENM005

Key Words: piglet survival, integrative biology, functional genomics

P2032 The differential transcriptome of the bovine Y-chromosome is associated with testis development in cattle. W.-S. Liu*[1], T.-C. Chang[1], and E. F. Retzel[2], *[1]The Pennsylvania State University, University Park, PA 16802, USA, [2]National Center for Genome Resources, Santa Fe, NM 87505, USA.*

The majority (95%) of the Y-chromosome is male-specific (MSY), characterized by the absence of recombination with the X during meiosis, enrichment of male-specific repetitive sequences, and predominant expression of MSY-genes in testis. MSY provides a peculiar genomic niche involved mainly in maleness, spermatogenesis and male fertility. However, the redundant nature and absence of recombination have impeded the sequencing of the Y-chromosome. MSY sequences are available only for a few species to date and their transcriptomes have not been analyzed. We have recently investigated the genomic structure and gene repertoire of the bovine MSY and found that it is composed of 3 sequence classes: X-degenerate (Xd,

2.5Mb), Y-ampliconic (Ya, 35Mb) and Y-transitional (Yt, 5Mb). Xd comprises 12 single-copy genes. Ya contains an array of ~80 ~420Kb inverted repeats, and is distinct from the primate and mouse Ya. Four major protein-coding gene families and ~400 novel transcripts (mainly ncRNAs) are present in Ya with copy numbers ranging from 80 to 320. Yt displays a transitional feature between Xd and Ya and consists of a bovid-specific Y-gene family. Further RNA-seq analysis of the bovine testicular transcriptome (~80M reads) at the age of 20 d, 8 mo, and 2 years old indicated that the Y-genes/transcripts are differentially expressed in 5 major different patterns. These differential transcriptomes of the bovine Y-chromosome are associated with testis development.

Key Words: bovine Y-chromosome, transcriptome, testis

P2033 Efficient production of transgenic chickens based on piggyBac. Xiaojuan Liu,* Ning Li, Xiaoxiang Hu, Ran Zhang, Qingyuan Li, Dainan Cao, Tongxin Liu, Yaqiong Zhang, and Xiaofang Liu, *China Agricultural University, Beijing, China.*

Transgenic techniques in chickens have been developed more slowly than in mammals due to chickens' unique reproduction mechanism. Retroviral methods have been the most successful. piggyBac (PB) can be inserted into TTAA sites and can also be precisely excised in mammals and insects. Therefore, we have selected PB as a candidate to establish a new method to produce transgenic chickens. We constructed 3 donor vectors (ZGl-neo, ZGm-neo and ZGs-neo) expressing a GFP marker gene and a Neomycin resistant gene based on PB. We co-transfected each donor vector with a helper vector (CAG-PBase). We found that ZGl-neo was the most efficient. This vector could insert into TTAA sites in DF-1 cells. PB vectors were microinjected into subgerminal cavity of newly laid eggs, and electroporation was then performed with a 20-V pulse for 5 cycles of 50 ms on and 100 ms off. GFP was expressed in different tissues of the embryos, including the gonads. Twenty-two chickens hatched after microinjection with compounds ZGl-neo and CAG-PBase(3:1). When we screened the blood DNA, 72.7% (16/22) of the individuals were positive. Thirteen of the chickens grew to adulthood, 11 of which were males. 40% (4/10) of the individuals were semen positive, and their copy numbers ranged from 0.21 to 0.05. We conclude that the PB system is a novel useful tool for the efficient production of transgenic chickens.

Key Words: piggyBac, transgenic chicken, nonviral method

P2034 Genome-wide DNA methylation analysis for porcine adipose and muscle tissues. Mingzhou Li[1], Li Zhu[1], Qi Zhou[2], Ning Li[3], Ruiqiang Li[4], and Xuewei Li*[1], *[1]Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, Sichuan, China, [2]Ya'an Vocational College, Ya'an, Sichuan, China, [3]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China, [4]Peking-Tsinghua Center for Life Sciences, Biodynamic Optical Imaging Center, and School of Life Sciences, Peking University, Beijing, China.*

DNA methylation is an epigenetic mechanism with important regulatory roles in obesity development. To investigate the systematic association between DNA methylation and obesity, we used pig as a model, and sampled 8 variant adipose and 2 distinct skeletal muscle tissues from 3 pig breeds living within comparable environments but display distinct fat level. We generated 1,381 Gb sequence data from 180 methylated DNA immunoprecipitation (MeDIP) libraries, and provided a genome-wide DNA methylation as well as gene expression map for adipose and muscle studies. The analysis showed global similarity and difference among breeds, genders and anatomic locations, and identified the differentially methylated regions (DMRs). The unsupervised clustering for all samples using DMRs of each category of genomic elements identified DMRs in promoter regions as best at characterizing phenotypic difference among the samples. We uncovered a wealth of information on candidate genes susceptible to epigenetic regulation and with a role in the obesity development, among which 80% of pig orthologs of the 282 known human obesity-related genes and 72.2% of the 2,311 pig genes located in QTLs affecting fatness and port quality were within the defined DMR regions. This comprehensive map provides a solid base for exploring epigenetic mechanisms of adipose deposition and muscle growth.

Key Words: pig, obesity, methylation

P2035 Using RNA-Seq for transcriptome profiling in liver of boar with divergent skatole levels. A. Gunawan, S. Sahadevan, C. Neuhoff, C. Große-Brinkhaus, D. Tesfaye, E. Tholen, C. Looft,* K. Schellander, and MU Cinar, *Institute of Animal Science, Unit of Animal Breeding and Husbandry, University of Bonn, Bonn, 53115, Germany.*

Boar taint is the offensive odour or taste that can be evident during the cooking or eating of porcine meat derived from non-castrated male pigs which is primarily due to high levels of androstenone and skatole. Skatole is metabolite of tryptophan and is produced by intestinal bacteria in gut and catabolised in liver. The aim of the present study was to perform a transcriptome profiling in liver of boars with divergent skatole levels by using RNA-Seq. Five boars with high skatole and 5 boars with low skatole were selected for RNA-Seq experiment.

A total of 1.620.570 expression sequence tags (ESTs) were generated from cDNA libraries design to represent the known pig transcriptome. The total number of reads produced for each liver sample ranged from 14.559.329 to 46.050.468. The results showed that 562 genes differentially expressed between the 2 groups of skatole level. Three hundred and three genes significantly upregulated in higher skatole group and 359 were significantly downregulated ($P < 0.01$, FC >1.5). Liver associated with higher skatole revealed group of genes, involved in the metabolic process, cellular activities, transcription and translation process. Interestingly, members of solute carrier family genes such as SLCO2A1, SLC5A6, SLC13A3, SLC22A7, SLC22A18, SLC25A25, SLC26A7, SLC37A2, SLC39A14 were upregulated (FC >2) in the higher skatole group. Further studies are warranted for proofing the roles of candidate genes to reduce the boar taint in pig breeding programs by using genomic selection.

Key Words: RNA-Seq, skatole, liver

P2036 Differential growth rate sea bass population gene expression study with SOLiD4–SuperSAGE sequencing. B. Louro*[1], R. Reinhart[2], D. J. De Koning[3,4], A. V. M. Canário[1], and D. M. Power[1], *[1]CCMAR, Universidade do Algarve, Faro, Portugal, [2]Max Planck Genome Centre, Köln, North Rhine–Westphalia, Germany, [3]The Roslin Institute and R(D) SVS, University of Edinburgh, Easter Bush, Midlothian, Scotland UK, [4]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

One of the major goals in the sea bass (*D. labrax*) aquaculture industry is to understand and control the complexity of growth associated traits. In the present study the question of high growth heterogeneity in sea bass reared under high density is addressed. Growth heterogeneity was identified in a batch of hatchery sea bass, individuals were tagged and specific growth rates (SGR) determined by phenotyping all fish at monthly intervals. Fish revealing the most divergent SGR (10 individuals per group) were killed to sample brain, liver and white muscle tissues. Gene expression for each tissue was assessed using SuperSAGE (Serial Analysis Gene Expression) combined with next generation SOLID4 sequencing. A total of approx. Twelve million edited tags (26bp), on average 2 million tags per SAGE library, that represented 47.725 unique transcripts were identified. Comparison of transcripts in fish with high and low SGR yielded 322, 683 and 596 differently expressed tags (1% false discovery rate and log2 threshold of 2) in brain, liver and muscle, respectively. The tags were mapped onto the sea bass genome and around a third of the tags could be assigned to annotated genes in sea bass. All genes have been mapped to Kegg orthology pathways aiming to establish the regulatory pathways most affected and give insight into the tissue specific changes underlying divergent SGR in sea bass.

P2037 The response of gene expression of HSP90AA to heat stress in broiler chickens. Z. Lowman,* C. Ashwell, and F. Edens, *North Carolina State University, Raleigh, NC, USA.*

Heat Shock Proteins (HSPs) are a group of highly conserved proteins that produced in organisms in response to stress including high ambient temperatures. The HSPs function as molecular chaperones, aiding the folding of newly synthesized and mis-folded proteins, as well as the degradation of denatured proteins. A novel HSP, 90AA has been shown in humans to be involved in the promotion, maturation, structural maintenance and proper regulation of specific target proteins involved in cell cycle control and signal transduction. HSP 90AA has been shown to undergo a functional cycle that is linked to its ATPase activity. The bulk of HSP research in chickens has involved HSP 60 and 70, while HSP 90AA is relatively uncharacterized. This work describes the development of an assay for measuring the gene expression of chicken HSP 90AA. Broiler chickens (3 weeks of age) were exposed to acute elevated temperatures, livers harvested, total RNA extracted, reverse transcribed, and amplified using SYBR green detection. Real time PCR detected a significant expression increase in heat stressed birds as compared with controls. This response agrees with observations in humans where HSP 90AA induces conformational changes in its client proteins, thereby causing their activation. Further studies need to be completed in the chicken to characterize the role of 90AA and verify its ATPase cycle and chaperone function.

P2038 The peripheral blood transcriptome dynamically reflects immunocompetence in swine. N. Mach*[1], Y. Gao[1,2], G. Lemonnier[1], J. Lecardonnel[1,3], J. Estellé[1], and C. Rogel-Gaillard[1], *[1]INRA, UMR1313, Laboratory of Animal Genetics and Integrative Biology, Jouy-en-Josas, France, [2]Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, USA, [3]INRA, Biological Resources Centre for Livestock Genomics, CRB GADIE, Laboratory of Animal Genetics and Integrative Biology, Jouy-en-Josas, France.*

Immune traits (ITs) are potentially relevant criteria to characterize individual's immunocompetence (IC). Thus, porcine ITs related to innate and adaptive immunity were studied by functional genomics approaches, with no initial focus on resistance to specific pathogens. Peripheral blood transcriptome was analyzed in 60 d old Large White pigs (n = 443) 3 weeks after vaccination against *Mycoplasma hyopneumoniae*. Groups of 4 to 10 animals classified in the extreme tails of white blood, CD4-CD8+ and TCRγδ+ cell counts, phagocytosis, in

vitro production of IL2, IL10, TNF and IFNG, and anti-Mycoplasma antibodies distributions were selected for transcriptome studies. A porcine generic array enriched with immunity-related genes (SLA-RI/NRSP8–13K) was used. Among ITs studied, transcriptome analysis revealed differentially expressed genes for white blood and CD4-CD8+ cell counts, phagocytosis, and in vitro production of IL2 and IL10. A subset of these genes was confirmed by real time qPCR. Gene set enrichment analysis showed a significant over-representation of immune response functional modules. In conclusion, we show that ITs' levels are associated with gene transcript profiling, therefore blood transcriptome is a relevant molecular phenotype. Lastly, blood transcriptome could be combined with genetic studies to identify candidate genes underlying heritable ITs.

Key Words: pig, blood transcriptome, immunocompetence

P2039 Enabling functional and comparative analysis of genomic data sets for agriculture. F. M. McCarthy*[1], C. J. Schmidt[2], K. V. Shanker[2], and S. C. Burgess[3], *[1]Mississippi State University, Mississippi State University, Mississippi, USA, [2]University of Delaware, University of Delaware, Delaware, USA, [3]University of Arizona, University of Arizona, Arizona, USA.*

New sequencing technologies enable us to generate genomic data not only for each agricultural species but also individual genomes. However using this data to understand how changes in the genotype affect phenotype is hindered by poor annotation. The Gene Ontology (GO) is increasingly used for analyzing functional genomics data, however the GO does not include all key aspects affecting agricultural production (e.g., GO does not capture information about disease states or tissue expression). Moreover, technologies such as RNASeq identify many novel genes that have no known function. We are developing new tools for functional annotation and developing ontologies to support annotation of anatomy and phenotypic data. For example, eGIFT (Extracting Gene Information From Text) identifies key functional terms for a gene or gene set based upon published literature and we are developing reference gene sets, standardized gene nomenclature and chicken anatomy and phenotype ontologies. We also facilitate functional modeling by simultaneously developing analysis tools to use these new data types and adapting existing tools to cope with the larger data sets generated by RNASeq experiments. Moreover, we are developing resources for comparative genomics so that functional annotation for closely related species can be leveraged to identify common traits. We will highlight and demonstrate the use of these tools for functional analysis of agricultural functional genomics data.

Key Words: bio-ontology, phenotype, functional modeling

P2040 Expression of adipose differentiation-related protein (ADFP) gene linked to adipogenesis in bovine intramuscular fat. Y. Mizoguchi* and M. Moriya, *Graduate School of Agriculture, Meiji University, Kawasaki, Kanagawa, Japan.*

To investigate the genes involved in intramuscular adipogenesis, we had analyzed 11 genes that were selected based on dramatically changed expression ($P <$ 10−12) after differentiation in a clonal bovine intramuscular preadipocyte (BIP) cell line by SAGE. Of the 11 genes identified previously we found that the ADFP expression level was upregulated by retinoic acid during adipogenesis. Therefore, in the present study we conducted further detailed studies to investigate the role of ADFP gene expression by transfection with siRNA. The effect of ADFP downregulation on the accumulation of Triglyceride (TG) was examined by transfecting the BIP cells with ADFP siRNA. At 2 d post-confluence, the transfected cells were induced to differentiate into adipocytes. We harvested the BIP cells 3 and 6 d after stimulation and measured the ADFP gene expression levels and the accumulation of TG. On the basis of the real-time PCR analysis the expression level of ADFP in the ADFP-siRNA transfected cells was 21.4% of that in the scr-siRNA transfected cells in BIP 3 d after induction ($P < 0.05$). Also, 6 d after induction, the accumulation of TG in the ADFP-siRNA transfected cells was 20% less than in the control cells ($P < 0.01$). This accumulation of TG in BIP cells strongly suggests that the ADFP gene is involved in bovine intramuscular adipogenesis.

Key Words: adipogenesis

P2041 Ontogeny of mRNA expression of somatostatin and its receptors in chicken embryos in association with methylation status of their promoters. Muhammad Moaeen-ud-Din*[2,1], Nosheen Malik[1], and Ruqian Zhao[1], *[1]Nanjing Agricultural University, Nanjing, China, [2]PMAS Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan.*

Somatostatin (SST) is an inhibitory regulatory peptide, to control cellular growth and development . The present study was designed to investigate the ontogeny and tissue distribution of somatostatin and its 5 receptor (SSTR1–5) mRNA expression in the brain, gonads, intestine, kidney, liver, muscle, stomach and yolk sac membrane (YSM) of chicken embryos on the embryonic (E) ages of 10, 16 and 21 d . To reveal the possible role of DNA methylation on ontogenic regulation of mRNA expression, bisulfite sequencing PCR was performed to determine the methylation status of the promoter region of all the 6 genes in the liver. SST was predominately expressed in intestine, brain and gonads with different ontogenic patterns. The highest expression in intestine was detected at E10, while in brain and gonads was detected at E16. The tissue distribution and

ontogenic patterns of SSTRs were distinct from that of SST. At E10, the highest expressed SSTR subtype was SSTR1 in stomach. At E16, the highly expressed subtypes were SSTR5 in the brain, as well as SSTR4 and 5 in the muscle. At E21, almost all the 5 SSTR subtypes reached the highest level in kidney and liver. The results indicate important role of SST in control of development of embryogenesis in chicken in a time and tissue specific manner.

Key Words: somatostatin, chicken, ontogeny and methylation

P2042 Utilizing spleen transcriptome analysis to identify responses to aflatoxin B1 in the domestic turkey. M. Monson*[1], R. Settlage[2], K. Mendoza[1], S. Rawal[3], R. Coulombe[3], R. Dalloul[2], and K. Reed[1], *[1]University of Minnesota, St. Paul, MN, USA, [2]Virginia Tech, Blacksburg, VA, USA, [3]Utah State University, Logan, UT, USA.*

Domestic turkeys (*Meleagris gallopavo*) are extremely susceptible to aflatoxicosis, caused by consumption of aflatoxin B1 (AFB1). AFB1 causes liver damage, hepatocellular carcinoma, and immunosuppression. Mycotoxin contamination of corn and grains is a worldwide food safety issue and adverse effects of AFB1 lead to over $140M in losses annually for the poultry industry. Feed additives such as *Lactobacillus* (LGG) have been investigated for their potential to mitigate AFB1 toxicity. Impacts of AFB1 and LGG on turkeys can be characterized through changes in gene expression after exposure. To obtain genome-wide effects in an immune context, RNA-sequencing (RNA-seq) of the spleen transcriptome was performed on the Illumina GA IIx. Twelve libraries (3 spleen samples per challenge group: control, AFB1, LGG, and LGG + AFB1) were sequenced to an average depth of 8.8M reads. RNA-seq data sets (7.2 Gb total sequence) were assembled 2 ways, short-read alignment (TopHat and Cufflinks) and de novo (Velvet and Oases), to produce predicted transcripts. The number of reads mapping to each transcript was compared with identify uniquely and significantly differentially expressed transcripts and to determine the effects of AFB1 and LGG on expression. Spleen transcriptome analysis provides gene targets to increase resistance in the domestic turkey and improve health and production.

Key Words: RNA-seq, aflatoxin, turkey

P2043 Identification of a functional variant of the porcine glucocorticoid receptor with a major effect on the activity of the HPA axis. Eduard Murani,* Henry Reyer, Siriluck Ponsuksili, Stephan Fritschka, and Klaus Wimmers, *Leibniz Institute For Farm Animal Biology (FBN), Dummerstorf, Germany.*

Cortisol, the effector hormone of the hypothalamic-pituitary-adrenal (HPA) axis, modulates various neurobiological, metabolic, and immune processes and plays an essential role in the stress response. Activity of the HPA axis thus influences animal productivity and well-being. To identify genes affecting acute and long-term regulation of HPA axis activity in the pig we performed a genome-wide association study for plasma cortisol level and adrenal weight, respectively. We detected a major QTL affecting both traits at the position of the glucocorticoid receptor gene (NR3C1) – a key regulator of the HPA axis. We resequenced coding region of NR3C1 and found a SNP c.1829C>T, leading to a p.Ala610Val substitution in the ligand binding domain. This SNP was reproducibly associated with large (about 0.6 × and 1.2 × phenotypic standard deviations for cortisol level and adrenal weight, respectively), and highly significant negative effects on both analyzed traits in 3 different commercial populations. In vitro analysis of transcriptional activity of porcine GR revealed that the p.Ala610Val substitution significantly increases its sensitivity to glucocorticoids. Examination of the impact of polymorphisms in linkage disequilibrium with SNP c.1829C>T on the function of NR3C1 revealed only a minor effect on its mRNA expression. Our findings provide compelling evidence that SNP c.1829C>T in NR3C1 is directly responsible for a major part of the variation in HPA axis activity in pigs. Experiments addressing effects of SNP c.1829C>T on traits related to productivity, and well-being are underway.

Key Words: GWAS, QTL, causal variant

P2044 Proteome analysis of skeletal muscle in high and low drip loss Duroc × Pietrain F2 pigs. C. Neuhoff,* C. Große-Brinkhaus, H. Heidt, D. Tesfaye, E. Tholen, C. Looft, K. Schellander, and M. U. Cinar, *Institute of Animal Science, University of Bonn, Germany.*

Water holding capacity measured as drip loss is the ability of the post-mortem muscle to retain water during application of external forces such as cutting, heating, grinding, or pressing. Proteolysis and protein oxidation are key factors influencing the loss of water in meat, and degradation of cytoskeletal proteins may result in increased shrinking of muscle cells and drip loss. The aim of this work was the global proteome analysis and to identify the relevant biological mechanism of porcine muscle proteins, with potential functional relevance for drip loss. Additionally, genotyping and transcriptome analysis were performed in Duroc × Pietrain (DuPi) F2 resource population (n = 100). Proteins of the m. longissimus dorsi were isolated from a subset of 42 F2 animals with high and low levels of drip loss. The relative protein quantification was done using isotope-coded protein labeling techniques (ICPL) and liquid-chromatography- tandem mass spectrometry (LC-MS/

MS). The data analysis was performed with the ICPL Quant software package. In total, 688 different proteins were identified. Approximately 187 suggestive proteins were found to be differentially expressed between high and low drip loss groups. Five proteins among them (TNNC2, HSPA2, TPM1, MYLPF and AK1) were found to be significantly differentially identified ($P < 0.07$). While, TNNC2, TPM1 and AK1 were down-regulated in high drip loss group of animals, HSPA2 and MYLPF were upregulated. First network analysis suggested 3 main functional areas (glycolysis, cytoskeleton, mitochondrial part). Quantification of candidate proteins in larger animal population is warranted for further experiments.

Key Words: proteome, drip loss, pig

P2045 Tissue-specific differential and preferential allele expression of KCNJ11 in cattle. V. Catoia[1], P. C. Tizioto[1], M. M. Souza[1], M. I. P. Rocha[1], S. S. Mello[1], L. C. A. Regitano[2], and S. C. M. Niciura*[2], *[1]PPGEEv - Universidade Federal de São Carlos, São Carlos, SP, Brazil, [2]Embrapa Pecuária Sudeste, São Carlos, SP, Brazil.*

To investigate differential (DAE) and parental preferential (PAE) allele expression in the KCNJ11 gene, related to energy metabolism and muscle development, 34 bovine fetus were genotyped for a C > T SNP on exon 1. Muscle, skin and liver from fetuses homozygous CC and TT and heterozygous CmTp and CpTm, where m indicates the maternal origin and p the paternal origin, were used. Relative gene expression normalized by RPS-9 was assessed with SYBR green, and hydrolysis probes were used for allelic-specific expression, which was considered significant when the ratio between alleles was greater than 2. KCNJ11 was 24 times more expressed ($P < 0.05$) in liver of heterozygous CpTm than in homozygous TT, showing the parental origin effect on gene expression. In heterozygous CT, the C allele was more expressed than the T allele in muscle, skin and liver, indicating the existence of DAE. The effect of parental origin was observed in skin, with higher expression of the Cp, and in liver, with higher expression of the Cm, indicating tissue-specific PAE. Then, we conclude that KCNJ11 gene expression in bovine fetus is subjected to differential and parental preferential allele expression in a tissue-specific manner. This study contributes to a better understanding of the regulation of a gene related to productive traits in cattle. Financial support: FAPESP.

Key Words: allele-specific expression, parent-of-origin effect

P2046 Changes in gene expression of DNA methyltransferases in chicken embryonic tissues. S. Nolin* and C. Ashwell, *North Carolina State University, Raleigh, NC, United States.*

The enzymes, known as DNA methyltransferases (DNMTs), are important for gene regulation and are one of the primary means of epigenetic modifications. While much research has been done regarding DNA methylation, it has focused extensively on mammals, with little work done in avian species. This experiment sought to examine differences in gene expression of chicken DNMT1, DNMT3a, and DNMT3b at various points of embryo development in 3 tissues. Heart, liver, and intestine samples were collected from chicken embryos on d 10, 12, 15, 18, and 21 of incubation. Total RNA was extracted from the tissues and reverse transcribed for use in real time PCR, which used SYBR green chemistry and gene specific primers. Results showed significant changes in gene expression in all 3 tissues, with a common pattern of high expression from d 10–15, followed by a reduction in expression on d 18, and a return to previous levels on d 21. In mammals DNMT3a and DNMT3b are implicated in the establishment of methylation patterns early in development, while DNMT1 has been shown to be primarily involved in maintenance methylation during DNA replication. The similarities between the de novo and maintenance DNMTs expression observed here may suggest that the methylation patterns of avians are not thoroughly established until later in life, as opposed to early embryonic development as shown in mammals.

P2047 NOTCH1 expression regulation by miR-449b in the bovine blastocyst. K. Goossens[1], P. Mestdagh[2], A. Van Soom[3], J. Vandesompele[2], and L. Peelman*[1], *[1]Department of Nutrition, Genetics and Ethology, Ghent University, Heidestraat 19, Merelbeke, Belgium, [2]Center for Medical Genetics, Ghent University Hospital, De Pintelaan 185, Ghent, Belgium, [3]Department of Reproduction, Obstetrics and Herd Health, Ghent University, Salisburylaan 133, Merelbeke, Belgium.*

Mammalian blastocyst development is characterized by 2 lineage segregations and a delicate balance between pluripotency and differentiation. These processes are regulated by transcription factors and microRNAs (miRNAs). An integrative analysis of matching miRNA and mRNA expression data in early bovine blastocysts (day 7 p.i.) and hatched bovine blastocysts (day 8 p.i.) revealed that miR-449b was downregulated in hatched blastocysts in line with upregulation of its predicted target gene NOTCH1, a transmembrane receptor is expressed in trophectoderm cells and is suggested to be involved in placental cell fate decision.. The regulation of NOTCH1 by miR-449b was tested using an in vitro 3′UTR luciferase assay. Luciferase reporter constructs containing either the wild- type 3′UTR or mutant 3′UTR miR-449b target seed were engineered. HEK293T cells were co-transfected with the luciferase reporter constructs and the miRNA precursor (pre-miR-449b) or

a scrambled pre-miR negative control. Pre-miR-449b significantly reduced the luciferase activity of the WT NOTCH1 reporter with 50%, compared to the negative control (paired, two-tailed t-test, $P = 0.008$). The mutant reporter was not repressed by pre-miR-449b, which confirms that the target site directly mediates the repression.

Key Words: NOTCH1, pluripotency, blastocyst

P2048 Developing biomarkers for bovine health using whole blood transcriptome profiling. A. Hosseini[1], K. Orsel[2], B. Wolfger[2], H. E. Barkema[2], L. L. Guan[1], and G. S. Plastow*[1], *[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.*

Transcriptome analysis of whole blood may help identify biomarkers associated with healthier animals that could help reduce costs associated with disease. The objective was to develop a molecular phenotype that classifies animals in terms of their disease response. A pilot study was performed analyzing total RNA extracted from blood collected from animals on entry (ENT n = 10) to the feedlot, from individuals identified as being potentially sick (PUL n = 8); and from healthy animals from adjacent pens as controls (CTR n = 10). After globin mRNA removal, expression profiling was performed using dual color microarrays. Data were analyzed using Genesifter, DAVID and Ingenuity System Pathway Analysis. Genesifter revealed 147 differentially expressed genes ($P \leq 0.01$; $\geq$ 2-fold change) between CTR and ENT, and 183 and 382 genes between CTR and PUL, and ENT and PUL respectively. Network analyses between the 3 groups indicated the highest expression of inflammatory biomarkers in PUL (42 score for 17 molecules), with CTR (27 score for 9 molecules) and ENT (24 score for 9 molecules) animals scoring lower. These preliminary results indicate that blood inflammatory biomarkers may be key molecules for diagnosis and prediction of health status of beef cattle. Such blood biomarkers might potentially help selection for reduced disease susceptibility.

Key Words: blood transcriptome, microarray, bovine health

P2049 MicroRNAs and functionally linked mRNAs affecting meat and carcass traits in pigs. S. Ponsuksili*[2], Y. Du[1], E. Murani[1], B. Brand[2], M. Schwerin[2], and K. Wimmers[1], *[1]Research Unit 'Molecular Biology', Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Research Group 'Functional Genome Analysis', Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

MicroRNAs regulate gene expression by translational repression, degradation, or deadenylation of target mRNAs. Their roles in organismal development indicate that they contribute to phenotypic differences in muscle. To examine their role in muscle mRNA expression and traits related to meat quality and body composition, M. longissimus dorsi necropsies of 207 performance tested crossbred pigs (PI × (DL × DE)) were studied for miRNA and mRNA expression by using the custom-designed miRNA and catalog mRNA microarrays (Affymetrix). We applied weighted gene co-expression network analysis (WGCNA) to identify groups of co-expressed genes correlated with muscle traits. We found members of 130 miRNA families showing expression levels associated with various meat and carcass traits. The expression pattern of mRNAs led to the assignment to 22 modules by WGCNA. Furthermore, target mRNAs of significant miRNA were predicted from different databases and the lists were filtered based on mRNA expression data. Lists of significantly correlated mRNA and miRNA with muscle traits were analyzed by querying the Ingenuity Knowledge Base. The integration of miRNA and mRNA expression analyses as well as network analysis enabled us to interpret the differentially regulated genes from a systems perspective, yielding new insight into several biological pathways underlying phenotype differences.

Key Words: miRNA, transcriptome, muscle

P2050 A systems-genetics analysis of bovine skeletal muscle iron content. J. E. Koltes[1], R. G. Tait Jr.[1], E. R. Fritz[1], B. P. Mishra[2], A. L. Van Eenennaam[3], R. G. Mateescu[4], D. L. Van Overbeke[4], A. J. Garmyn[4], Q. Liu[1], G. Duan[1], D. Nettleton[1], D. Beitz[1], D. J. Garrick[1], and J. M. Reecy*[1], *[1]Iowa State University, Ames, Iowa, USA, [2]National Bureau of Animal Genetic Resources, Karnal, India, [3]University of California, Davis, California, USA, [4]Oklahoma State University, Stilwater, Oklahoma, USA.*

Improper iron homeostasis results in disease both in excess (hemochromatosis) and deficiency (anemia). Since beef is an excellent source of dietary iron, our objective was to investigate the genetic mechanisms responsible for variation in skeletal muscle iron content. To identify genomic regions associated with skeletal muscle iron content, we analyzed 54k bovine SNP genotypes from 2259 head of Angus-sired calves using BayesC. GWAS results indicated that markers with the largest effect were located on chromosomes1, 7, 15, and 17. Markers on chromosomes 7 and 17 were near GDF15 and SMAD1, both genes with known roles in human hemochromatosis. To investigate the transcriptional control of skeletal muscle iron content, mRNA levels in high and low total iron content Longissimus dorsi samples were compared. Sequence tags were aligned to the UMD3 genome build using Cufflinks and

analyzed in R assuming a Poisson distribution and accounting for fixed effects of iron, sex, age and contemporary group. RNA-seq identified 3010 differentially expressed genes ($q < 0.05$). Pathway Studio identified pathways that included known regulators of iron homeostasis: BMP6 and STAT1, as well as, novel ones: SMAD3, as potential novel regulators of iron homeostasis. Interestingly, our initial results indicate that many of the same genes involved in human hemochromatosis may also regulate bovine skeletal muscle total iron content.

P2051 High throughput RNA-sequencing of the domestic turkey liver transcriptome: Response to aflatoxin B1. Melissa S. Monson[1], Robert E. Settlage[2], Kristelle M. Mendoza[1], Sumit Rawal[3], Roger A. Coulombe[3], Rami A. Dalloul[2], and Kent M. Reed*[1], *[1]University of Minnesota, St. Paul, MN, USA, [2]Virginia Tech, Blacksburg, VA, USA, [3]Utah State University, Logan, UT, USA.*

Aflatoxin B1 (AFB1) contamination of feed leads to major economic losses for the poultry industry. After ingestion and absorption, AFB1 is bio-activated into a hepatotoxic epoxide form (AFBO) in the liver. Exposure to AFB1 is especially detrimental for domestic turkeys (Meleagris gallopavo), since these birds cannot detoxify AFBO. In the liver, AFB1 causes inflammation, cell remodeling, necrosis, carcinoma, and reduced organ weight, resulting in decreased performance and increased mortality. Addition of probiotic Lactobacillus (LGG) to feed has been examined as a technique to reduce AFB1 uptake and toxicity. Gene expression in the liver can be used to determine the genome-wide effects of AFB1 and LGG on domestic turkeys. Sequencing of the liver transcriptome (RNA-seq) was performed for 4 pooled libraries (PBS control, AFB1, LGG, and LGG + AFB1) on the Illumina GA IIx platform. A total of 13.9 Gb of sequence at an average depth of 80.6M reads/library was obtained. Predicted transcripts were assembled with Velvet and Oasis (de novo) and with TopHat and Cufflinks (short-read alignment to genome). Qualitative differential expression analysis was used to identify transcripts impacted by the AFB1, LGG and combined treatments. RNA-seq of the liver transcriptome provides insight into aflatoxicosis and gene targets to improve the health of domestic turkeys.

Key Words: transcriptome, poultry, aflatoxin

P2052 ASL2 sense/antisense expression in the chicken testis and their relationship to ASL activity and testes weight. T. Shimogiri*[1], K. Shibata[1], M. Chiba[2], S. Yamamoto[1], K. Mizuta[1], S. Murata[1], M. Nishibori[3], K. Kawabe[1], S. Okamoto[1], and H. Yasue[4,5], *[1]Kagoshima University, [2]Hirosaki University, [3]Hiroshima University, [4]NIAS, [5]Tsukuba Gene Technology Laboratories.*

Argininosuccinate lyase (ASL) is one of the urea cycle enzymes. In our previous study, we showed expression of the ASL2 mRNA (sense) and 2 antisense RNAs in the chicken testis. In the present study, we investigated the relationships of the antisense RNA expression to ASL activity and testes weight. Testes from 36 RIR chickens of 10- to 22-week-old were measured by weight and used for determination of antisense expression and ASL activity. Presence/absence of the antisense RNA was determined by RT-PCR. The presence of the antisense RNAs showed heavier testes weight ($P < 0.05$) and lower ASL activity ($P < 0.01$) than the absence. Real-time PCR for testes of twenty 14-week-old WL chickens revealed that the expression amounts of 2 antisense RNAs were strongly related to each other ($R > 0.9$), but not significantly related to the mRNA amount. The amount of the antisense RNAs was positively correlated with the testes weight ($0.6 < R < 0.8$). That of the sense RNAs was negatively correlated with the testes weight ($-0.4 < R < -0.5$). Currently, we perform ISH for cellular localization of the sense/antisense RNAs in the testis.

Key Words: ASL, chicken testis, sense/antisense RNA

P2053 Adipogenic expression profiling in bovine preadipocytes during differentiation. B. Soret,* P. Tiberio, C. Manso, L. Alfonso, and A. Arana, *Departamento de Produccion Agraria, Universidad Publica de Navarra, Pamplona, Navarra, Spain.*

The molecular events that take place during adipogenesis have been widely studied but, although species specificity is well known, there is less information referring ruminants. The aim of this work was to study the molecular cascade of genes involved in adipogenesis using a bovine primary cell culture model. Preadipocytes were isolated by collagenase digestion of subcutaneous adipose tissue and allowed to proliferate. Cells were challenged to differentiate and harvested for mRNA extraction on d 0, 1, 3, 6, 8 and 10. Relative gene expression was quantified by RT qPCR and calculated by normalizing against βactin, calibrator was d 0 (ΔCt method). C/EBPα, a master transcription factor (TF) of adipogenesis, increased on the first day of differentiation and decreased by day 6. PPARγ, the other master adipogenic TF, reached its higher level of expression on day 3 and maintained a high level of expression thereafter. On the contrary Wnt 10 b, associated with inhibition of adipocyte differentiation, decreased during the first 3 days; this was not mirrored by antiadipogenic NR2F2. Lipogenic enzymes LPL and ACC increased as well by day 1 and ACC maintained high level of expression during the differentiation period. These results show coordinate expression of adipogenic and antiadipogenic TFs that leads to preadipocyte differentiaton and target genes activation.

Key Words: adipogenesis, gene expression, bovine

P2054 Metabolomic and lipidomic analysis of equine serum in acute laminitis. S. Steelman*[1], P. Johnson[2], and B. Chowdhary[1], *[1]Texas A&M University, College Station, TX, USA, [2]University of Missouri, Columbia, MO, USA.*

Equine laminitis often occurs secondary to gastrointestinal (GI) disorders, although it is unclear how the GI system affects the laminae of the foot. Many authors have postulated that proteins, lipids, or other metabolites are transported via the bloodstream from the GI tract to the foot, where they trigger laminar inflammation. To test this hypothesis, we performed metabolomic and lipidomic analyses on serum collected from horses (n = 6) before (CON) and after induction of carbohydrate overload induced laminitis (LMN). Using mass spectrometry, we identified 256 metabolites and 922 lipids in equine serum. In CON samples, the serum metabolome was similar to that described in humans, although the presence of microbial-derived metabolites in horses suggests a link between the gut microflora and the serum metabolome. Laminitis altered serum levels of 32 lipids and 18 metabolites, many of which were involved in lactic acid metabolism. Some of the upregulated metabolites were of microbial origin, including a bacterial quorum-sensing signal that has been shown to have pro-inflammatory effects on epithelial cells. Our results support the hypothesis that bacterial metabolites escape from the GI tract during carbohydrate-induced laminitis and could initiate laminar inflammation. Further analyses are required to determine the specific effects of these metabolites on laminar tissue.

Key Words: metabolomics

P2055 Identification of differentially expressed genes in anagen and telogen of Inner Mongolian Cashmere goat. Rui Su,* Wenguang Zhang, Yanjun Zhang, Ruijun Wang, Yanhong Zhao, Zhihong Liu, and Jinquan Li, *Animal Science Department, Inner Mongolian Agricultral University, Hohhot, Inner Mongolia, China.*

The objective of this study was to characterize the mode of gene expression in skin in anagen and telogen. Gene expression profile of Cashmere goat skin in anagen and telogen were studied via Functional Classification Microarray and in situ hybridization in this study. The results revealed that, compared with the newborn goats on March, 33 genes were differentially 2-fold or greater. Among these 33 genes differentially expressed, 8 genes were upregulated, the percentage was 3%, whereas 25 genes were downregulated with the percentage 9.5%; Compared with the adult goats on September, 35 genes were differentially 2-fold or greater. Among these 35 genes, 4 genes were upregulated; the percentage was 1.5%, whereas 31 genes were downregulated with the percentage 11.8%. Compared with the adult goats on September, 49 genes were differentially 2-fold or greater. Among these 49 genes, 5 genes were upregulated; the percentage was 1.9%, whereas 44 genes were downregulated with the percentage 16.8%. Results of in situ hybridization indicated that BMP2 expressed at hair shaft in secondary hair follicle in telogen. However, it was found to be absent in anagen. It suggested that BMP2 gene could be recognized as the reference gene in hair follicle development in future study.

Key Words: Cashmere goat, hair, expression

P2056 Do bovine plasma exosomes contain plant miRNA? S. Briscoe and R. L. Tellam,* *CSIRO Livestock Industries, St Lucia, QLD, Australia.*

Exosomes are 30–90 nm lipid vesicles secreted by a range of cell types. They contain select proteins and RNA derived from the secretory cells. Exosomes are present in body fluids, including blood and milk, where their functions remain unclear. The RNA present in exosomes include pre-miRNA and these have been shown by others to undergo shuttling between different cells thereby affecting the functions of the recipient cells through the actions of mature miRNA. Recently, it was demonstrated that samples of human, mouse and cow serum contain at least 10 plant miRNA (Zhang et al. (2011) Cell Research 1-20). Moreover, humans and mice fed a diet enriched with rice produced circulating serum exosomes containing the abundant rice miRNA, MIR168a, which was shown to target the mammalian transcript LDLRAP1. These observations raise the intriguing possibility that ingested plant miRNA may have direct roles in regulating cell function in mammals. In the current investigation bovine plasma exosomes were purified using extensive differential centrifugation and filtration. Specific bovine miRNA were shown to be present within these exosomes using TagMan miRNA assays. Small RNAs (<150 bp) within exosomes were isolated and sequenced using an Illumina GAIIX sequencer (paired end 65 bp reads). Several bovine miRNA have been identified and current efforts are aimed at identification of plant miRNA.

Key Words: exosome, miRNA

P2057 Histone deacetylase 9 is a negative regulator of myogenesis. T. Vuocolo[1], K. Byrne[1], N. E. Cockett[2], T. Hadfield[2], C. A. Bidwell[3], and R. L. Tellam*[1], *[1]CSIRO Livestock Industries, St Lucia, QLD, Australia, [2]Utah State University, Logan, Utah, USA, [3]Purdue University, West Lafayette, Indiana.*

Callipyge sheep are noted for their increased muscling, which develops postnatally in a rostro-caudal gradient. The causal point mutation lies in an intergenic region within a cluster of imprinted genes on the telomeric end of OAR18. Analysis of gene expression in

skeletal muscle samples from callipyge sheep using microarrays revealed that the expression of histone deacetylase 9 was suppressed in the paternal heterozygote, the genotype associated with enhanced muscling, and this suppression was specific to only those muscles affected by the mutation. RT-qPCR analysis confirmed the microarray results. Based on these data and published information we hypothesized that histone deacetylase 9 (HDAC9) was a negative regulator of muscle hypertrophy. To test this hypothesis, HDAC9 expression in a murine model of myogenesis (C2C12 cells) was suppressed using a variety of transfected siRNA targeted to different splice variants of HDAC9. Three siRNA markedly accelerated myogenesis and induced the formation of large highly multinucleated myofibers. These data suggest that mutation-induced muscle specific suppression of HDAC9 could directly contribute to the muscle hypertrophy observed in callipyge sheep.

Key Words: callipyge, muscle, epigenetics

P2058 Determination of reference microRNAs for relative quantification in porcine tissues. Oriol Timoneda,* Ingrid Balcells, Sarai Córdoba, Anna Castelló, and Armand Sánchez, *Centre de Recerca en AgriGenòmica (CRAG), uUniversitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.*

RT-qPCR is the most used method for microRNAs (miRNAs) expression measurement, performing relative quantification as strategy to process the data, being necessary reference genes to normalize expression data. However, reference miRNAs have not been widely studied in livestock species. The aim of this work is the study of the expression stability of 10 porcine miRNAs (Let-7a, miR-103, miR-17-3p, miR-25, miR-93, miR-106a, miR-191, miR-16, miR-26a and miR-17-5p) to evaluate if they can be used as reference miRNAs. Samples included different tissues (skeletal muscle, kidney, liver, ovary and uterus) and different pig breeds (Iberian, Landrace, Large White, Meishan and Vietnamese). Stability values were calculated with geNorm and NormFinder algorithms ($r^2 = 0.99$). All 10 miRNAs had good stability values being able to be used as reference miRNAs. When all tissues are considered, miR-93 was the most stable miRNA, followed by miR-25, miR-106a, miR-17-5p and miR-26a. Dividing data set by tissues, Let-7a was the most stable in skeletal muscle and ovary, miR-17-5p in kidney, miR-26a in liver and miR-103 in uterus. The optimal number of reference miRNAs to be used for normalization data was determined suggesting the use of 5 reference miRNAs (cited above) in multi-tissue experimental designs and the use of the 3 most stable miRNAs in single tissue studies.

Key Words: microRNA, reference gene, RT-qPCR

P2059 Notch1-mediated signaling is required for proliferation of porcine satellite cells (PSCs). Lili Qin[1], Jian Xu[1], Qiaoming Long[2], Zhenfang Wu[1], Jiaqi Li[1], Zhe Zhang[1], and Chong Wang*[1], *[1]College of Animal Science/Guangdong Provincial Key Lab of Agro-animal Genomics and Molecular Breeding, South China Agricultural University, Guangzhou, Guangdong, People's Republic of China, [2]Department of Animal Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, New York, USA.*

Notch signaling is an evolutionarily conserved cell-cell communication mechanism involved in the regulation of cell proliferation, differentiation and fate decisions of mammalian cells. In the present study, we investigated the possible requirement for Notch signaling in the proliferation and differentiation of porcine satellite cells. We show that Notch1, 2 and 3 are expressed in cultured porcine satellite cells. Knock-down of NOTCH1, but not NOTCH 2 and NOTCH3, decreases the proliferation of porcine satellite cells. In contrast, enhancement of NOTCH1 expression via treatment of porcine satellite cells with recombinant NF-κB increases the proliferation of porcine satellite cells. The alteration of porcine satellite cell proliferation is associated with significant changes in the expression of cell cycle related genes (cyclin B1, D1, D2, E1 and p21), myogenic regulatory factors (MyoD and Myogenin) and the Notch effector Hes5. In addition, alteration of Notch1 expression in porcine satellite cells causes changes in the expression of GSK3β-3. Taken together, these findings suggest that of the 4 notch-related genes, Notch1 is likely to be required for regulating the proliferation and therefore the maintenance of porcine satellite cells in vivo, and do so through activation of the Notch effector gene Hes5.

Key Words: porcine satellite cell, Notch, proliferation

P2060 Fine-mapping of a QTL for milk fat percentage on bovine chromosome 5 based on imputed sequencing data. Xiaolong Wang,* Hubert Pausch, Michal Wysocki, Sandra Jansen, Bernhard Aigner, and Ruedi Fries, *Chair of Animal Breeding, Technische Universität München, Freising-Weihenstephan, Germany.*

Genotypes were imputed at 12 million polymorphic sites in 3668 animals based on whole-genome resequencing of 43 key ancestors of the Fleckvieh population. A genome-wide association study with these imputed genotypes identified a highly significant fat percentage QTL region on chromosome 5 ($P = 3.19 \times 10^{-14}$ for top SNPs). A previous study with array-based markers yielded a top signal of $P = 4.83 \times 10^{-13}$. The QTL region contains MGST1, the gene encoding microsomal glutathione S-transferase 1. The 17 most significant SNPs are located within 2532 bases of a putative

regulatory region of the gene. MGST1 is about 0.5 Mb distance from EPS8, which we have previously identified as the underlying gene for a fat percentage QTL in the German Holstein-Friesian population. The product of MGST1 is localized to the outer mitochondrial membrane and the endoplasmic reticulum, the site of lipid synthesis, and is presumed to protect these membranes from oxidative stress. It is likely that this protective function of MGST1 affects the lipid synthesis capacity in the mammary gland.

Key Words: fat percentage, GWAS, MGST1

P2061 Identification of novel miRNAs and pathways in myoblast differentiation. Wei Wei,* Shuhong Zhao, Jianhua Cao, and Xinyun Li, *Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Wuhan, Hubei Province, China.*

Recently, more and more miRNAs were proved involving in cardiac or skeletal muscle development, whereas there are still many muscle-related miRNAs need to be uncovered. To explore potential novel miRNAs in skeletal muscle developmental process, C2C12 myoblasts were induced to differentiation for 0 d, 1 d, 2 d and 4 d. Total RNAs were isolated from biological replicates of each time point. High-throughput Solexa sequencing was used to search differentially expressed miRNAs among the above samples. A total of 43 downregulated and 12 upregulated miRNAs was obtained with total clean reads more than 100. In addition to known muscle-related miRNAs, 48 miRNAs were selected as candidates for further functional verification. Also, digital gene expression tag profiling was used to find differentially expressed mRNAs. Several differentially expressed genes were involved in muscle-related pathways according KEGG database. Next we tried to establishing links between candidate miRNAs and gene pathways. For example, we have found that the expression of miR-365 was constantly downregulated in differentiating cells. Subsequently, we confirmed one potential target gene of this miRNA which participates in a pathway related to muscle development. Further studies are ongoing to illustrate the mechanism of these miRNAs in regulating muscle development in mouse and pigs.

Key Words: miRNAs, myoblast, differentiation

P2062 Cattle QTL hotspots for amino acids, phosphocholines and sphingomyelines on BTA9 & 10. P. Widmann*[1], R. Weikard[1], K. Suhre[2], H. Hammon[3], E. Albrecht[4], and C. Kühn[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Research Unit Molecular Biology, Dummerstorf, Germany, [2]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany, [3]Leibniz Institute for Farm Animal Biology (FBN), Research Unit Nutritional Physiology "Oskar Kellner," Dummerstorf, Germany, [4]Leibniz Institute for Farm Animal Biology (FBN), Research Unit Muscle Biology and Growth, Dummerstorf, Germany.*

The identification of genetic loci that act on metabolites will reveal modulators of metabolic processes relevant for the expression of important phenotypes. This was demonstrated by a recent study combining metabolomics with genomics, which had put a mutation in the bovine NCAPG gene affecting postnatal growth into the context of the growth regulating functions of arginine. In the present study we performed genome-wide QTL analyses across all bovine autosomes with metabolites serving as phenotypes. We applied a targeted metabolic approach to quantify the 244 plasma metabolites in a F2 population of Charolais x German Holstein. To find genotype-metabolite associations, we focused on genomic regions with QTL accumulations, because these display promising loci for the genetic basis of key modulators in metabolic pathways. Our data revealed a cluster of QTL affecting phosphocholine and sphingomyeline concentrations on BTA9. We suggest that this region represents a crucial locus in phospho- and sphingolipid metabolism. On BTA10, we detected an accumulation of QTL affecting amino acid concentrations. The patterns indicate that putatively important regions of amino acid metabolism are located in the centro- and telomeric region of BTA10. Further analyses will be carried out to elucidate candidate genes for the QTL and to embed our findings into current metabolic pathway networks.

P2063 Genome-wide expression and association analyses to identify genes either affecting or responding to plasma cortisol in pigs. K. Wimmers*[1], E. Murani[1], Y. Du[1], and S. Ponsuksili[2], *[1]Research Unit 'Molecular Biology', Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Research Group 'Functional Genome Analysis', Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Variation of the afferent and efferent axis of glucocorticoid signaling affects growth, health and well-being. To identify candidate genes of liver and muscle that impact or respond to plasma cortisol levels, we addressed the 3-way relationship of (1) gene expression (Affymetrix microarray), (2) genotype (Illumina 60k SNP chip), and (3) phenotype (plasma cortisol, ELISA) by estimating genome-wide association (GWAS), trait-correlated expression and detecting eQTL. We further integrated the data by causality modeling (NEO). In liver, 990 genes showed transcript abundances associated with cortisol levels and exhibited eQTL. According to NEO, 26 were predicted to be causal to plasma cortisol levels; 70 were predicted to be responsive. In muscle, out of 593 genes 2 were predicted to affect plasma

cortisol levels; 25 were found to be responsive. These were genes related to lipid metabolism, cell death, inflammatory response, glucocorticoid receptor signaling, and transcription regulation. Comprehensive data integration has helped to elucidate molecular networks contributing to cortisol levels and its subsequent metabolic effects. The discrimination of up and downstream effects of transcripts affecting or responding to plasma cortisol levels improves the understanding of the biology of complex traits.

Key Words: eQTL, transcriptome, cortisol

P2064 Transcriptome variance of porcine renal epithelial cells induced by viral dsRNA stimulatory and DNA methyltransferases inhibitor. Wang Xiaoshuo,* Bai Lijing, Zhai Liwei, Yu Ying, and Wang Chuduan, *Department of Animal Breeding and Genetics, China Agricultural University, Beijing, 100193, P.R. China.*

Porcine viral-diseases cause great economic losses in the pig-raising industry. Since the low heritability of antiviral diseases, it is hard to improve the resistance of viral-diseases in swine population. DNA methylation builds up the crosstalk between virus and host, while DNA methyltransferases inhibitor 5-Aza-2′-deoxycytidine (5-Aza-dC, Aza) has demethylating effect. Here, we tested the hypothesis that genome-wide demethylation can influence the host gene expression induced by Aza and viral dsRNA stimulatory (Poly(I:C), Poly). We treated normal porcine renal epithelial cells with Poly and Aza agents to analyze genome-wide expression variation with Microarray techniques. A total of 860 (Poly), 5563(Aza) and 874 (Poly+Aza) porcine genes were showed differential expression compared with the controls (fold change ≥ 1.5, $P \leq 0.05$). Pathway analysis disclosed that the significant pathways were mainly concerned with antigen processing and presentation, long-term depression and T cell receptor signaling pathway via compared with 2 groups of Poly versus Control and Poly+Aza versus Poly. The results found that Aza has the negative effects on viral replication. Additionally, porcine *CD4*gene expression was upregulated by DNA demethylation in the cell line, which is warranted to validate by bisulfite cloning sequencing and EMSA.

Key Words: porcine, gene expression, DNA methylation

P2065 FADD-mediated activation of an apoptosis program in bovine FGCs. R. Yang*[1], Z. Zhao[1], J. Li[2], and S. Xu[2], *[1]College of Animal Science and Veterinary Medicine, Jilin University, Changchun, Jilin province, P.R. China, [2]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China.*

FADD is a signal connection protein in the Fas/FasL system, which may play a key role in follicular development. To reveal the signal protein involved in the process of bovine follicular development, FADD gene without the stop codon was amplified using RT-PCR and directly cloned into the plasmid pAcGFP-N1. The recombinant plasmid pAcGFP-FADD was then transfected into bovine follicular granulosa cells (BFGCs).Expression of AcGFP was observed, and the transcription and translation of FADD were detected by RT-PCR and Western blot. The MTT assay, Hoechst33342 staining and DNA ladder method were performed to determine the growth inhibition and apoptosis of the cells. The results showed that GFP expression was detected as early as 24 h after transfection. The FADD fusion gene was successfully expressed in BFGCs as evidenced by the detection of a 654 bp fragment corresponding to the FADD mRNA and a 51.4 kD band corresponding to the Fas fusion protein. BFGCs viability decreased significantly at 72 h after transfection, and the apoptosis rate of the cells transfected with pAcGFP-FADD was significantly higher than control group. Cells in the FADD transfection group showed ladder patterns characteristic of apoptosis, and the nuclei were shrunken and densely hyperchromatic or fragmented, suggesting that FADD is capable of inhibiting the proliferation of BFGCs and inducing cell apoptosis when overexpressed.

Key Words: FADD, apoptosis, bovine

P2066 Epigenetic impacts associated with early phosphorus (P) conditioning on intestinal gene expression may result from NOTCH signaling networks. A. Zavelo* and C. Ashwell, *North Carolina State University, Raleigh, NC, US.*

Evidence in mammals suggests that a poor in utero environment resulting from maternal dietary or placental insufficiency may program susceptibility in the fetus to metabolic disorders. We have observed similar programming in chickens when stressed by dietary manipulation by limiting P. These birds better utilize P later in life, which can partially be explained by an enduring increase in the expression of the intestine-specific Na/P cotransporter gene. We have used next- generation sequencing technologies to better understand the genes and gene networks that are involved in this conditioning process chicken. Comparisons of dietary treatments (Control and Low) at ages 90 hours and 35 days were made for the duodenum. The most significant gene network identified by the RNAseq data involved NOTCH signaling. NOTCH has been shown to induce the demethylation of genes, and may be functioning in a similar matter to modulate intestinal gene expression. With significant impacts on bird performance it is not surprising to observe broad changes in gene expression. It is the accumulation of these changes and shifts in gene networks that holds the key to understanding the mechanism of this phenomenon.

Key Words: gene expression, NOTCH, chicken

P3000–P3076

Genetic diversity and polymorphisms

P3000 Assignment of SLA class II haplotypes and outcome of the heterozygous breeding in microminipigs. Asako Ando*[1], Naoki Kaneko[2], Noriaki Imaeda[3], Shino Ohshima[1], Masaki Takasu[3], Hisako Kawata[4], Hidetoshi Inoko[1], and Hitoshi Kitagawa[3], *[1]Department of Molecular Life Science, Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Isehara, Kanagawa Prefecture, Japan, [2]Fuji Micra Inc., Fujinomiya, Shizuoka Prefecture, Japan, [3]Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu Prefecture, Japan, [4]Education and Research Support Center,Tokai University School of Medicine, Isehara, Kanagawa Prefecture, Japan.*

Extremely small sized novel miniature pigs designated as Microminipigs (MMPs) have recently been developed by Fuji Micra Inc. for laboratory use. A population of MMPs was established by successive breeding of an initial female and other miniature male pigs. Body weight of the young mature MMPs at 6 mo of age ranges from 7 to 8 kg, comparable to the weight of mature Beagle dogs. MMPs provide an advantage over miniature pigs due to their smaller body sizes, easier handlings, smaller rearing spaces, less expensive feedstuff cost, and lower doses of test articles. To establish swine leukocyte antigen (SLA)-defined MMP lines, we assigned 8 SLA class II haplotypes from selective breeding of MMPs; Hp-0.11, -0.17, -0.23, -0.37, and other 4 haplotypes. Major haplotypes were Hp-0.23 and -0.37, and their frequencies were 44% and 23%, respectively in 58 MMPs. In this herd, SLA class II homozygotes with Hp-0.23, -0.17, and -0.11 have been obtained by the breeding of heterozygous parents with each of the haplotypes. Although the frequency of SLA class II heterozygous MMPs with Hp-0.37 was high, no homozygous individual has been obtained in 9 deliveries by heterozygous mating. This suggests the presence of some interfering factors at developmental stages of homozygotes with Hp-0.37.

Key Words: SLA, haplotype, miniature pig

P3001 Functional diversity arising from intra and inter haplotype combinations of DQ loci within the sheep MHC. P. Steele, M. Rocchi, and K. Ballingall,* *Moredun Research Institute, Penicuik, Midlothian, UK.*

Animals with fully characterized MHC regions are often used to explore the interactions underpinning the induction of adaptive immunity to pathogen infections. While developing such a resource for sheep we have characterized diversity associated with the class II DQ subregion. Full length DQA and B transcripts were amplified from MHC homozygous animals representing 4 haplotypes. Two DQA genes representing either DQA1 and DQA2 or DQA2 and DQA2-like combinations were identified in each. Similarly, 2 DQB genes representing either DQB1 or DQB2 genes were identified in each haplotype. Functional A/B gene combinations were verified by surface expression following co-transfection within or across haplotypes. DQA1 genes were restricted to surface expression with DQB1 genes. In contrast, the DQA2 genes expressed with DQB2 genes in both cis and trans combinations as well as with some but not all DQB1 genes. Only intracellular expression of the DQÎ ± 2-like protein was detected. These experiments indicate that the number of DQ molecules available for presentation of antigen to T cells may range from 2 in MHC homozygous animals to potentially 12 in MHC heterozygous animals. Such a large variation in the number of available class II MHC DQ molecules has the potential to significantly alter the range of antigens presented to CD4+ T cells.

Key Words: sheep, MHC, DQ

P3002 Characterization and exploitation of the unique genomes and adaptations of Ethiopian cattle to changing environments. Zewdu Edea Bedada*[1,2], Kwan-Suk Kim[2], Hailu Dadii Melka[3], and Tadelle Dessie[1], *[1]International Livestock Research Institute (ILRI, Addis Ababa, Ethiopia, [2]Chungbuk National University (CBN, Cheongju, Korea, [3]Konkuk University, Seoul, Korea.*

The genetic diversity and the genetic merits of most of Ethiopian indigenous cattle populations is not yet well understood, exploited and known to decision makers and development actors. As a result, some of the indigenous cattle populations are already extinct and endangered while the risk status of many of them is unknown. This study is a collaborative project between Korean Government, Ethiopian Institute of Agricultural Research and ILRI designed with the objectives to estimate genetic diversity using high density panel of SNP markers and test the hypothesis that adaptability can be detected at the DNA level by linking molecular data with environmental variables. A total of 254 individual nasal samples were collected from the following indigenous cattle populations: Borana (50), Arsi (52), Horro (50) and Danakil (52) inhabiting in different agro-ecologies, production environments and raised for different production objectives by diverse ethnic groups. DNA samples will be genotyped using the Illumina Bovine SNP50 BeadChip. Observed and expected heterozygosities, estimate of FIS per population, FST per locus and population pairs will be calculated. Univariate logistic regression analysis will be carried out to test for association between allelic frequencies at marker loci and environmental variables.

Key Words: Ethiopian cattle breeds, genetic diversity, unique genome

P3003 A genome-wide association study identifies "tiger" eye color variation locus on ECA1 in Puerto Rican Paso Fino horses. Elizabeth Kowalski[1], Elizabeth Staiger[2], Samantha Brooks[2], and Rebecca Bellone*[1], *[1]University of Tampa, Tampa, FL, USA, [2]Cornell University, Ithaca, NY, USA.*

The Puerto Rican Paso Fino is a gaited horse breed that segregates for iris color. The irises of Puerto Rican Paso Finos can vary from a dark brown color to a dynamic color breeders call "tiger" eye. "Tiger" eye is characterized by shades of orange, yellow, and amber. Pedigree analysis implicated a recessive mode of inheritance for this phenotype. To map this trait and identify candidate genes for further investigation, a genome-wide association study (GWAS) was conducted using an Illumina Equine SNP70 BeadChip and 24 unrelated DNA samples. To determine significant allelic associations, we used gPLINK (version 2.050) and applied an adaptive permutation approach to correct for multiple testing in this small sample set. The highest association detected was on ECA1 ($P = 9.0 \times 10^{-6}$). The association of SNPs in 2 functional candidate genes from this region was confirmed in a larger sample set (n = 47) by RFLP analysis ($P = 3.91 \times 10^{-7}$ and $P = 2.14 \times 10^{-8}$, respectively). To identify a causative mutation, DNA sequencing of these candidate genes is ongoing.

Key Words: GWAS, Puerto Rican Paso Fino

P3004 Novel mutations controlling ovulation rate in sheep. L. Bodin*[1], J. Demars[2], W. Drobik[3], S. Fabre[4,2], J. P. Hanrahan[5], O. Keane[6], E. Martyniuk[3], P. Mulsant[2], Z. Nowak[3], L. Persani[7], R. Rossetti[8], J. Sarry[2], and G. Tosser-Klopp[2], *[1]INRA-SAGA, Castanet-Tolosan, France, [2]INRA-Génétique Cellulaire, Castanet-Tolosan, France, [3]Warsaw University of Life Sciences, Warszawa, Poland, [4]INRA-Physiologie de la Reproduction et des Comportements, Nouzilly, France, [5]Teagasc Mellows Campus, Athenry, Co. Galway, Ireland, [6]Teagasc, Animal & Bioscience Department, Grange, Dunsany, Co. Meath, Ireland, [7]IRCCS, Milano, Italy, [8]University of Milan, Milano, Italy.*

In the frame of a European program (3SR), a case study aiming to identify major genes for ovulation rate was set up in 3 sheep populations: the Irish Cambridge, the French Grivette and the Polish Olkuska. Animals were genotyped with the 50K SNP chip and analyzed within each population. For Cambridge; the inheritance pattern of sterile cases suggested a new autosomal recessive gene unlinked to GDF9. Homozygosity mapping localised a unique genomic area on chromosome 2 for which sterile ewes presented large homozygous segments. In this area it was possible to determine 2 putative regions (112 kb and 1.2Mb) carrying the causative mutation. The smallest encodes 2 strong candidate genes which are being sequenced. For Grivette and Olkuska, a GWA study revealed significant signals close to the BMP15 gene. Sequencing this gene identified a new mutation in each population, both resulting in a non-conservative amino-acid substitution, T317I in Grivette and N337H in Olkuska. In Grivette, 151 adult ewes chosen at random in the population were genotyped for the new mutation. Mean prolificacy of wild type, heterozygous and homozygous carriers were 1.93, 1.98 and 2.31. The functional effect of this mutation was tested in vitro after directed mutagenesis. In Olkuska, none of 32 control ewes were homozygous for the mutated allele and only 1 of the 22 cases was homozygous wild-type. Mean OR were 1.63, 1.93 and 4.34 for the respective 3 genotypes.

Key Words: mutation, ovulation, sheep

P3005 New opportunities and new objectives for the ISAG-FAO Advisory Group on Animal Genetic Diversity. P. Boettcher*[1], J. A. Lenstra[2], and P. Ajmone-Marsan[3], *[1]FAO-AGAG, Rome, Italy, [2]Utrecht University, Utrecht, the Netherlands, [3]Università Cattolica del S. Cuore, Piacenza, Italy.*

Since 1995, FAO and ISAG have collaborated on issues regarding management of animal genetic resources (AnGR) through the ISAG-FAO Advisory Group on Animal Genetic Diversity. Among the main contributions of the Advisory Group has been the establishment of standard panels of microsatellites for the molecular characterization of AnGR. Over the past decade, these panels have been used for characterization of more than 500 breeds in 9 species. However, the utility of microsatellites in breed characterization is generally limited to estimating within-breed genetic variation and similarity across breeds, while providing inferences on breed origin and development. For major livestock species, microsatellites have been superseded in research and selection by genomic tools such as SNP and sequencing. These technologies offer new opportunities for breed characterization as well. FAO will soon implement "Production Environment Descriptors" for characterization of production systems and will integrate geographic information into its Global Data Bank on AnGR. Complementary genomic data would allow for the deeper study of adaptation. Genomic tools may also enhance measures to add value to local breeds, by improving the understanding of the genetic basis underlying unique traits and characteristics of typical products. Enhanced activity of the Advisory Group will be necessary to provide direction in establishing standards for the use of the new technologies to meet these objectives.

Key Words: breed, characterization, genomics

P3006 Mitochondrial DNA D-Loop diversity of Tibetan pig populations. Yuan Cai* and Shengguo Zhao, *Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China.*

The Tibetan pig, a plateau type pig breed, is recognized as one of the most important pig breeds in China and a valuable genetic resource worldwide. As part of an initial step to investigate the genetic structure and diversity of its populations, phylogenetic analysis was carried out using 417bp mitochondrial DNA (mtDNA) D-loop sequence variations. The mtDNA breed sequences of 26 Hezuo Tibetan pigs were found to be distributed in 20 haplotypes, 19 of which were unique to this breed. The nucleotide diversity within the control region sequences of Hezuo Tibetan pigs (0.82%) was found to be higher than those of the other Tibetan pig populations sampled in this study. Based on the results, conservation of Hezuo Tibetan pigs should be a priority as the breed serves as a vector of unique genetic resources. Rooted neighbor-joining tree and median joining network procedures carried out on the data showed that the Tibetan pig breed has 3 major ancestral maternal origins and different domestication histories.

Key Words: Tibetan pig, genetic diversity, phylogenetic

P3007 Investigation of parasite infectious status for 4 indigenous goat breeds in Southern China. Jianhua Cao,* Zhenyang Wu, Dan Huang, Yuhua Fu, and Shuhong Zhao, *Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Wuhan, Hubei, P.R. China.*

Because of high humidity and temperature, the loss caused by parasite infection in goat production is significant in South and Southwest China. To evaluate the risk of parasitosis for indigenous goat breeds, the investigation of parasite infectious status for 4 local breeds was first conducted by fecal egg counting (FEC). According to the statistical requirements, 362 individuals from 4 goat breeds (NJY, YCW, ESB, HYB) in Hubei province (located central China, E108.81 N29.83) were randomly sampled, in which each breed was sampled at least 30 individuals including ear tissue and faces sample. FEC was characterized by McMaster's method. The results showed that different breeds have extremely different ability to resist parasite infection, in which ESB has a minimum infectious ratio 57%, HYB has a maximum ratio 76% as well. Interestingly, the average eggs per gram faces (EPG) which indicates the infectious intensity was not ESB (0.83E4) but YCB breed (0.098E4), which imply a potential capacity underlying genetic variation for infectious disease. Furthermore, the 2-tailed individuals on the distribution were chosen for genomic SNP scanning to identify the genes associated with parasitosis. The study will benefit for improving goat productivity, and shed light on the mechanism of parasitosis on goats.

Key Words: parasitosis, goat

P3008 Genetic relationships between the Garfagnina Bianca sheep and other Italian sheep breeds. A. Carta*[1], M. G. Usai[1], G. Mulas[1], T. Sechi[1], F. Pilla[2], G. Ferruzzi[3], and S. Pieroni[4], *[1]AGRIS-Sardegna, Olmedo, Italy, [2]Animal Breeding and Genetics, Dip Scienze Animali e dell Ambiente, Universitá degli Studi del Molise, Campobasso, Italy, [3]Universitá Degli Studi di Pisa, Pisa, Italy, [4]Comunitá Montana della Garfagnana, Castel Nuovo di Garfagnana, Italy.*

The Garfagnina Bianca (GF) is a dual-purpose Italian breed probably originated from the Appeninic Mountains. Spread in the past over a large region of north Italy, it experienced an important population size contraction. In the framework of the project -VAGAL: PO Italia-Francia Marittimo 2007–2013-, aimed at the valorization of autochtonous genotypes, 24 GF animals, sampled from 3 flocks, were genotyped with the Ovine50K Beadchip. Genotypes of 24 individuals of other 21 Italian breeds were collected and genotyped by the Biovita Consortium. Reynolds genetic distance estimates indicated the Massese, Bagnolese and Sopravissana breeds as the closest to GF. The highest genetic distance was estimated with the Nera di Arbus, from the Sardinian island. The extent of haplotypes sharing between breeds, defined as the correlation of r statistic for SNP separated by different intervals, confirmed the high genetic similarity between GF and Massese, despite different color of their fleece

Key Words: Ovine50K Beadchip, haplotypes sharing

P3009 Genetic relationships between Corse, Sarda, and Sardinian Nera di Arbus sheep breeds. S. Casu*[1], M. G. Usai[1], M. Piras[1], T. Sechi[1], G. Secchi[1], F. Casabianca[2], V. Nunziatini[3], and A. Carta[1], *[1]AGRIS-Sardegna, olmedo, Italy, [2]INRA - SAD, Laboratoire de Recherches sur le Développement de l'Elevage, Corte, France, [3]Provincia di Grosseto, Grosseto, Italy.*

Genotypes of 44 300 SNP were obtained from the Ovine50K Beadchip for 94 Sarda (SW), 24 Corse (C) and 116 Nera di Arbus (PN). SW and C rams derived from the 2 AI centers, PN were sampled from 32 flocks. The degree of LD was calculated as r2. The extent of haplotypes sharing between breeds was taken as the correlation of r statistic for SNP at different intervals 0–10kb, 10–25kb, 25–50kb, 50–100kb, 100–250kb, 250–500kb, 500–1000kb. The genomic relationship matrix (Hayes et al., 2009) revealed a higher average coefficient within C (0.1839) than PN (0.1037) and SW (0.1289); average relationship between PN and the other 2 breeds was as high as within PN. LD decay with SNP distances was similar for SW and C and sharper than for PN. The shortest genetic distance resulted between PN and SW (Dr = 0.0163), the highest between C and SW (0.0410). C resulted genetically closer to PN (Dr = 0.0248) than SW. Genetic distance estimates were in

agreement with the level of haplotypes sharing: average correlation between r ranged from 0.7 to 0.3 for SW and lower than 0.2 for SNP 100 kb apart in both SW-C and PN-SW comparisons. To identify divergently selected regions, average difference in allelic frequencies (VAD) was calculated in overlapping sliding windows of 11 SNP (Hayes et al., 2009b). Work funded by the project VAGAL PO Italia-Francia Marittimo 2007-2013

Key Words: Ovine50KBeadchip, genetic distance

P3010 The use of medium-density SNP array for parentage verification and genetic traceability in cattle. Silvia Cenadelli,* Anna Maria Frana, Luca Mario Gandini, Graziella Bongioni, and Andrea Galli, *Istituto Sperimentale Italiano Lazzaro Spallanzani, Rivolta d'Adda (CR), Italy.*

The employ of SNPs as a simple, low cost and high density genetic markers has introduced the use of new techniques in parentage and traceability analysis. The aim of this work was to develop and validate a new medium density cattle SNPs chip using TaqMan probes in real time PCR based OpenArray system. For this purpose, 88 SNPs were selected from the list used in the ISAG Bovine Comparison Test 2011, and 40 SNPs were identified by screening the NCBI database, based on allele frequencies, chromosome position and validation status. Overall, a total of 128 SNPs, equally distributed troughout the bovine autosomes, was selected. We genotyped 356 bulls from 25 different breeds and allele frequencies was estimated. Among the tested markers, 120 were resulted polymorphic, with 35 of these showing allele frequencies between 0,46 and 0,54. The estimated identity of this sub set SNP panel power was 9.80×10^{-23}. Parentage exclusion probabilities, when both (p2) suspected parents' genotypes were known and when only one (p1) suspected parent was genotyped, were estimated as p2 = 0.9999 and p1 = 0.9998. The panel of SNPs reported in this study should provide useful tool to identify individuals, using a minimal set of markers. This highly informative SNPs chip could be useful for different applied research livestock project.

Key Words: SNP, cattle, traceability

P3011 Genotyping of the SNP AY428575.1:g.346G>A of the bovine *TCAP* gene by PCR-RFLP and its occurrence in Brazilian beef cattle. L. A. L. Chardulo*[1], B. O. Borges[3], A. Tamanaha[2], and R. A. Curi[2], *[1]Instituto de Biociências - UNESP, Botucatu, São Paulo, Brazil, [2]Faculdade de Medicina Veterinária e Zootecnia - UNESP, Botucatu, São Paulo, Brazil, [3]Faculdade de Ciências Agrárias e Veterinárias - UNESP, Jaboticabal, São Paulo, Brazil.*

Genetic studies have identified candidate genes to meat quality on beef cattle, including the titin-cap gene (*TCAP*). The objective of this research was genotyping the SNP AY428575.1:g.346G>A of the bovine *TCAP* gene by PCR-RFLP, and reports its use for the first time. Though the primers 5′GGAGTGAGCAGTCATCATGGC3′ and 5′AGAGGCAGCACCCGCTGGT3′ were acquired products of 517bp that digested with *Bts*CI resulted in the genotypes AA (177, 154, 128 and 58bp), AG (305, 177, 154, 128 and 58bp) and GG (305, 154 and 58bp). 118 Nelore (*Bos indicus*) and 8 Angus × Nelore (*B. taurus* × *B. indicus*) animals were genotyped. The use of the PCR-RFLP for the genotyping of the SNP has shown inexpensive and robust, which will greatly facilitate its analysis by basic laboratory when compared with the single-base extension method. There were slight variation in Nelore that had just one genotype AG and 117 genotypes GG. Differently, there were found 6 Angus × Nelore with genotype AG and 2 GG. These preliminary results suggest the worthlessness of SNP AY428575.1:g.346G>A of the bovine *TCAP* gene to association studies with traits of interest in Nelore breed and, probably, in the subspecie *B. indicus*. On the other hand, they show the viability of these studies with the crossbreed Angus × Nelore, with Angus and, possibly, to all subspecies *B. taurus.*

Key Words: *Bos indicus*, DNA, meat quality

P3012 ISAG recommended microsatellite marker analysis among 5 Korean native chicken breeds. Nu-Ri Choi,* Dong-Won Seo, M. D. Rashedul Hoque, Cheorun Jo, and Jun-Heon Lee, *Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea.*

The objective of this study was to determine genetic variation of 5 Korean native chicken breeds using 30 microsatellite markers, which were previously recommended by ISAG. The initial study indicated that 2 microsatellite markers, MCW0284 and LEI0192, were not amplified in these breeds and excluded for further analysis. Twenty-eight microsatellite markers were investigated in 88 birds from 5 Korean native chicken breeds, which were classified with feather colors (Gray, Black, Red, Yellow, and White). The identified mean number of alleles was 5.54 per locus. Also, the observed and expected heterozygosity values ranged from a minimum of 0.045 to a maximum of 0.898 and from 0.293 to 0.869, respectively. This result presented here will help the decision of conservation strategies in these native breeds. Also, the use of ISAG recommended microsatellite markers may indicate that the global comparison with other chicken breeds is possible.

Key Words: genetic variation, Korean native chicken, microsatellite marker

P3013 Global variation in copy number in the chicken genome. R. Crooijmans*[1], M. Fife[2], T.

Fitzgerald[3], C. Schmidt[4], H. Cheng[5], P. Kaiser[6,2], R. Redon[7,3], and M. Groenen[1], *[1]Animal Breeding and Genomics Centre, Wageningen University, Wageningen, The Netherlands, [2]Institute of Animal Health, Avian genomics group, Compton, Berkshire, UK, [3]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambrigde, UK, [4]Department of Animal & Food Sciences, University of Delaware, Newark DE, 19717, USA, [5]Avian Disease and Oncology Laboratory, East Lansing, MI, 48823-5338, USA, [6]The Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Scotland, UK, [7]L'institut du thorax, Inserm UMR915, Nantes Cedex 1, France.*

Although many analyses of genetic variation have focused on single nucleotide polymorphisms (SNPs), there is a growing appreciation for the roles of structural polymorphism as a cause for phenotypic variation. While the sizes of genetic variants range from a single base to whole chromosomes, historically only the extreme ends of the spectrum have been explored. DNA copy number variants (CNVs) lie between these 2 extremes, ranging in size from thousands to millions of bases. To study global CNV patterns in chicken we have sampled 60 samples from 15 diverse breeds, varying from experimental lines to commercial breeds. All samples have been compared with the single reference individual UCD001 - also used to generate the chicken genome assembly - with the Agilent chicken whole genome 244K array. In total 3234 CNVs are detected, covering 1573 CNVR, of which 1196 were losses and 377 gains. In the examined chicken the average genome coverage of CNVRs is 60Mb which represent almost 5.6% of the genome. As a result we have constructed the first global CNV map of the chicken genome, not only showing variations between breeds but also between individuals from the same breeds.

Key Words: chicken, CNV, CGH

P3014 Genome characterization of Quarter Horse using a high-density equine SNP array. R. A. Curi*[1], C. T. Meira[2], A. Tamanaha[1], J. A. II V. Silva[1], H. N. de Oliveira[2], and M. D. S. da Mota[1], *[1]Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brazil, [2]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

Designed to identify SNPs and genes that contribute to traits of interest in the major horse breeds raised today in the world, the Equine SNP50 BeadChip (Illumina, USA), represents a powerful platform for genetic studies. However, most of the 54,602 SNPs used for its development, have not been investigated in detail for almost all horse breeds in the world. The objective of this study was to investigate the distribution and density, in the 31 autosomal chromosomes of the species, of the 42,058 informative SNPs (MAF ≥0.05) that passed quality control of genotyping (cluster separation ≥0.3; call frequency ≥0.9; *P*-value ≥0.001 for EHW) in a sample of 184 Quarter Horse (120 of racing and 68 of cutting line). The largest number of informative SNPs was detected on chromosome 1 (3,502), the longest chromosome (ECA1) containing 186 Mb, and the smallest number on chromosome 31 (492), the shortest chromosome (ECA31) containing 25 Mb, indicating that the quality control of genotyping did not affect the uniformity of distribution of SNPs across the genome. With respect to genome coverage, the density of SNPs was 18.72 SNPs/Mb or one SNP at an interval of 53,419 bp. This density varied between chromosomes, ranging from 16.82 SNPs/Mb (ECA12: average of one SNP at an interval of 59,453 bp) to 19.91 SNPs/Mb (ECA16: average of SNP at an interval of 52,356 bp). Supported: FAPESP.

Key Words: horses, polymorphisms, DNA

P3015 Developing of method for routine identity testing using STR loci in *Accipiter gentilis*. Martina Safrova, Marketa Dajbychova,* Barbora Blahova, Katerina Saskova, and Katerina Stampachova, *Genomia s.r.o., Pilsen, Czech Republic.*

We built a panel of markers for DNA identity testing for *Accipiter gentilis*, species protected under CITES Appendix II. This panel was used to create a methodology to uniquely identify individuals and their mapping in the wild and to control rearing of offspring in captivity. Based on the comparison of published work, we chose eight STR loci for which we assumed a high genetic variability: Age10, Age9, Age7 (di repeat motif), Age5, Age11 (tri repeat motif), µAge1 (tetra repeat motif), Age4, Age2 (penta repeat motif). To validate the methodology we used a set of 70 samples from wild individuals collected in 2010 and 2011. Blood samples were preserved in ethanol. We analyzed using optimized two multiplex PCR reactions with fluorescently labeled primers. Size analysis of all eight STR markers was performed simultaneously in a single injection in capillary electrophoresis device. We have determined number of repeat units by direct sequencing, which allowed to create control sample and bin sets for routine DNA identity testing using Genemapper 4.0 software. We have created a methodology for routine DNA identity testing *Accipiter gentilis*. Methodology is used in Czech Republic for populations screening to determine genetic biodiversity of wild *Accipiter gentilis*.

Key Words: *Accipiter gentilis*, identity testing

P3016 Identification of allelic variants in melanocortin-1 receptor gene in llama (*Lama glama*). Maria S. Daverio,* Florencia di Rocco, and Lidia Vidal Rioja, *Instituto Multidisciplinario de Biologia Celular.*

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Genes responsible for coat color variation have been identified in a wide variety of mammals. Among them, MC1R (melanocortin-1 receptor), plays an essential role in pigmentation of the fiber. In llamas (*Lama glama*), segregation of coat color have been assessed by classical genetic studies, but molecular bases of coat color variation remain unknown. The aim of this study was to identify allelic variants in Argentinean llamas and determine if they are associated with different color patterns. The MC1R gene was amplified by PCR in 48 llamas, including 10 guanacos (*Lama guanicoe*) and then amplicons were purified and automatically sequenced. The llama MC1R gene has 3 alleles (MC1R*1; MC1R*2 and MC1R*3). The wild-type allele (MC1R*3) was observed in all color groups. MC1R*1 allele was not found in white llamas whereas the allele MC1R*2 was absent in red-brown with black face and trim phenotype. In contrast with data published by Feeley et.al. (2009) for alpacas, haplotype A82/T126/C901 was not associated to eumelanin production in llamas. Although direct correlation between MC1R alleles and eumelanic/pheomelanic patterns could not be established, significant allelic frequencies differences between red-brown with black face and white llamas suggest a possible role of MC1R polymorphisms in coat color variation.

Key Words: MC1R, *Lama glama*, coat color

P3017 Conservation of the fibrillin gene family: Understanding the origin of FBN3. M. R. Davis* and K. M. Summers, *The Roslin Institute, The University of Edinburgh, Edinburgh, Scotland, United Kingdom, EH25 9RG.*

Fibrillin is a major component of the 10nm microfibrils that provide strength and elasticity in the extra cellular matrix of connective tissue. There are 3 fibrillin genes (FBN1, FBN2 and FBN3), encoding fibrillin-1, -2 and -3 proteins, in humans, most mammals and other higher vertebrates. In addition, fibrillin-1 and -2 are thought to be involved in sequestering transforming growth factor β, through interactions with latent transforming growth factor β binding proteins, while interactions with fibrillin-3 are not confirmed. FBN1 and FBN2 mutations are associated with connective tissue disorders Marfan syndrome and congenital contractual arachnodactyly respectively. Both FBN1 and FBN2 mutation phenotypes have been further characterized through mouse models. FBN3 is expressed at low levels in human fetal and embryonic tissue and cell types. The gene is degenerate in rodents and fibrillin-1 or -2 may take over the functions of fibrillin-3 in mice. We analyzed conservation of fibrillin genes in vertebrates and identified transcripts in multiple mammals with conserved FBN3 genes.This study indicates that degeneration of the rodent gene postdates the separation of the rodent lineage. In addition, we have detected a region of sequence homology in the FBN3 promoter region among mammals which contains transcription factor binding motifs that will elucidate the regulation and function of the gene.

P3018 Genetic diversity and disease resistance of horses exposed to equine infectious anemia (EIA) in Argentina. S. Diaz*[1], S. A. Sadaba[1,3], C. M. Corbi-Botto[1], J. P. Liron[1], R. A. Lopez[2], M. H. Carino[1], E. E. Villegas-Castagnasso[1], G. Giovambattista[1], and P. Peral-Garcia[1], *[1]Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (IGEVET-CCT La Plata, CONICET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CP1900, La Plata, Buenos Aires, Argentina, [2]Departamento de Clínica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CP1900, La Plata, Buenos Aires, Argentina, [3]Cátedra de Nutrición, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata., CP1900, La Plata, Buenos Aires, Argentina.*

A well registered history of survival of a group of horses exposed to the equine infectious anemia virus (EIA), gave rise to questions concerning their genetic composition as regards of the possible resistance to the disease. For this purpose, whole blood samples were retrieved from 50 horses residing in Chaco province, Argentina, and fractionated for biochemical, serological and molecular analysis. EIA serological condition was assessed by the Coggins's test, and re-sampling of the same horses were performed in 3 different occasions throughout 2 years. Only 4 horses became seropositive during the time of the investigation, and no evidence of EIA acute infection or clinical symptoms were observed. DNA was isolated from whole blood and 15 molecular markers comprising horse STRs (ISAG panel), single nucleotide polymorphisms (SNPs) from candidate cytokines and the equine lymphocyte antigen (ELA), were typed by PCR-multiplex, PCR-Pyrosequencing and direct DNA sequencing methodologies. Population genetic analysis allowed describing the genetic profile of the horse sample, and polymorphic immune markers revealed elevated levels of polymorphism. To preliminary investigate the association to the EIA epidemiological condition, candidate cytokines SNPs (TNF-α, IL-12) and MHC class II alleles (DRA, DRB) were evaluated by odds ratio calculation, and values from 1.7 to 3.30 for some alleles, suggests that some polymorphisms of the cytokines and antigen presenting molecules could be related to the virus capability of infection and/or later immune response stages concerning the disease progression.

Key Words: polymorphism, immune genes, equine infectious anemia

P3019 Mitochondrial DNA sequencing detected additional variability in tench (*Tinca tinca* L.). R. Lo Presti[1], K. Kohlmann[2], P. Kersten[2], C. Lisa[1], and L. Di Stasio*[1], *[1]Dept. Animal Science, Turin University, Grugliasco, Italy, [2]Dept. Aquaculture and Ecophysiology, Leibniz Institute, Berlin, Germany.*

Previous RFLP studies on ND1, ND6, cytb and D-loop segments of mtDNA revealed the existence of 9 haplotypes, H1-H9, with H1 corresponding to the RefSeq. With the aim of analyzing in more detail the genetic variability in tench, the same 4 segments were sequenced in 14 individuals covering all the composite haplotypes found with the RFLP procedure. Eighty-four polymorphic sites were identified, 42 located in the coding regions and 42 in the control region. All the differences were single nucleotide substitutions, except for 3 indels found in the D-loop. Compared with the RFLP technique, the sequencing revealed additional variability, with the H2 haplotype subdivided into 4 different haplotypes (named H2a-H2d), so that a total of 12 composite haplotypes were found. Moreover, the sequencing showed that the H1 haplotype differed from the reference sequence for 3 base substitutions in the D-loop. The Neighbor-joining tree confirmed the existence of 2 deeply differentiated haplogroups, one including H1, H3, H4, H5 and H6 haplotypes (haplogroup A), and the other including H2a, H2b, H2c, H2d, H7, H8 and H9 haplotypes (haplogroup B). The Median-joining network, illustrating the evolutionary branching of the observed haplotypes and the potential connecting haplotypes, showed a star-like phylogeny for the haplogroup A and a less clear situation for the haplogroup B.

Key Words: tench, mtDNA, sequencing

P3020 Identification of shared epitopes in the light chain of avian immunoglobulins. Mateja Bancina, Ivanka Cizelj, Mojca Narat, Dusan Bencina, and Peter Dovc,* *University of Ljubljana, Biotechnical Faculty, Domzale, Slovenia.*

Immunoglobulins (Ig) play the essential role in the vertebrate immune defense system. In the phylum Aves, the Ig structures have been determined for several species at molecular level only. In the present study, we report the genomic DNA sequence encoding Ig light chain (LC), namely joining LC (jLC) and constant LC (cLC) regions in turkey (*Meleagris gallopavo*), ring-necked pheasant (*Phasianus colchicus*) and gray partridge (*Perdix perdix*). Their deduced amino acid (aa) sequences of jLC (13 aa) and cLC (103 aa) contain the same number of aa as homologous regions in chicken (*Gallus gallus*). However, comparison with chicken cLC revealed deletion of an asparagine (N 19) and extension for an additional Serine. The LC of *P. colchicus* is most similar to that of *G. gallus*, with 99% and 94% nucleotide sequence identity in jLC and cLC region, respectively. The N-terminal and internal sequences obtained by direct aa sequencing of *P. colchicus* LCs were identical to the chicken sequences. The high level of similarity between different avian species was also confirmed using monoclonal antibodies (3C10/F6 and CH31 recognizing chicken LC, which also reacted with LCs from 7 species belonging to the phylum Aves. The highest similarity was found among LCs of 3 genera of pheasants and *Tragopan melanocephalus*.

Key Words: immunoglobulin, light chain, avian species

P3021 Transcriptome-wide investigation of genomic imprinting in chicken. L. Fresard*[1], S. Leroux[1], D. Gourichon[2], M. San Cristobal[1], N. Marsaud[3], O. Bouchez[3], P. Dehais[1], C. Beaumont[4], T. Zerjal[5], P. Kaiser[6], S. Lagarrigue[7], A. Vignal[1], M. Morisson[1], and F. Pitel[1], *[1]INRA, ENVT, LGC, Castanet-Tolosan, France, [2]INRA, PEAT, Tours, France, [3]GeT-PlaGe, Genotoul, INRA Auzeville, Castanet-Tolosan, France, [4]INRA, URA, Tours, France, [5]INRA, GABI, Jouy-en-Josas, France, [6]The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, United Kingdom, [7]INRA, Agrocampus Ouest, PEGASE, Rennes, France.*

The question of evolution of imprinting in vertebrates and its existence in birds is evoked in the literature, but not yet definitely answered. Genomic imprinting is an epigenetic modification leading to parent-of-origin-specific expression of certain genes. It has been observed in eutherian mammals and marsupials, but not in birds. So far, the allelic expression of imprinted gene orthologs has been analyzed in the chicken, without any reliable evidence of imprinting. Several imprinted QTL have been found in poultry; as some of them may finally be considered as not relevant to genomic imprinting, others appeared to be consistent, when using appropriate animal design and methodology. Our main objectives are to detect genes for which variation in expression is observed according to the allele, either because of an allele-specific expression or a parent-of-origin dependent expression. We screened the entire genome for allele-specific differential expression on whole embryonic transcriptomes by using high-throughput sequencing. Two chicken lines were used, as inbred and as genetically distant as possible, to unquestionably identify the parental origin of each observed haplotype. Two families from 2 reciprocal crosses were produced and transcripts from 20 embryos (4.5 d) have been tagged and sequenced through 6 HiSeq2000 lanes. About 200 Gb have been generated and are under analysis.

Key Words: imprinting, chicken, RNAseq

P3022 Direct resequencing reveals further variability within the bovine casein genes. J. L. Gallinat*[1], S. Quanbari[2], C. Drögemüller[3], E. Pimentel[4], G. Thaller[1], and J. Tetens[1], *[1]Institute of Animal Breeding*

and Husbandry, Christian-Albrechts-University, Kiel, Germany, [2]Department of Animal Science, Georg-August-University, Göttingen, Germany, [3]Institute of Genetics, University of Bern, Bern, Switzerland, [4]Department of Animal Breeding, University of Kassel, Kassel, Germany.

In cattle, at least 39 variants of the 4 casein proteins (αS1-, β-, αS2- and κ-casein) have been described to date. Many of them are known to affect milk production traits, cheese making properties and the nutritive value of milk. So far, the majority of studies to explore genetic variability of bovine caseins considered European taurine breeds and applied electrophoretic techniques on protein level. This only allows the identification of variants that differ in their electric charge, molecular weight or isoelectric point. Here, we resequenced the open reading frames of the casein genes CSN1S1, CSN2, CSN1S2, CSN3, which are tightly linked on BTA6. We used 354 animals from 13 taurine and 4 indicine breeds. With this approach we identified 23 alleles including 5 new sequence variants. The new variants were only found in indicine breeds and in one local Iranian breed, which has been phenotypically classified as a taurine breed. Based on available SNP-chip data, however, we were able to assign it to the indicine breeds. Subsequent haplotype reconstructions using PHASE 2.1 revealed 90 different casein haplotypes. One of those was found to be restricted to taurine, while 2 were unique to indicine breeds. Specific indicine alleles/haplotypes were also identified in a few European taurine breeds indicating the introgression of indicine breeds into these populations.

Key Words: milk proteins, caseins, genetic variability

P3023 Selection, diversity and bottlenecks in Hanwoo cattle. C. Gondro*[1], G. Jang[2], and S. Lee[3], *[1]University of New England, Armidale, NSW, Australia, [2]National Institute of Animal Science, Pyeongchang, Gangwon, Korea, [3]National Institute of Animal Science, Suwon, Gyeonggi, Korea.*

Characterization of genetic diversity and evolutionary history provides a low level handle on population structure which can be used to make decisions on how these populations will be managed. This has immediate implications for natural populations in conservation programs but is also highly relevant for Agricultural species. We used 29,844 SNPs to estimate genetic variability in 3 subpopulations of Hanwoo cattle (Jeju Black, Brindle and Brown) and their genetic distances to 7 other breeds (Chinese Yeonbyun, Brahman and 5 European breeds). Jeju Black and Brindle are evidencing clear signs of island population effects with high FST = 0.06 between them and 0.03 with the continental Brown. This may lead to negative consequences and even endanger these subpopulations in the future. Eastern Asian breeds show a minor contribution (~2%) of Bos indicus to their genetic architecture. Brown Hanwoo and Yeonbyun are genetically highly related with the latter showing higher levels of diversity which may be due to lack of intense artificial selection. This makes Yeonbyun cattle worthy of attention since they could potentially act as proxies to the ancestral Hanwoo before selection. This close relationship can reduce noise and lead to more robust identification of recent signatures of selection due to breeding for production traits.

Key Words: genetic diversity, drift, Hanwoo

P3024 Concerted evolution of the keratin-associated protein-1 (KAP1) genes. H. Gong,* H. Zhou, and J. Hickford, *Lincoln University, Lincoln, New Zealand.*

Keratin-associated proteins (KAPs) are a structural component of hair and wool fibers, and form the matrix between the keratin intermediate filaments. Up to 27 KAP families have been reported, of which KAP1 is probably the best characterized and diverse family. While the KAP1 genes have been characterized in several species, the orthologs in many other species remain un-identified and the evolution of these genes is poorly understood. In this study, the KAP1 genes from cattle, horses, rabbits and elephants were identified in the respective genome sequences. Sequence analyses revealed that the coding regions of the specific KAP1 family members in each species were much more closely related to each other, than to the same family members in other species. In contrast, the 5′ and 3′ regions flanking the genes were clustered across species into specific family members. Unique strings of amino acid residues were shared between family members from within a given species, or at times between closely related species. Shared nucleotide polymorphisms were also found across family members in the coding region in sheep. This suggests that the coding region of the KAP1 genes has been subjected to concerted evolution and that gene conversion-like events may be the driving force that leads to homogenization of the KAP1 coding sequences.

Key Words: keratin-associated protein, concerted evolution

P3025 Identifying equine MHC haplotypes with microsatellites. K. T. Graves*[1], P. J. Henney[1], B. Mealy[2], E. Bailey[1], L. B. Daugherty[1], K. L. Davies[1], and R. Leach[1], *[1]University of Kentucky, [2]Washington State University.*

The major histocompatibility complex (MHC) plays an important role in regulation of the immune response and identification of MHC genes is important for many types of research, from infectious disease to cancer.

Equine MHC haplotypes were originally defined serologically but a system was recently devised to assess the MHC using haplotypes based on 5 linked microsatellite markers. This study examined the MHC microsatellite genotypes among serotyped and non-serotyped horses, including Arabian horses. A large number of new haplotypes were identified. The haplotypes previously identified for Arabian horses were not found in this study. There was good correspondence of ELA serotype with the COR110 microsatellite.

Key Words: MHC, haplotype, equine

P3026 Identification and screening for long deletions in BF1 gene of Silkie chicken. Wen-Juan Yang[1] and Jian-Lin Han*[1,2], *[1]CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100193, China, [2]International Livestock Research Institute (ILRI), PO Box 30709, Nairobi 00100, Kenya.*

Chicken has 2 copies of BF genes in MHC-B region, namely the weakly expressed BF1 and the predominantly expressed BF2. Previous studies and genomic DNA sequences of chicken MHC-B region show that inbred chicken lines carrying specific blood types had 3 forms of long deletions in the BF1 gene: normal functional BF1 in complete length (A), handicapped BF1 missing 269 bp including enhancer A in 5′-UTR (Ad) and pseudo-BF1 missing its nearly complete genomic DNA sequence (B). They define 6 genotypes of AA, AdAd, AAd, AB, AdB and BB. However, it is difficult to characterize duplicated BF genes due to their highly homologous genomic DNA sequences. In this study, we developed 2 sets of specific primers to identify the 3 forms of BF1 gene in Silkie chicken. The primers flanked the region of complete pseudo-BF1 gene and amplified a minimal 3511 bp long fragment. The results showed that all 3 forms of BF1 gene were present in this breed with an allelic frequency of B up to 0.618 followed by A as 0.220 and Ad as 0.0162, and genotype frequencies of BB as 0.352, AB and AdB as 0.265 each, AA and AAd as 0.059 each. It is expected that these PCR arrays can be applied to screen indigenous chickens with unknown and highly heterogonous MHC-B genotypes and generate quality DNA sequences for a better understanding of the function and interaction of duplicated BF genes.

Key Words: long deletion, MHC BF1, chicken

P3027 The use of single nucleotide polymorphism array data to analyse the genetic structure of a cattle herd. B. Harrison,* R. Bunch, R. McCulloch, P. Williams, W. Sim, N. Corbet, and W. Barendse, *CSIRO, St. Lucia, QLD, Australia.*

Genetic progress depends on accurate knowledge of the genetic composition of a population or herd including level of inbreeding and parentage. However, in most herds, especially in the developing world, the genetic relationships between animals may be uncertain. We genotyped 938 animals from a herd in Queensland using an Illumina Bovine SNP50 array. Animals in the study were of the Senepol, Belmont and Bonsmara breeds. We used principal components to separate animals into breed groups and found that precise principal component values were only achieved when a few thousand SNPs are used. We calculated the genome relationship matrix (GRM) between individuals and found that at least 3000 SNPs were required for accuracy. Approximately 19% of comparisons between individuals showed a relationship equivalent to sharing a great-grandparent. Approximately 8% of the individuals showed more than 10% inbreeding. We counted the tick burden on each animal several times and then used the GRM to calculate a heritability of tick burden of h^2 = 0.46 (±0.08). Our results suggest that a low density SNP array of several thousand elements will be extremely useful for herds where it is currently impossible to estimate basic statistics of heritability and the (co)-variance of traits.

Key Words: SNP, population, structure

P3028 Identification of SNPs with high-resolution genotyping in the MHC region in Korean native chicken. M. D. Rashedul Hoque*[1], Seung-Hwan Lee[2], Bertrand Bed'hom[3], Olympe Chazara[3], Kang-Nyeong Heo[4], Bo-Seok Kang[4], and Jun-Heon Lee[1], *[1]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea, [2]Hanwoo Experiment Station, National Institute of Animal Science, RDA, Pyeongchang, Korea, [3]INRA, UMR de Genetique Animale et Biologie Integrative, Jouy-en-Josas, France, [4]Poultry Science Division, National Institute of Animal Science, RDA, Cheonan, Korea.*

The major histocompatibility complex (MHC) is a genomic region containing immune related genes and has relatively high genetic variations compared with other regions. A total of 480 chicken samples from 12 chicken populations in Korea were investigated for the 77 SNPs in the chicken MHC region using Illumina GoldenGate genotyping assay. Based on the Fst distance value, unrooted neighbor-joining (NJ) and the unweighted pair-group method with arithmetic mean (UPGMA) phylogenetic trees were constructed among the breeds. Also, the population structure has been derived to characterize the Korean native chicken populations. The results indicated that 8 SNPs showed strong LD among the 77 SNPs. The haplotype information suggests that 16 haplotypes were observed and these haplotypes are sharing among the breeds. Our study indicated that the SNPs in the chicken MHC region can provide valuable information for the genetic variability among the chicken populations in Korea.

Key Words: Korean native chicken, MHC, SNP

P3029 Polymorphism of adipocyte fatty acid-binding protein gene (A-FABP) in Yak. J. Hu,* J. Cao, Y. Luo, S. Cheng, and X. Liu, *Faculty of Animal Sci-tech/Gansu Key Laboratory of Herbivorous Animal Biotechnology, Gansu Agricultural University, Lanzhou, Gansu, China.*

Adipocyte fatty acid-binding protein (A-FABP), play a pivotal role in regulating intracellular fat concentration and then affect intramuscular fat (IMF) in mammalian. So A-FABP was suggested as a candidate gene of IMF in some livestock and poultry. In this study, SNPs were investigated at A-FABP gene intron 3, exon4 and 3′-UTR in total 432 yaks (Gannan yak, Qinghai yak, and Tianzhu white yak) in China by PCR-SSCP. Five novel SSCP patterns, representing 5 different alleles A-E were identified in 3 yak populations and allele A was the most common allele (76–80%). With alignment of the bovine A-FABP gene sequences, 6 SNPs including c.4174–161G>A, c.4174–160T>C at intron 3 and synonymous mutation (c.4222A>G) at exon4 and c.*6G>A, c.*53 C>T, c.*94T>A at 3′-UTR were checked in yak. SNP c.*94T>A detected only in yak A-FABP represented one of genetic characteristics of yak that differ from bovine. Alleles distributed disproportionably among 3 yak populations, such as only alleles A-C were observed in Tianzhu white yak and alleles A-D in Qinghai yak. This may be related to geographical distribution and breeding methods of different yak populations. The mutations in amplified region of A-FABP gene would become a potential locus for genetic markers of meat quality traits as it showed moderate polymorphism with PIC in the range of 0.29–0.36 in yak.

Key Words: yak, A-FABP, SNPs

P3030 Sequence variants, haplotypes and combined genotypes in the NPM1 gene and their associations with growth traits. Yong-Zhen Huang*[1], Hua He[1], Jing Wang[1], Jia-Jie Sun[1], Zhuan-Jian Li[1], Xian-Yong Lan[1], Chu-Zhao Lei[1], Chun-Lei Zhang[2], and Hong Chen[1], *[1]Northwest A&F University, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China, [2]Xuzhou Normal University, Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu 221116, China.*

The nucleophosmin 1 gene (NPM1) encodes a multifunctional nucleolar phosphoprotein that plays a crucial role in cell growth and homeostasis. Seven sequence variants (SVs) were identified in the coding region of the bovine NPM1, five of which were in complete linkage disequilibrium. Eight different haplotypes were identified, of which two major haplotypes have a frequency of 23.2 and 20.4%. Three SVs were significantly associated with body weight in the Nanyang population as analyzed at different ages. No significant association was detected between 18 combined genotypes and body weight at five different ages. Our results suggest that some polymorphisms in NPM1 are associated with body weight at some ages, and may be used as a possible candidate for marker-assisted selection and management in beef cattle breeding program. This study was supported by the National 863 Program of China (Grant No. 2008AA101010), National Natural Science Foundation of China (Grant No. 30972080), National Key Technology R&D Program (Grant No. 2008ADB2B03-19), Keystone Project of Transfergene in China (Grant No. 2009ZX08009-157B, 2008ZX08007-002, and 2009ZX08007-005B-07), Program of National Beef Cattle Industrial Technology System (Grant No. CARS-38).

Key Words: cattle, combined genotypes, growth traits

P3031 Phylogenetic relationship among sheep breeds from Pakistan and India compared to Central Asia and Eastern Europe. T. Hussain*[1,2], A. Nadeem[1], A. Ali[1], M. Javed[1], H. Sadia[1], A. Wajid[1], S. A. Shah[1], K. Abbas[1], M. Al Abri[2], M. De Donato[2], S. O. Peters[2], I. G. Imumorin[2], and M. E. Babar[1], *[1]Institute of Biochemistry & Biotechnology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, [2]Dept. Animal Science, Cornell University, Ithaca, NY, USA, [3]IIBCA, Universidad de Oriente, Cumana, Venezuela.*

We analyzed CYTB sequence in 49 samples from 10 Pakistani sheep breeds: 5 of each of Awassi, Dumari, Kaghani, Karakul, Lohi, Salt Range, Shenwari, Thali, and 4 Kachi breed, and compared with published sequences from India (132), China (20), Turkey (31), Australia (2), Israel (1), wild sheep (9), and related species *Ovis vignei* and *O. ammon*. Analysis revealed presence of 2 major groups: the biggest containing most of the analyzed sequences which is also subdivided into 2, one contains only 2 Turkish sequences from Morkaraman breed and the other with most of sequences from different breeds and geographical origins, showing different degrees of differentiation. In this subgroup, a group of sequences from Pakistan (34), Turkey (4), Australia (2), China (3) and India (72) were almost identical among them and to 3 of the wild sheep. Other Pakistani sequences were distributed into 3 clusters closely related to Indian clusters. The other group was also divided into 2 subgroups, one with sequences from China (2) and Turkey (7) breeds and other with sequences from Turkey (2), China (1), Pakistan (1), Israel (1) and a wild sheep. This suggests some interbreeding among sheep breeds of Pakistan with breeds originated in other regions. These results will help in conservation of Pakistani sheep breeds and to plan genetic improvement programs.

Key Words: cytochrome b, sheep, phylogenetic

P3032 Phylogenetic re-analyzing of Iran's Jebeer based on cytochrome b sequence. M. R. Nassiri[1,2], M. Mahdavi[1], and A. Javadmanesh*[3], *[1]Department of Animal Science,Faculty of Agriculture, Ferdowsi University of Mashhad, PO Box 91775-1163, Mashhad, Khorasan, Iran, [2]Department of Agricultural and Animal Biotechnology, Institute of Biotechnology, Ferdowsi University of Mashhad, PO Box 91775-1163, Mashhad, Khorasan, Iran, [3]JS Davies Epigenetics and Genetics group, School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, Roseworthy SA 5371, Adelaide, South Australia, Australia.*

There are 3 species of Gazelle genus living in Iran: *Gazella gazella*, *Gazella subgutturosa* and Jebeer. Among them taxonomic position of the Jebeer is ambiguous. Jebeer lives mainly in central and southern desert areas of Iran. Some reports considered Jebeer to be conspecific with *Gazella gazella*. Also, others found it to be closer to *Gazella dorcas* and *Gazella bennetti*. The goal of this study was to analysis genetic and phylogenetic of cytochrome b region in Jebeer of Iran. In this regard 5 biological samples from Shirahmad's Wildlife Refuge of Sabzevar (Khorasan, Iran) were collected (36°5′6"N and 57°53′4"E). After DNA extraction, Cytochrome b region of mitochondrial DNA has been amplified and sequenced with ABI 3130 automated device. Sequenced fragments were compared with 190 sequences from *Gazella genus* that have been classified based on species with regard to homology and evolutionary divergence using MEGA v5, CLC Main Workbench v5.5 and BioEdit v7.1 software packages. Our results showed that among all *Gazelle* sp., Jebeer had the shortest distance (0.003) with *G. bennetti* and longest distances with *G. dorca* and his sister taxon *G. saudiya* that are equal to 0.073 and 0.074, respectively. These findings might underscore the necessity of establishing Jebeer as a *Gazella bennetti* species.

Key Words: mitochondrial DNA, Jebeer, Iran

P3033 The use of DNA to reveal a potentially deadly package! Two wildlife forensic case studies from the Australian Museum. R. N. Johnson*[1], A. G. King[1], C. Vockler[1], and G. M. Cooke[1,2], *[1]Australian Museum, Sydney, NSW, Australia, [2]University of NSW, Kensington, NSW, Australia.*

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.

Key Words: wildlife forensics, DNA-diagnostics, law enforcement

P3034 Sequence-based genotyping of the SLA class I and II loci in Asian wild boars. Woo-Young Jung*[1], Dong-Won Seo[1], Hyun-Tae Lim[2], Chak-Sum Ho[3], and Jun-Heon Lee[1], *[1]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea, [2]Department of Animal Science, College of Agriculture, Gyeongsang National University, Jinju, Korea, [3]Gift of Life Michigan, Histocompatibility Laboratory, 3861 Research Park Drive, Ann Arbor, MI, USA.*

The porcine MHC (major histocompatibility complex), called SLA (swine leukocyte antigen), has been known to play very significant roles in controlling immune responses to foreign infectious agents such as bacteria and virus. It has also been known to affect vaccine responsiveness. Knowledge of SLA diversity in domestic pigs is rapidly growing while it remains largely unknown in wild pigs. In this research, we investigated the 2 highly polymorphic SLA class I (SLA-1 and SLA-2) and 3 class II (DRB1, DQB1 and DQA) loci using the cDNA SBT (sequence-based typing) method in 4 Asian wild boars. We identified a total of 12 novel SLA alleles consisting of 7 class I (arbitrarily designated SLA-1*wy06, SLA-1*wy07, SLA-1*wy08, SLA-1*wy09, SLA-1*wy10, SLA-2*wy11 and SLA-2*wy12) and 5 class II (arbitrarily designated SLA-DRB1*wy01, DQB1*wy02, DQB1*wy03, DQA*wy04 and DQA*wy05) sequences. On the other hand, we also detected 4 class II alleles (DRB1*0102, DRB1*0401, DQB1*0601 and DQA*0301) which are frequently observed in the domestic pigs. This sharing of SLA alleles may indicate a very recent divergence between the domestic and wild pig populations in Asia. These results will provide valuable information for understanding the SLA allelic architecture in swine.

Key Words: MHC, SLA, Asian wild boar

P3035 Genetic markers on ECA20 associated with equine erythrocyte antigen A system. H. Kakoi,* T. Tozaki, H. Gawahara, I. Kijima-Suda, and M. Kurosawa, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

Monitoring of horse blood group A (EAA) system is critical to prevent neonatal isoerythrolysis. Due to the lack of enough amount of anti-serum, identification of responsible genes for EAA would be useful for the monitoring. Significant linkage between EAA and equine MHC mapped to ECA20 was reported. As a first step to find responsible genes for EAA, we try to identify genetic markers associated with EAA by using STRs and SNPs on ECA20. We phenotyped 1,607 thoroughbreds by EAA reagents, anti-Aa, -Ab and -Ac, and then selected 38 (phenotype A-) and 52 (phenotype Aab) horses. For the horse groups, 15 STRs within 20.29–29.24M of ECA20 were analyzed. Fine mapping was performed using 9 SNPs. One STR (ABGe8996, g.24396936–64) and 2 SNPs (g.24029308T>G and g.24257326A>C) indicated the most significant chi-squared probabilities for association with EAA phenotypes, respectively. However, 3 predictive genes located near the region including these markers, HFE, BTN2A2 and BTN1A1, showed lower association probabilities. In the analysis of thoroughbreds possessing other phenotypes and other breeds, genotype of g.24257326A>C corresponded with each phenotype, suggesting that this SNP is closely associated with EAA. The SNP is located in the cluster of putative genes homologous to butyrophilin gene family according to the NCBI/UCSC genome database. Further investigation of such a candidate region is needed.

Key Words: horse, EAA

P3036 Evaluation of genetic diversity in Japanese Black cattle population using SNP-MaP strategy. T. Kato*[1], H. Matsumoto[1], S. Sasazaki[1], T. Akiyama[2], M. Fukushima[2], E. Yoshida[2], T. Nomura[3], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan, [2]Northern Hyogo Prefecture Institute of Agriculture, Asago, Hyogo, Japan, [3]Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan.*

Closed Japanese Black cattle (JB) population in Hyogo prefecture, where well known Kobe beef has been produced, is highly inbred, and is required for maintaining genetic diversity. The aim of this study is to evaluate change of genetic diversity over the genome based on large numbers of SNP markers. We performed an SNP-MaP (single nucleotide polymorphism microarrays and pooling) analysis for 2 JB groups sampled in different times (2000 and 2010). Two sets of 96 individuals were analyzed by pooled genotyping on the bovineSNP50v2 Bead Chip. For each of SNPs, the effective population size (Ne) is calculated from difference of allelic frequency between the 2 groups. The Ne is also evaluated in each chromosome using 200-loci sliding window average differences (SWAD). The result shows that average Ne (14.6) over whole-genome SNP analysis is almost consistent with Ne (15.3) estimated by pedigree information. From analysis by SWAD, some regions show extremely low Ne on specific chromosomes. For example, the regions with low Ne (<5.0) are 67.5–77.4Mb on BTA2, and 3.9–4.8Mb on BTA20. The diversity on these chromosomal regions is decreased dramatically during the last 10 years. SNP-MaP gives detailed picture for monitoring diversity over the genome in closed population.

Key Words: Japanese Black cattle, SNP-MaP, diversity

P3037 Whole genome sequencing of 75 sheep for variant detection and design of an HD chip. J. Kijas*[1], K. C. Worley[2], R. A. Gibbs[2], J. Reid[2], F. Yu[2], S. L. Lee[2], Y. Wu[2], D. M. Munzy[2], S. McWilliam[1], J. Yu[1], H. D. Daetwyler[3], B. J. Hayes[3], R. Brauning[4], J. McEwan[4], The International Sheep Genomics Consortium[5], *[1]CSIRO Livestock Industries, Brisbane, QLD, Australia, [2]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA, [3]Department of Primary Industries, Melbourne, VIC, Australia, [4]Invermay Research Center, AgResearch, Mosgiel, New Zealand, [5]ISGC, www.sheephapmap.org.*

The International Sheep Genomics Consortium (ISGC) has embarked on a whole genome sequencing project which aims to identify approximately 50 million SNP and use them to design a high density genotyping platform. To initiate the experiment, animals were selected from 41 breeds originating in Africa, Asia, Europe and the Americas. Most animals were drawn from the recently completed ISGC HapMap experiment. Sequencing has been completed for 75 genomes using Illumina paired end reads and guided assemblies constructed using the sheep genome assembly version 3.0 as the reference. On average, 10-fold mapped read coverage was obtained for each genome. Variant detection was performed separately by multiple groups before an overlapping set of SNP were defined. Functional annotation was performed to sort variants into classes including intergenic, intronic, synonymous and non–synonymous. The design for a high density genotyping platform for the sheep research community will be presented.

Key Words: sheep, genome sequencing, HD chip

P3038 Genetic diversity of Ethiopian indigenous cattle for adaptation to local environments. Zewdu Edea and Kwan-Suk Kim,* *Chungbuk National Unversity, Cheongju, Chungbuk, Korea.*

The genetic diversity and the genetic merits of most of Ethiopian indigenous cattle populations is not yet well understood, exploited and known to decision makers and development actors. As a result, some of the indigenous cattle populations are already extinct and endangered while the risk status of many of them is unknown. This study is a collaborative project between Korean Government, Ethiopian Institute of Agricultural Research and ILRI designed with the objectives to estimate genetic diversity using high density panel of SNP markers and test the hypothesis that adaptability can be detected at the DNA level by linking molecular data with environmental variables. A total of 254 individual nasal samples were collected from the following indigenous cattle populations: Borana (50), Arsi (52), Horro (50) and Danakil (52) inhabiting in different agro-ecologies, production environments and raised for different production objectives by diverse ethnic groups. DNA samples will be genotyped using the Illumina Bovine SNP50 Bead Chip. Observed and expected heterozygosities, estimate of FIS per population, FST per locus and population pairs will be calculated. Univariate logistic regression analysis will be carried out to test for association between allelic frequencies at marker loci and environmental variables.

Key Words: Ethiopian cattle, genetic diversity, SNP

P3039 Assessing the origins of Norfolk Island feral chickens using mitochondrial DNA. S. Langford[1], B. Baskerville[2], S. Ho[3], S. Kraitsek*[1], and J. Gongora[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW 2006, Australia, [2]Kingston & Arthur's Vale Historic Area, Norfolk Island Government, Australia, [3]School of Biological Sciences, University of Sydney, Sydney, NSW 2006, Australia.*

European chickens were introduced into Norfolk Island by Europeans after their arrival in the 18th Century. Some chickens escaped and/or were abandoned to establish feral populations. Although some historical data suggest that Pacific people visited Norfolk Island between the time of Captain Cook (1774) and colonisation from Australia (1788), there is no evidence of chickens predating the presence of European people. With few records of chicken introductions into this island, there is ongoing debate about their origins. To study the origin of these feral populations we generated partial mitochondrial DNA control region sequences from 75 Norfolk Island feral and Australian mainland domestic chickens. We compared these with a database of ~3,000 published chicken sequences from across the world. Preliminary median-joining network analysis shows that Norfolk Island chickens cluster into 2 documented major mitochondrial haplogroups corresponding to (i) European, Indian subcontinental, and Southeast Asian chickens and (ii) Indonesian, Japanese, and Chinese chickens. The former group is consistent with European introductions, whereas 2 major scenarios can be considered for the latter group: either early European settlers introduced chickens from Asian sources, or chickens were brought in from Melanesia after 1866, when numerous Melanesian students came to the Norfolk Island.

Key Words: chicken, Norfolk Island, mtDNA

P3040 Defining SLA class I and II combined haplotypes using genomic DNA-based high-resolution genotyping. Minhthong Le,* Hojun Choi, Kyooyeol Lee, Kyung-Tae Kim, and Chankyu Park, *Department of Animal Biotechnology, Konkuk University, Hwayang-dong, Kwangjin-gu, Seoul, South Korea.*

The swine leukocyte antigen (SLA) is one of the most polymorphic regions of the pig genome, and not only is the important determinant of immune responses but affecting other phenotypic characteristics. We have successfully developed high resolution genotyping methods using genomic PCR and direct sequencing for 6 SLA class I and II loci including SLA-1, -2, -3, DRB1, DQB1 and DQA, respectively. Haplotype analysis from 270 animals using the genomic sequence-based typing (GSBT) of 6 SLA loci allowed us to identify 37 SLA class I (SLA-1, -2 and -3) and 34 SLA class II (DRB1, DQB1 and DQA) haplotypes. 34 and 25 haplotypes were new for SLA class I and II, respectively. To investigate the patterns of linkage disequilibrium (LD) between SLA class I and II genes which are located separately across the centromere of pig chromosome 7, we combined and analyzed the genotyping results of SLA class I and II from 225 pedigreed pigs. We identified 84 SLA class I and class II combined haplotypes. Our result shows that there is strong LD between SLA class I and II regions which is consistent to previous report, and demonstrate that the genetic analysis of pig MHC using SLA GSBT method using field samples is extremely useful to study MHC polymorphisms and could be utilized to evaluate their possible association with immune responses to specific antigens or infectious pathogens.

Key Words: GSBT, haplotype, SLA

P3041 The swine leukocyte antigen (SLA) nomenclature system: Current status after 10 years of its establishment. Chak-Sum Ho[1], Asako Ando[2], Sabine Essler[3], Claire Rogel-Gaillard[4], Jun-Heon Lee*[5], Joan Lunney[6], Lawrence Schook[7], and Douglas Smith[8], *[1]Gift of Life Michigan, Ann Arbor, MI, USA, [2]Tokai University School of Medicine, Isehara, Kanagawa, Japan, [3]University of Veterinary Medicine Vienna, Vienna, Austria, [4]INRA, Jouy-en-Josas, France, [5]Chungnam National University, Daejeon, Korea, [6]USDA, Beltsville, MD, USA, [7]University of Illinois, Urbana, IL, USA, [8]Ann Arbor, MI, USA.*

The SLA Nomenclature Committee was established in 2002 at the 28th ISAG Annual Conference in Göttingen, Germany. It currently comprises a total of 8 members from 3 continents, and its primary functions 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 which is the repository for maintaining a list of all recognized SLA genes and their allelic sequences. To date, there are 131 class I (SLA-1, SLA-2, SLA-3, SLA-6) and 174 class II (DRA, DRB1, DQA, DQB1, DMA) alleles officially designated. There are also 31 class I (SLA-1–3-2–6) and 26 class II (DRA-DRB1-DQA-DQB1) high-resolution (allele level) haplotypes designated, while designation of low-resolution (group level) haplotypes is in progress. The SLA system is among the most well characterized MHC systems in non-primate species. Continuous efforts on studying of SLA diversity in various pig populations will further our understanding of the allelic architecture and polymorphism of the SLA system, which may ultimately facilitate the research on swine immunology, vaccine development, and the use of swine as a large biomedical animal model.

Key Words: MHC, SLA, nomenclature

P3042 Withdrawn

P3043 Genetic and phenotypic fine characterizations of French porcine reference populations. M. Sancristobal[1], S. Boitard[1], M. I. Fariello-Rico[1], H. Gilbert[1,5], B. Laurent[4], L. Liaubet*[1], A. Paris[6], C. Rogel-Gaillard[5], F. Rohart[1,4], J. Riquet[1], B. Servin[1], N. Villa-Vialaneix[7], M. P. Sanchez[5], T. Tribout[5], D. Milan[1], *[1]INRA, UMR444 Cellular Genetics, F-31326 Castanet Tolosan, France, [2]INRA, UE450 Unité Expérimentale de Testage de Porcs, F-35653 LE RHEU, France, [3]INRA, UMR1331 ToxAlim, F-31027 Toulouse, France, [4]CNRS, UPS, UMR5219 Institut de Mathématiques de Toulouse & INSA Toulouse, F-31077 Toulouse, France, [5]INRA, UMR1313 GABI, F-78352 Jouy-en-Josas, France, [6]INRA, UR1204 Food Risk Analysis Methodologies, Mét@risk, F-75231 Paris, France, [7]Université Paris I Panthéon-Sorbonne, EA4543 SAMM, F-75634 Paris, France.*

Fine study of the porcine genome. The porcine industry has to face new societal stakes, and propose a more sustainable production. Genetics is one of the control levers. The objective of the DéLiSus project (ANR-07-GANI-001) was, based on recent developments in genomics, to improve our knowledge on the porcine genome structure, on genetic mechanisms controlling traits, but also connected to the development of new systems of production (such as food efficiency, quality of meat). High throughput genotyping and phenotyping strategy laid on accumulating information on a large number of animals of various porcine breeds, from the gene to the phenotype, and looking for the links between these informations. Thereafter the main results: (1) Large-sized databases with genotypes, transcriptomic, metabolomic and phenotypic data; (2) Contribution to the improvement of the assembly of the porcine genome and to the mapping of 60K SNP; (3) QTL detection (25 phenotypes); (4) Revealing the genetic variability clearly structured; (5) New methodology to detect footprint of selection revealing of genomic regions presenting a strong local decline of variability; (6) Metabolomic profiles allow predicting phenotypes of interest, such as the rate of muscle; and (7)Differential expression between races of numerous genes in the post-mortem muscle (among 44K).

Key Words: diversity, genomics, haplotype

P3044 Diversity studies of SLA haplotypes by targeted high-throughput resequencing. Núria Mach*[1], Marco Moroldo[1,2], Sylvain Marthey[1,2], Asako Ando[3], Jun-Heon Lee[4], Chak-Sum Ho[5], Joan Lunney[6], Jordi Estellé[1], and Claire Rogel-Gaillard[1], *[1]INRA, Laboratory of Animal Genetics and Integrative Biology, F-78350 Jouy-en-Josas, France, [2]INRA, Biological Resources Centre for livestock genomics, CRB GADIE, Laboratory of Animal Genetics and Integrative Biology, F-78350 Jouy-en-Josas, France, [3]Department of Molecular Life Science, Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan, [4]Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Korea, [5]Histocompatibility Laboratory, Gift of Life Michigan, Ann Arbor, MI, USA, [6]Animal Parasitic Diseases Laboratory, BARC, ARS, USDA, Beltsville, MD, USA.*

The swine MHC (SLA complex) is a gene-dense region that plays a key role in self versus non-self immune recognition. It consists of 3 major gene clusters (class I, III and II), and the diversity of the SLA class I and II genes has a fundamental role in susceptibility to infectious diseases. Characterizing and maintaining SLA diversity at the population level is crucial to maximize the capacity to face a large range of pathogens. Sequencing and annotation of the entire Hp-1a.1 haplotype, and of 2 class I haplotypes Hp-28.0 and Hp-62.0, revealed that haplotype variability relies also on copy number variation. Since genetic variation of other haplotypes is largely unknown we decided to analyze 11 SLA haplotypes by studying the genetic variability of the whole SLA region. A 385K solid phase array targeting a region of 2.8 Mb based on the Hp-1a.1 haplotype

without DNA repeat sequences was designed to capture a region of 2.4 Mb for re-sequencing. The average values of coverage and specificity (% reads on target sequences) were 130X and 30%, respectively, whereas the number of polymorphisms was 11,330 ± 393. Our results show that despite a decrease in hybridization efficiency in the most polymorphic exons of SLA genes, the targeted resequencing approach is appropriate and effective to study SLA diversity and will contribute to refine reference sequence database on SLA haplotypes.

Key Words: SLA, diversity, resequencing

P3045 The aggrecan (ACAN) gene polymorphisms in horses of different morphological types. M. Mackowski,* A. Klimowska, G. Cholewinski, and J. Cieslak, *Poznan University of Life Sciences. Department of Horse Breeding. Horse Genetic Markers Laboratory, Poznan, Poland.*

Aggrecan is a major proteoglycan found in the extracellular matrix of cartilage. Dwarfism in Dexter cattle is caused by mutations in the ACAN gene. Therefore, this gene is considered as strong candidate for dwarfism in horses. It is well known that horse breeds present a variety of morphological phenotypes, but the genetic background of this variability remains unclear. The aim of this study was an analysis of the ACAN gene, including screening for polymorphism in selected gene fragments and an evaluation of genotypes interbreed distribution. Searching for polymorphism was performed by sequencing in a panel of 96 unrelated horses from 12 breeds: Thoroughbred, Arabian, Wielkopolski, Silesian, Percheron, Polish Heavy Horse, Haflinger, Norwegian Fjord, Hucul, Polish Primitive Horse, Welsh and Shetland Pony. Altogether 5 polymorphisms were identified. Two of them (g.94349700T>C and g.94376486G>C) were already described in EquCab 2.0 database, whereas remaining 3 SNPs (g.2406G>A, g.5379C>T and g.5775C>T) turned out previously unknown. Initial results showed uneven distribution of identified SNPs among horse breeds representing different morphological types. Further analyses will be focused on their putative association with selected phenotypic traits of horses. Study was funded by the Polish Ministry of Science and Higher Education. Grant: N N311 241438.

Key Words: horse, polymorphism, morphological traits

P3046 Genetic diversity and structure in goat analyzed by newly developed SNPs. H. Mannen,* B.Z. Lin, T. Kato, M. Kaneda, H. Matsumoto, and S. Sasazaki, *Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan.*

A series of novel single nucleotide polymorphisms (SNPs) within intron region were detected in goat (*Capra hircus*) by utilizing genomic information of cattle and sheep, due to poor available genomic information of goat at present. A total of 65 high polymorphic RFLP-SNP markers, which preconsidered no linkage with each other, were developed. The orthologous loci were covered for all autosomes of cattle. Using the novel SNP markers, genetic analyses of genetic diversity and structure were carried out for 10 goat populations collected from across 9 Asian countries (Cambodia, India, Mongolia, Laos, Myanmar, Vietnam, Bhutan, Bangladesh and Philippine). Analyses of PCA and Structure illustrated well separation, admixture and relationships among populations: -Bangladesh, Bhutan-, -Mongol, India- and -Myanmar, Laos, Vietnam, Cambodia (Plane area)- were clustered. In contrast, Philippine and Cambodia (Mountain area) populations were separated from other clusters. The results clearly reveal genetic diversity, phylogenetic relationship and population structure for the goat populations. Our data also demonstrates the strongly significant correlation between genetic diversity and geographic distance from domestication center, and well reflects the migration history of Southeast Asian goat. The developed markers could probably be applied to non-Asian goat.

Key Words: goat, genetic diversity, genetic structure

P3047 Compilation and analysis of high-density SNP and genomic sequence data for investigation of livestock diversity. J. A. Lenstra[1], P. Boettcher[2], and P. Ajmone Marsan*[3], *[1]Utrecht University, Utrecht, the Netherlands, [2]FAO-AGAG, Rome, Italy, [3]Catholic University of the Sacred Heart, Piacenza, Italy.*

High-density SNP analysis and complete genomic sequences renew investigations into livestock genetic diversity by providing reproducible and standardized genotypes, accurate individual genetic distances, localization or breed-specific adaptive variation and feasibility of sophisticated algorithms. However, published HD SNP studies so far cover a small part of the genetic resources available. Here we address 2 obstacles to a global-wide systematic comparison of livestock breeds. (1) For the major farm species international microsatellite-based studies used to attract funding. In contrast, there is little coordination of the several individual laboratories that generate HD-SNP or genomic data with various purposes: comparison of local breeds, whole-genome association studies, breeding value estimations, etc. These independent projects are now called, under the auspices of the FAO and ISAG, to collaborate in a broad study of genetic diversity. (2) The high information content of the genomic data sets requires new software for data handling and analysis. Large-scale investigations of genetic diversity will benefit from a sharing of experience with available general-purpose and specialized programs and from a further development of comprehensive program packages.

Key Words: genetic diversity, SNP, genomic sequence

P3048 Polymorphism information content as a measure of the usefulness of microsatellites for genetic analysis. Leroy H. McClean,* *The University of the West Indies, Cave Hill, St. Michael, Barbados.*

The mean statistical values for all of the genetic parameters measured at the 19 microsatellite loci in the Barbados Blackbelly sheep were all significantly different in the 3 populations ($P < 0.001$) that were investigated, thus providing evidence that analysis at microsatellite loci is a reliable method for differentiation between populations of Barbados Blackbelly sheep. Comparison of the extent to which gene diversity was influenced by polymorphism, heterozygosity and polymorphism information content (PIC) indicates that, of these parameters, PIC is the most reliable means of determining the usefulness of a microsatellite for genetic analysis. The PIC values of 18 of the 19 microsatellite loci investigated were greater than 0.5 and are therefore highly informative for the genetic analysis of Barbados Blackbelly sheep, while the values at 4 loci were greater than 0.25 but less than 0.5 and are therefore reasonably informative. At locus MAF214 the PIC value of 0.111 indicates that this locus is not useful for the genetic analysis of the BBB sheep breed. However, this locus is of interest since the BBB sheep analyzed were also fully inbred at this locus (f = 1) and homozygous in the 3 populations that were investigated. Therefore, of the 19 polymorphic microsatellite loci investigated, 18 are useful for the genetic identification of purebred Barbados Blackbelly sheep and for the differentiation and identification of individuals in different populations of the breed.

Key Words: polymorphism information content (PIC), hair sheep, microsatellites

P3049 Quality control in genotype data using Equine SNP50 BeadChip for genomic studies in Quarter Horse. C. T. Meira*[2], M. D. S. da Mota[1], N. A. R. Beltran[2], J. A. II V. Silva[1], H. N. de Oliveira[2], and R. A. Curi[1], *[1]Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brazil, [2]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

The quality genotype data for 188 equine Quarter Horses (120 racing and 68 cutting line) and 54,602 SNPs, genotyped with the Illumina Equine SNP50 BeadChip was investigated using the Genome Studio program (Illumina, USA). The filtering procedures are important to reduce computational effort, the number of false results, and to improve precision of the estimates of the remaining polymorphisms. Animals with call rate <0.95, heterozygosity ± 3 standard deviations from the mean, and errors in gender estimation were excluded. Agreement between 4 replicates and parentage concordance between 4 stallion/progeny and 3 stallion/mare/progeny pairs were evaluated. With respect to the quality of SNP genotypes, SNPs located on the X chromosome, with low genotyping quality (cluster separation <0.3), call rate <0.9, MAF <0.05, and Hardy-Weinberg p-value <0.001 were excluded. Quality control of individual genotype data led to the exclusion of 4 cutting animals from the sample because of low call rate. Samples genotyped in duplicate showed agreement ≥99.8%. Parentage concordance between stallion/progeny and stallion/mare/progeny pairs was very high (0.998 to 0.999). Considering the whole population (n = 184) that passed quality control, 12,544 SNPs were excluded by the filtering process and 42,058 remained for further analysis. Supported: FAPESP.

Key Words: polymorphisms, DNA, horses

P3050 Evaluation of genetic diversity and population structure of Ryukyu wild boar (RWB) on Iriomote Island, Japan. K. Murakami*[1], S. Watanabe[2], and Y. Mizoguchi[1], *[1]Graduate school of Agriculture, Meiji University, Kawasaki, Kanagawa, Japan, [2]Tropical Biosphere Research Center, University of Rkyukyus, Taketomi-cho, Okinawa, Japan.*

In this study we evaluate the genetic diversity and population structures of RWB on Iriomote Island in Japan. We used a genetically based approach using a combination of mitochondrial DNA (mtDNA) D-loop region polymorphisms and 24 microsatellite (MS) markers. RWB samples (n = 122) were examined with reference to 66 European and Asian domestic pigs. mtDNA variants (part of the D-loop region; 596 bp) were utilized to develop a phylogenetic tree using the neighbor-joining method. We identified 25 distinct haplotypes in the RWB population. The phylogenetic data were divided into 2 branches, one representing the RWB group and the other the domestic lineage. Interestingly, 9 of the 122 RWBs (0.7%) belonged to the domestic lineage. The values of heterozygosity and the mean number of alleles of the RWB population were lower than that of the European domestic pigs based on the polymorphisms of the 24 MS. The index of the FIS in the RWB population indicated that this population was more inbred than the domestic pigs ($P < 0.05$). The clustering pattern observed in the STRUCTURE analysis using the 24 MS showed 11 RWBs including the domestic cluster; this was similar to the 9 RWBs shown to belong to the domestic lineage in the mtDNA analysis. These results suggest that gene flow could have occurred from domestic pigs to RWB and that the RWB population has low genetic variation.

P3051 A genome-wide study of selection signatures in five cattle breeds. R. Negrini*[1], P. Ajmone Marsan[1], E. L. Nicolazzi[1], L. Bomba[1], N. Bacciu[1], M. Milanesi[1],

G. Mancini[2], L. Pariset[3], A. Valentini[3], and A. Nardone[3], *[1]Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy, [2]CASPUR, Roma, Italy, [3]DIBAF, Università della Tuscia, Viterbo, Italy.*

The availability of genome-wide catalogs of genetic variation permits the genome wide investigation of within and across breed signatures of past and recent selection. In total 3220 animals from Italian Holstein (1088), Italian Brown (775), Simmenthal (493), Marchigiana (485) and Piedmontese (379) breeds were genotyped with the Illumina 54K SNPchip in the frame of the Italian livestock genomic project "SelMol." SNP datasets were edited and genotypes phased using the fastPHASE program. To identify genomic regions under selection, we scanned individual breed datasets, searching core haplotypes having high frequency and high relative extended homozygosity (rEHH). Significant core haplotypes were aligned across breeds to identify those specific for an individual breed, for dairy/beef production, or shared by all breeds. Bioinformatics analysis discovered genes mapping in these genomic regions. We hypothesize that genes subjected to natural and/or anthropogenic selection in the past still carry useful variation for modern cattle breeding.

Key Words: selection signature, SNP, cattle

P3052 Genealogical and genetic analysis of the "Paso" Colombian Creole horse population census. M. A. Novoa* and E. Bernal, *Genética Animal de Colombia Ltda., Bogotá, Cundinamarca, Colombia.*

The study of the zoogenetic resources based on both genealogical and genotypic data are crucial to understand the genetic dynamics of the populations and to identify potential threats. In this study we analyze both data (near 88,000 microsatellites genotypic data and 172,000 genealogical registers) of the census of the "Paso" Colombian Creole horse populations - CCC as the first report based on census of a large horse population. The genealogical analysis showed low levels of genetic diversity and high levels of inbreeding corresponding to a small effective size, which is below the recommended value by FAO, in particular at "Paso Fino," which threatens the population viability in terms of genetic erosion. The genetic structure analysis established the presence of significant distinct genetic groups among the populations of CCC. Besides, there are genetic differences between the last 2 generations analyzed, which allows to hypothesize the speed of evolutionary changes in these populations. Therefore, these results suggest the importance in making decisions, at administrative and breeding level, on the control and monitoring of inbred mating in the CCC, to conserve the genetic diversity as base to carry out genetic improvement programs in a sustainable manner, and finally to avoid a possible inbreeding depression process.

Key Words: equine genetics, inbreeding depression, Creole breeds

P3053 Finding selection sweeps through variation in linkage disequilibrium of taurine and indicine cattle. A. M. Perez O'Brien*[1], Y. T. Utsunomiya[2], J. F. Garcia[2], J. McEwan[4], C. P. VanTassell[3], T. Sonstegard[3], and J. Sölkner[1], *[1]University of Natural Resources and Life Sciences, Vienna, Austria, [2]São Paulo State University, Araçatuba, Brazil, [3]USDA - ARS, Beltsville, Maryland, USA, [4]AgResearch Invermay, Mosgiel, New Zealand.*

Selection pressure on genes linked to favorable phenotypes decreases the polymorphism of the regions associated until reaching fixation of the desired genes, and sweeps nearby nucleotides in close regions due to strong linkage disequilibrium (LD). The process leaves a Signature of Selection (SS) identifiable by its high monomorphism and a strong LD pattern. This work aimed to assess SS by comparison of genome-wide variations in regional LD patterns between Indicine (IND) and Taurine (TAU) cattle used for dairy (DA) and beef (BE) production using the varLD methodology in pairs of breeds to look for differences between TAU/IND, and DA/BE breeds. Four breeds were included in the analysis (Nelore, Gir, Angus, and Brown Swiss) representing a DA and BE breed for IND and TAU subpopulations respectively. Approximately 30 males for each breed were genotyped with the Illumina Bovine HD Beadchip. The results show important differences in LD patterns in newly and previously reported genomic regions, validating the approach and suggesting interesting regions for further evaluation.

Key Words: signatures of selection, linkage disequilibrium variation, indicine cattle

P3054 Development of Y-chromosomal microsatellite markers in Japanese macaque (*Macaca fuscata*) population of Fukushima. Toshinori Omi*[1], Masayuki Shito[1], Chihiro Udagawa[1], Naomi Tada[1], Young Hwa Chon[1], Kazuhiko Ochiai[1], Atsushi Sakamoto[2], Shuichi Tsuchida[1], and Shinichi Hayama[1], *[1]Nippon Veterinary and Life Science University, Musashisakai, Tokyo, Japan, [2]Jichi Medical School, Shimotsuke, Tochigi, Japan.*

The Japanese macaque (*Macaca fuscata*) is known to represent the current northernmost distribution of non-human primates in the world. To improve the genetic features of the Japanese macaque, we developed of Y-chromosomal microsatellite markers using the DNA samples collected in Fukushima population. We have here identified 10 male-specific loci for Japanese

macaque from 82 selected human Y-chromosomal microsatellites referred to the published studies. Polymorphism was detected at 6 loci in the first panel consisted with 23 animals from 23 subpopulations. In the second panel of 163 animals from 23 subpopulations, we detected the 23 alleles and 28 haplotypes in 6 loci. The haplotype diversity of Y-chromosomal microsatellites was 0.925 in 163 animals. These results showed that the developed Y-chromosomal microsatellites makers would be valuable for investigation of the genetic features of the Fukushima population before or after of earthquake, tsunami and Fukushima nuclear crisis.

Key Words: Y-chromosomal, microsatellites, Japanese macaque

P3055 Leptin and leptin receptor gene expression profiles across different porcine tissues. D. Pérez-Montarelo*[1], A. Fernández[1], J. M. Folch[2], R. Pena[3], C. Rodríguez[1], L. Silió[1], C. Óvilo[1], and A. I. Fernández[1], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain, [2]Universitat Autònoma de Barcelona, Barcelona, Spain, [3]Institut de Recerca i Tecnologia Agroalimentàries, Lleida, Spain.*

The leptin hormone drives food intake and energy expenditure through the interaction with its receptor (LEPR). Despite their relevant role in porcine growth and fatness, their expression profiles have not been characterized so far in pig. In the present study we analyzed by Q-PCR the expression levels of the LEP gene and different isoforms of the LEPR gene across 4 porcine tissues: backfat, liver, longissimus dorsi and diaphragm muscles. Samples from 20 males of an (Iberian × Landrace) × Landrace backcross were analyzed. The primer pairs were designed between exons 2 and 3 of the LEP gene, and between exons 8 and 9 for the short isoforms and exons 19 and 20 for the long isoform of the LEPR gene. Q-PCR reactions were carried out in a LightCycler device, using SYBR Green. Two housekeeping genes (B2M and ACTB) were used for normalization. Significant differential expression of LEP gene was detected between tissues. Backfat LEP expression was around 100 times higher than in the other tissues. In contrast, liver showed the highest short LEPR isoforms expression, > 10-fold higher than in the other tissues. The long LEPR isoform showed very low, almost undetectable, expression in all the analyzed tissues. Moreover, we investigated their expression differences conditional on the genotypes for the LEPg.1387C>T and LEPRc.1987C>T polymorphisms, previously associated with porcine growth and body composition. We observed LEP expression differences in backfat conditional on LEPg.1387C>T genotype (C > T). As well, short LEPR isoforms expression differences conditional on LEPRc.1987C>T genotype were observed in liver (T < C).

Key Words: LEP, LEPR, expression

P3056 Genome-wide characterization of Italian ovine biodiversity. E. Ciani[1], F. M. Sarti[2], F. Napolitano[3], A. Carta[4], D. Matassino[5], P. Crepaldi[6], R. Ciampolini[7], S. Bordonaro[8], P. Modesto[9], N. P. P. Macciotta[10], P. Ajmone Marsan[11], B. Portolano[12], and F. Pilla*[13], *[1]Dipartimento Fisiologia Generale Ambientale Uniba, Bari (BA) Italy, [2]Dipartimento Biologia Applicata Unipg, Perugia (PG) Italy, [3]Consiglio Ricerca Agricoltura, Centro Produzione Carni e Miglioramento Genetico, Monterotondo Scalo, Roma, Italy, [4]DiRPA – AGRIS, Olmedo (SS) Italy, [5]ConsDABI, San Giorgio del Sannio, Benevento, Italy, [6]Università degli Studi di Milano, Milano, (MI), Italy, [7]Dipartimento di Produzioni Animali, Pisa (PI) Italy, [8]DACPA-Unict, Catania (CT) Italy, [9]Istituto Zooprofilattico Sperimentale PLV, Torino (TO) Italy, [10]Dipartimento Agraria-Uniss, Sassari (SS) Italy, [11]Istituto Zootecnia-Unicatt, Piacenza (PC) Italy, [12]Demetra-Unipa, Palermo (PA) Italy, 13AAA Unimol, Campobasso (CB) Italy.*

Genome-wide SNP panels provide an affordable tool to assess within-breed genetic diversity and between breed relationships, also among closely related populations. A set of 496 animals, representative of 20 local and cosmopolite sheep breeds from different geographic and agro-ecological areas of Italy were genotyped with the Illumina Ovine SNP50 BeadChip. On average, 97% of the loci were polymorphic within breed, with slight differences among breeds. SNP with MAF < 0.01, outside H-W equilibrium, with too many missing data and carrying signatures of selection were excluded from the working data set that comprised 41953 of the original 45068 markers Sardinian Ancestral Black is the breed displaying the highest proportion of monomorphic loci (0.05) and among the lowest gene diversity values (0.33), likely resulting from a combination of genetic isolation and demographic events. Cluster and Network analysis, Multi Dimensional Scaling (MDS), Structure analysis and haplotype sharing analysis highlighted known and unexpected relationships between breeds. MDS also revealed a clear geographical component of Italian sheep genetic diversity. Comparison with a panel of international breeds (International Sheep Genome Consortium) revealed a clear geographical component of genetic diversity only in non Merino-derived breeds.

Key Words: sheep, biodiversity, single nucleotide polymorphisms

P3057 SNP detection for QTL analysis of social behaviour and production traits in quail. F. Pitel*[1], S. Leroux[1], P. Dehais[1], A. Vignal[1], O. Bouchez[2], D. Gourichon[3], S. Rivière[3], A. Bertin[3], B. Bed'hom[4], F. Minvielle[4], J. Recoquillay[5], E. Duval[5], C. Beaumont[5], C. Arnould[3], C. Leterrier[3], *[1]INRA, ENVT, LGC, Castanet-Tolosan, France, [2]GeT-PlaGe, Genotoul, INRA*

Auzeville, Castanet-Tolosan, France, [3]INRA, PEAT, Tours, France, [4]INRA, GABI, Jouy-en-Josas, France, [5]INRA, URA, Tours, France.

Social behavior is critical when rearing animals in large groups as observed in poultry production. This project aims at finding QTL responsible for social motivation in birds and to decipher the genetic relationships between social behavior characteristics and the main production parameters. It is carried out in quail to take advantage of 2 experimental lines which have been selected divergently for social reinstatement (motivation to join flockmates) over 47 generations. An F2 design has been produced, and 940 individuals were measured for production traits (body weight, laying traits), and behavioral traits between 1 and 12 weeks of age (social reinstatement, response to social isolation, general activity, response to human, sexual behavior...). For a species without million of polymorphisms available, a way to obtain SNP informative in a specific cross is to directly develop these markers by sequencing the parental individuals of the population. Whole genome sequencing was thus performed on F0 parents of each line, to observe line-specific SNPs. From 2 HiSeq2000 lanes, more than 42 billion bases were obtained. Analyses were performed using the chicken genome as a reference for sequence alignment. From the most discriminating SNPs between the 2 lines, 6,000 markers were selected to perform individual genotyping through an Infinium iSelect beadchip.

Key Words: quail, SNP, social behaviour

P3058 Mitochondrial DNA lineage sorting from one diverse founder population can explain extant domestic sheep haplotypes. S. Hiendleder[1], A. Javadmanesh[1], P. L. Hind[2], M. R. Nassiri[3], M. Pirastru[4], P. Mereu[4], B. Masala[4], and Y. Plante*[2], *[1]JS Davies Epigenetics and Genetics group, School of Animal and Veterinary Sciences and Robinson Institute, University of Adelaide, Roseworthy Campus, Roseworthy, SA 5371, Australia, [2]Canadian Animal Genetic Resources Program - Programme Canadien des Ressources Genetiques Animales, AAFC - AAC, and Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada, [3]Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, PO Box 91775-1163, Iran, [4]Universita degli Studi di Sassari, Dipartimento Scienze Biomediche, Via Muroni, 25 - 07100 Sassari, Italy.*

Domestic sheep (*Ovis aries*) display major mtDNA haplotypes originally assumed to reflect multiple domestication events, involving different subspecies of Eurasian wild sheep, including mouflon (*O. orientalis*), urial (*O. vignei*) and argali (*O. ammon*). Subsequent analyses excluded mtDNA contributions, from urial and argali, and pointed to domestication of mouflon subspecies. However, mtDNA lineage sorting from a highly diverse population could provide an alternative explanation for the 5 major haplotypes. We sequenced the complete mtDNA control region (CR) of mouflon from Sardinia (n = 5) and Cyprus (n = 3) - both now classified as feral Neolithic domesticates of *O. orientalis* -, of domestic sheep from these islands (n = 4), and of urial (*O. vignei arkal*, n = 23) from a single location in north-east Iran, Tandoureh National Park. We combined these 35 novel sequences with complete CR sequences from the database, including Anatolian mouflon (*O. orientalis anatolica*, n = 8), other mouflon with Sardinian/Corsican ancestry from Central Europe (n = 5) and domestic sheep from Eurasia (n = 33). Pair-wise nucleotide differences for domestic sheep, mouflon and urial were 26.9 ± 12.1, 27.6 ± 12.6 and 26.6 ± 12.1, respectively. Phylogenetic analyses (MEGA) revealed 2 major clusters formed by *O. vignei arkal* and *O. orientalis*/*O. aries* with similar deep branching. Anatolian, Cyprus, Sardinian/Corsican mouflon grouped with or near all 5 major domestic sheep haplotypes: A, C, D and E were found in or close to Anatolian and Cyprus mouflon, while haplotype B was only found among Sardinian/Corsican mouflon. The position of Cyprus mouflon and Sardinian/Corsican mouflon is consistent with the expansion of *O. orientalis* from mainland Asia Minor to Cyprus, Sardinia and Corsica.

Key Words: sheep, mtDNA lineage sorting, domestication

P3059 Mitochondrial genome haplogroups associated with Thoroughbred racing performance. Aladaer Qi*[1], Li Wen[3], Shi Zhou[2], Bin Liu[1], Yong Zhang[3], and Allan Davie[2], *[1]Center of Systematic Genomics, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China, [2]School of Health and Human Sciences, Southern Cross University, Lismore, Australia, [3]Tianjin Key Lab of Exercise Physiology and Sports Medicine and Department of Health and Exercise Science, Tianjin University of Sports, Tianjin, China.*

Mitochondria are the powerhouse in cellular energy metabolism and breeding evidence suggested matrilineal inheritance has strong influence on aerobic endurance of racing horses. The aim of this study is to examine the association between the mitochondrial genomes and the elite racing performance of Thoroughbred horses. Blood samples were collected and the mitochondrial genome sequences were obtained to determine the haplogroup structures. Case and control groups were set up among 160 thoroughbred horses. The racing performance is ranked by the value of lifetime earnings divided by life time wins: horses with zero life time wins were selected as poor performance group and top ranking 30 horses were selected as elite performance group.

Eight mitochondrial genome haplogroups A, B, G, H, I, L, M, N were identified, in which haplogroups I (25%), L (47%) and N (8%) were among the main genotypes. The haplogroup distribution of the elite group differed significantly from the poor ($P < 0.05$), with the elite showing a greater proportion of haplogroup I (elite = 37%, poor = 16%) and lesser proportion of haplogroup L (elite = 30%, poor = 43%) and haplogroup N (elite = 3%, poor = 16%). In the entire sample set, the distribution of haplogroup I is positively correlated to the elite racing performance with r = 0.99 ($P = 0.05$) while haplogroup N and L showed negative correlation with r = −0.93 and −0.54, although not statistically significant. Our results suggested a genetic connection between mitochondrial haplogroups and elite racing performance of Thoroughbred horses. These findings may have practical implications in the training, selection and breeding of racing horses.

Key Words: Thoroughbred, mitochondria, elite performance

P3060 Characterization of the duck MHC class II genes. Liming Ren,* Yaofeng Zhao, and Ning Li, *State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, P. R. China.*

In birds, 2 classical class II β genes have been found in the chicken MHC B locus, but no α gene was found nearby. A single classical class II α gene (B-LA) has been found in chicken, and is located roughly 5.6 cM far from the MHC B locus which encodes the classical class II β-chains. Using the duck bacterial artificial chromosome (BAC) library that was previously constructed in our laboratory, we obtained and sequenced a BAC clone containing the duck MHC class II genes. A single classical class II α gene (Anpl-DRA) and 5 classical class II β genes were identified in the region. The presence of 5 β genes in ducks was also confirmed by Southern blot. The class II α gene is located next to class II β genes, which is the same as in humans but different from the situation in chicken, turkey and quail. RT-PCR detection of class II β genes in eight ducks showed that all the five genes were expressed but at different levels. Polymorphisms of these 5 genes are shown to be concentrated in the antigen-recognition domain. Whereas β1and β3 genes were nearly monopolymorphic, β5 gene, which was expressed in the lowest level, was shown to have 4 alleles including a total of eleven amino-acid variations. β2 and β4 genes were more complex as there were other five sequences in 8 ducks different from the sequenced BAC. More studies are still ongoing to characterize these duck genes.

Key Words: duck, MHC class II gene, polymorphism

P3061 Comparison of potential traits for reducing methane emissions in sheep. D. L. Robinson*[1,2], J. P. Goopy[1,2], R. Woodgate[1], A. Donaldson[1], R. S. Hegarty[2,1], and V. H. Oddy[1,2], *[1]Agriculture NSW, Armidale, NSW, Australia, [2]University of New England, Armidale, NSW, Australia.*

In 2007, Australian livestock emitted 57.6 million tonnes of CO_2-eq as methane - 65% of all agricultural and 10.5% of all Australian emissions. We evaluated the potential to breed animals for lower emissions by comparing 5 CH_4 measurement protocols in sheep: P1, 1-h emissions adjusted for liveweight (LW) under a management strategy intended to reduce variation in feed intake of grazing animals (overnight fast then access to hay from 120 to 60 min before a 1-h CH_4 measurement period); P2, P1 with better quality more abundant pasture; P3, P2 adjusted for LW and hay eaten 120–60 min before measurement; P4, 1-h emissions adjusted for LW of animals grazing normally until the morning of the test; P5, 22-h respiration chamber measurements on a standardized diet fed at 20 g/kg LW. For non-pregnant ewes from the genetically diverse sheepGENOMICs flock, individual measurement protocols were moderately repeatable (0.33–0.68) with varying heritabilities (0–47%). Genetic correlations were low to moderate suggesting the protocols measure different genetic aspects of CH_4 emissions, which are also influenced by feed quality and availability, feed intake, digestion efficiency and rumen microbial population. Heritabilities, costs and relationships with the trait of interest (national emissions under normal management) will help identify ways to reduce CH_4 without affecting production.

Key Words: CH_4, ruminants, measurement protocol

P3062 Nucleotide variability in 5′cis-regulatory regions of lipid metabolism related genes in taurine and indicine breeds: Evidence of selection pressure. A. Sanz[1], C. Serrano[1], O. Uffo[2], L. Ordovás[1], A. C. Acosta[2], P. Zaragoza[1], R. Osta[1], and C. Rodellar*[1], *[1]Universidad de Zaragoza, Zaragoza, Spain, [2]Centro Nacional de Sanidad Agropecuaria., La Habana, Cuba.*

The identification of polymorphisms in noncoding regions is a very valuable issue since it could define phenotypic variation, making them potential targets for natural selection. Studying the variability of 5′regulatory regions in genes related to fat synthesis, lipid metabolism and energy homeostasis pathways such as fatty acid synthase (FASN), stearoyl-CoA desaturase (SCD), perilipin (PLIN), glycerol-3-phosphate acyltransferase mitochondrial (GPAM); and melanocortin-4 receptor (MC4R) in taurine and cebuine breeds we found 42 polymorphisms. The number of SNPs per species evaluated in the study ranged between 12 in *Bos taurus* and 34 in *Bos indicus* showing as patterns of diversity vary among species. The higher number of

polymorphic points was observed in *Bos indicus* breed in FASN, SCD, PLIN and MC4R genes; only *Bos taurus* GPAM promoter contains more variations. The variability across species suggests that the diversity of progenitors of indicine cattle is greater than for taurine. These marked differences in variability across species also must be influenced by the different selection pressures exerted mainly in *Bos taurus* commercial breeds to improve some economic and productive traits, which might have negative consequences.

Key Words: lipid metabolism, nucleotide diversity, bovine

P3063 SNP marker technologies and applications for genetic improvement in the South African abalone (*Haliotis midae*). R. Roodt-Wilding,* S. Jansen, S. Blaauw, J. du Plessis, J. Vervalle, C. Rhode, and A. Bester-van der Merwe, *Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

Single nucleotide polymorphisms (SNPs) have become the molecular markers of choice in animal genetic improvement programmes using marker assisted breeding, due to their abundance, ease and potential for medium and high throughput genotyping. A similar trend is seen worldwide in commercial fish and shellfish species. Currently in South Africa, *Haliotis midae* is the primary aquaculture species in terms of revenue and the only commercially cultivated abalone species in the region. Similar to various other economically important aquaculture species, the advent of next-generation sequencing has led to an increase in the number of molecular markers generated in a short period of time at reduced costs compared with traditional marker isolation methods. In this study, the generation of SNP markers from the transcriptome of the South African abalone with the use of the Illumina and 454 pyrosequencing platforms are discussed, as well as the use of the Goldengate Genotyping Assay for medium throughput genotyping. Applications of these markers in population genetics and -genomics studies of wild and cultured abalone populations will be elaborated upon as well as their utility in linkage mapping in this species.

Key Words: single nucleotide polymorphisms, next-generation sequencing, Goldengate Genotyping Assay

P3064 New alleles detection in BoLA-DRB3.2 locus of Sistani cattle by molecular based typing method. Balal Sadeghi* and Mohammad Reza Nassiry, *Department of Animal science, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran.*

This study was carried out for identification new alleles in BoLA-DRB3.2 locus of Sistani cattle. For mentioned purpose, bovine DNA was isolated from aliquots of 200 samples blood. Then amplification of the BoLA-DRB3 exon 2 was done by using Hemi Nested PCR, in 2-step, and then followed by digestion with restriction endonucleases RsaI, HaeIII and BstX2I. As a whole, we identified 24 alleles in BoLA-DRB3.2 locus of Sistani cattle, that 21 of these alleles were similar to those reported in past studies. But the remaining 3 alleles including DRB3.2 *obc,*ibc, and *eac with respectively, 0.33%, 1.33% and 8.1% frequency, had not been identified in studies that carried out before. The obtained sequence of new patterns were submitted to the NCBI Gen Bank and registered with accession numbers as DQ486519, EU259858, EU259857 respectively. These results demonstrated that the BoLA DRB3.2 locus is highly polymorphic in Sistani cattle and the 3 new alleles, can be used as a selective index or breed marker in this species.

Key Words: Sistani cattle, BoLA-DRB3.2, sequence based typing

P3065 Intracellular localization and antiviral activity depending on position 631 amino acid polymorphism of chicken Mx protein. Keisuke Sasaki,* Akihiro Yoneda, Akinori Ninomiya, Manabu Kawahara, and Tomomasa Watanabe, *Laboratory of Animal Breeding and Reproduction, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan.*

Mx protein is known to inhibit the multiplication of several RNA viruses. In chickens, the antiviral ability of Mx protein against vesicular stomatitis virus (VSV) and influenza virus depends on a single amino acid substitution in the position 631 (631aa) using mouse 3T3 cells, indicating that the substitution in 631aa from Ser to Asn provides with the antiviral ability. However, the mechanism how it contributes to the antiviral activity still has been unknown. In this study, we investigated the localization pattern of chicken Mx protein fused with green fluorescent protein and the antiviral activity of chicken Mx protein against recombinant VSV (VSVΔG*-G) in mouse 3T3 cells. As a result, Mx protein containing Asn in 631aa distributed in a granular state in cytoplasm and inhibited the multiplication of VSVΔG*-G. Whereas Mx protein containing Ser in 631aa diffusely localized and did not inhibit the multiplication of VSVΔG*-G. Furthermore, mutant chicken Mx protein that was artificially replaced in 631aa from Ser to Asn showed granular distribution, and acquired the antiviral activity against VSVΔG*-G. These results demonstrated that the substitution in 631aa of chicken Mx protein was responsible for the intracellular localization and the antiviral activity against VSVΔG*-G.

Key Words: chicken, Mx protein, intracellular localization

P3066 Porcine TLR polymorphisms affecting the recognition of *Salmonella enterica* serovar Choleraesuis. Hiroki Shinkai*[1], Rintaro Suzuki[1], Masato Akiba[2], Naohiko Okumura[3], and Hirohide Uenishi[1], *[1]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, [2]National Institute of Animal Health, Tsukuba, Ibaraki, Japan, [3]JATAFF-Institute, Tsukuba, Ibaraki, Japan.*

Toll-like receptors (TLRs) recognize various molecular patterns derived from microorganisms and play a major role in host defense against infections such as diarrhea. *Salmonella enterica* serovar Choleraesuis (SC) is a highly invasive pathogen that causes enteric and septicemic diseases in pigs. In this study, we showed the involvement of TLR5 and the TLR2–TLR1 heterodimer in the recognition of SC by nuclear factor-κB-dependent luciferase reporter assay. We had previously found 12, 10, and 20 amino acid polymorphisms in TLR5, TLR2, and TLR1, respectively, by searching for single nucleotide polymorphisms (SNPs) in various pig populations. As a result of reporter assay by using expression constructs carrying each of these polymorphisms, we demonstrated that the polymorphisms resulting in amino acid changes TLR5R148L, TLR5P402L, and TLR2V703M attenuated the responses to stimulation with SC. Homology modeling using the crystal structure of the dimer of human TLR4–MD-2 complex as a template predicted the R148 and P402 residues in porcine TLR5 were situated on the interaction surface between TLR5 and ligand. The distribution of 3 SNPs causing TLR5R148L, TLR5P402L, and TLR2V703M was restricted only in Jinhua, Landrace, or Berkshire breeds, respectively. These results may be of great importance for the pig industry in terms of breeding for disease resistance and vaccine development.

P3067 Prospecting selection signatures in the cutting and racing lines of Quarter Horse. Marcilio Dias Silveira da Mota*[1], Camila Tangari Meira[2], Josineudson Augusto Vasconcelos Silva[1], Henrique Nunes Oliveira[2], and Rogério Abdala Curi[1], *[1]University of Sao Paulo State, Botucatu, Sao Paulo, Brazil, [2]University of Sao Paulo State, Jaboticabal, Sao Paulo, Brazil.*

The fixation index, FST, was used to identify genome regions in the cutting and racing lines of Quarter Horse that have been modified by selection. 54,602 SNPs were genotyped using the Illumina Equine SNP50 BeadChip in 120 racing and 64 cutting horses. The θ = FST parameter was estimated for 42,058 SNPs that passed quality control using a Bayesian method. The mean FST estimated in the 2 lines was 0.0342 ± 0.0403, with θ ranging from 0.0025 to 0.3556. The low FST value found was expected since fixation indices tend to be lower when lines instead of breeds are studied because of a theoretically lower genetic distance. The distribution of posterior means of the θ values (FST) in the 2 lines permitted classification of the loci (SNPs) into 7 clusters. The expectation is that these clusters are representative of different processes that occur in the populations such as balancing, directional selection and neutrality. Cluster 4, with 2,558 loci, presented the highest values of θ. The conditional probability of membership to this cluster was 1 for 271 loci. Fifteen of these loci, mapped to chromosomes 1, 2, 4, 5, 8, 9, 10, 16, 21 and 30, presented the highest values of θ (0.3013 to 0.3556), indicating genome regions that were more likely to have been subjected to divergent selection between lines. Supported: FAPESP.

Key Words: equine, SNP, selection

P3068 Characterization of diversity in the sheep MHC class II antigen presenting genes. Michael Stear,* *Glasgow University, Glasgow, Scotland, UK.*

The MHC contains some of the most important genes for disease resistance. However, the MHC is seldom used in breeding schemes because of concerns that breeding for resistance to one disease may reduce its diversity and increase resistance to other diseases. As part of a project to explore the effect of selective breeding for disease resistance on MHC diversity, we characterized the MHC class II antigen presenting loci in the Scottish Blackface breed by testing over 1000 animals from 16 farms. There were 33 alleles at DRB1, 14 at DQA1, 14 at DQB1, 12 at DQA2, 20 at DQA2, and 6 at the DQA2-like loci. The haplotypes are complex, with copy number variation in the DQ loci. Linkage disequilibrium was much higher than that observed in human populations. Diversity can be measured in several ways. After consultation with category theorists, we adapted Renyi's entropy to provide a composite measure of diversity.

Key Words: sheep, MHC, diversity

P3069 Prehistoric cattle breeding. E. M. Svensson*[1,2], F. Johnsson[1], P. Skoglund[1], Y. Telldahl[3], S. McGrory[4], J. Mulville[5], D. Anthony[6], M. Nussbaumer[7], M. Vretemark[8], S. Davis[9], C. Ginja[10], M. Jakobsson[1], and A. Götherström[1], *[1]Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden, [2]Department of Animal Genetics, Swedish University of Agricultural Sciences andDPI, Biosciences research division, Uppsala, Sweden and Melbourne, Victoria, Australia, [3]Osteoarchaeological Research Laboratory, Department of Archaeology and Classical Studies, Stockholm University, Stockholm, Sweden, [4]Dept of Archaeology, University of York, York, United Kingdom, [5]Cardiff School of History, Archaeology and Religion, Cardiff, United Kingdom, [6]Hartwick College, Oneonta, NY, USA, [7]Natural History Museum Bern, Bern, Switzerland, [8]Västergötlands museum, Skara, Sweden,*

[9]Instituto Português de Arqueologia, Lisbon, Portugal, [10]Centre for Environmental Biology University of Lisbon, Lisbon, Portugal.

There are close to a 1000 different cattle breeds in the world today. Cattle are the most important of our livestock animals, but the knowledge of how and when this diversity arose is obscured by lack of written documentation before the last 200 to 300 years. It is more than 10,000 years since cattle were domesticated and the need for specialized animals is likely to have invoked selection for specific traits already early on in history. In Europe the Middle Ages was a time with a lot of dynamic change in society, which likely affected the cattle. Here we genotype a large amount of aurochs and cattle remains ranging in time from Bronze Age to the 18th century, as well as modern cattle, for several nuclear SNPs. We use coalescent simulations to control for the effect of genetic drift on allele frequency changes over time. Small changes were observed from the Iron Age to medieval time, and also during medieval time, but the largest change is that observed in the coat color gene MC1R when all ancient samples are compared with modern cattle. It shows clear signs of having been under selection, and to account for the radical change in allele frequency a drastic reduction in effective population size must have occurred, consistent with the formation of breeds

Key Words: cattle breeding, ancient DNA, domestication

P3070 Genetic structure of domestic chicken populations. Ryo Tadano*[1] and Masaoki Tsudzuki[2], *[1]Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu, Japan, [2]Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan, Higashi-Hiroshima, Hiroshima, Japan.*

A wide variety of chicken breeds have been generated through long-term artificial selection by human since their domestication. The aim of this study was to investigate the genetic structure of chicken populations. A total of 3119 individuals from one population of Red Junglefowl (Gallus gallus) and 74 various types of domestic chicken populations (Gallus gallus domesticus) widely differing in their phenotypes, breeding histories, and current management were genotyped for 40 autosomal microsatellite loci. Bayesian model-based clustering was conducted to assess population structure based on multilocus genotypes of microsatellites. The major findings of this study were summarized as follows: (1) White-Egg Layers were genetically distinct from the other chicken populations; (2) some Japanese native breeds showed complicated genetic structure, which indicates that these breeds have multiple origins; (3) in Japanese native breeds, Chabo (Japanese Bantam) was characterized as quite unique gene pool; and (4) Red Junglefowl, which is one of the probable wild ancestors of domestic chickens, shared its genome with several populations of modern domestic chickens. These findings will provide new insight into phylogenetic relationships of chicken populations at the molecular level.

Key Words: domestic chickens, genetic structure, microsatellite

P3071 The diversity of caprine MHC DQA1 and DRB3 alleles in three Chinese goat breeds. J. Wang,* Y. Luo, J. Hu, S. Cheng, X. Liu, S. Li, and Y. Wang, *Gansu Key Laboratory of Herbivorous Animal Biotechnology, Gansu Agricultural University, Lanzhou city,Gansu province,China.*

Goat lymphocyte antigen (GoLA) have been extensively uesd as markers for goat diseases and immunological traits. In this study, we sequenced alleles of the GoLA-DQA1 and DRB3, from 1323 Chinese goats from 3 breeds(Hexi cashmere, Inner Mongolia cashmere and Liaoning cashmere) using PCR-single-strand conformational polymorphism (SSCP). First, 9 previously reported distinct DQA1 alleles were identified in the 3 breeds, with gene frequencies ranging from 2.6% to 21.8%. Second, in addition to 13 previously reported DRB3 alleles, 2 new DRB3 alleles were identified(DRB3*12 and DRB3*13). Third, a population tree based on the frequency of GoLA-DQA1 and DRB3 alleles in each breed suggested that Hexi cashmere and Inner Mongolia cashmere were the most closely related, and that Liaoning cashmere is different from both these breeds. In addition, Wu-Kabat variability anslysis indicated that the DRB3 gene was more polymorphic than the DQA1 gene in all breeds, and that the magority of the hypervariable positions both loci corresponded to pock-forming residues. Finally, the results of Chi-squared test showed that GoLA-DQA1 and GoLA-DRB3 gene in 3 breeds did not fit with Hardy-Weinberg equilibrium ($P < 0.01$). In conclusion, the results of this study demonstrated that DQA1 and DRB3 are highly polymorphic loci in 3 Chinese goat breeds.

Key Words: GoLA-DQA1, GoLA-DRB3, diversity

P3072 Genetic structure and diversity of Australian sheep breeds. W. M. S. P. Weerasinghe*[1], C. Gondro[1,2], M. G. Jeyaruban[3], J. M. Henshall[2,4], J. Kigas[5], and J. P. Gibson[1], *[1]Centre for Genetic Analysis and Applications,University of New England, Armidale, NSW, Australia, [2]Cooperative Research Centre for Sheep Industry Innovation, Armidale, NSW, Australia, [3]Animal Genetics and Breeding Unit, Armidale, NSW, Australia, [4]CSIRO Livestock Industries, FD McMaster Laboratories, Armidale, NSW, Australia, [5]CSIRO Livestock Industries, Brisbane, QLD, Australia.*

Australian sheep consist of numerous breeds, crosses and strains within breeds. Implementation of traditional and molecular-enabled genetic breeding benefits from understanding the between/within breed genetic diversity; and individual breed compositions. This study identified the genetic composition of Australian sheep breeds in the sheep CRC Information Nucleus. SNP genotypes for 7,169 CRC animals (most other than Merino being first crosses) and 880 animals from 16 Australian or related breeds from the Sheep HapMap were used. Principal component analysis was used to determine breed architecture and breed compositions of individual animals. Breed signatures clustered tightly and were well defined. As expected the F1 clustered densely halfway between their parental breeds while the back crosses showed more variability than their F1 counterparts. Coopworth in the CRC and HapMap were genetically distinct; probably due to the composite nature of the breed which shows larger variation than pure breeds. Similarly Australian White Suffolk evidence large genetic diversity with contributions from Suffolk, Poll Dorset and Border Leicester. This suggests that in relation to pure breeds, more animals have to be sampled in composites to get a better representtion of their genetic background. PCA is also accurate for identification of outliers and should be used in QC work.

Key Words: sheep diversity, population structure, principal component analysis

P3073 Polymorphisms of some candidate genes for litter size in the Mong Cai pig breed. Hoan Tran Xuan,* Mai Pham Phuong, Toan Tran Xuan, Nhan Giang Thanh, and Tuan Luong Nhan, *National Institute of Animal Sciences, Hanoi-Vietnam.*

Efficiency of pig production is influenced by reproductive traits, especially by litter size. It is important to identify individual genes or anonymous genetic markers associated with reproductive traits, could contribute to an increased rate of genetic gain in populations. Some major genes affecting reproductive traits in pig have been successfully identified such as the estrogen receptor (ESR) gene, the properdin (BF) gene, the ring finger protein 4 (RNF4) gene and the α (1,2) fucosyltransferase (FUT1) gene. The aim of this reaserch was investigated polymorphisms of some candidate genes for litter size in total 145 Mong Cai pigs (138 sows and 7 boars). The Mong Cai pigs were rearing in Vietnam for reproduction due to they have high number of piglets born alive. The polymorphisms in these genes were detected by the polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP) method. Two different alleles of ESR and BF and RNF4 gene were identified: alleles A (0.19) and B (0.81) of the ESR gene and alleles A (0.26) and B (0.74) of the BF gene and alleles C (0.36) and T (0.64) of the RNF4 gene. Only allele G (1.0) of the FUT1 gene was identified. Results here suggested that further studies were needed to confirm association between polymorphisms of ESR, BF and RNF4 gene with litter size of Mong Cai pig.

Key Words: polymorphism, Mong Cai pig, ESR-BF-RNF4-FUT1 genes

P3074 Nutritional composition analysis of milk and meat from human lactoferrin transgenic cows. Jie Zhao,* JianXiang Xu, JianWu Wang, ShunChao Sui, and Ning Li, *China Agricultural University, Beijing, China.*

Here we analyzed the compositions of milk and meat from human lactoferrin (hLF) transgenic cows. On the one hand, including lactose, fatty acids, amino acids, minerals and vitamins, more than 50 nutrient parameters of colostrum and mature milk were compared between 3 hLF transgnic cows and 27 wild type (WT) cows. In addition, average daily milk production of them in each month was compared between them. On the other hand, 6 hLF transgenic bulls and 3 WT bulls of 10 mo age were slaughtered for meat composition analysis of them. To determine the comparative health of hLF bulls for meat analysis, hematological analysis, organ/body weight analysis and pathology analysis were made. Results showed that the nutrition compositions of milk from hLF cows and WT cows did not have significant difference ($P \geq 0.05$). The average daily milk production in each month of hLF cows was a little lower than that of WT cows, but they did not show significant difference ($P \geq 0.05$). The hematological parameters, organ/body weight values of hLF and WT bulls for meat analysis did not show significant difference ($P \geq 0.05$), and histopathological examination revealed no abnormalities. Nutrient parameters of meat compositions of hLF and WT bulls did not show significant difference ($P \geq 0.05$).

Key Words: human lactoferrin cows, fatty acid composition, amino acid composition

P3075 Phylogeny and assessment of conservation priority for Asian native chicken genetic resources. Shengguo Zhao*[1,2], Jianlin Han[2,3], and Olivier Hanotte[3], *[1]Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China, [2]CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [3]International Livestock Research Institute (ILRI), Nairobi, Kenya.*

Chicken genetic resources are faced with a gradual decrease in genetic diversity thus there is need to provide theoretical basis for conservation. The genetic diversity and phylogeography for native chicken from 10 countries in Asia, commercial chickens and the red jungle

fowls were investigated by analyzing mtDNA D-loop HVS. The order of the conservation priorities of native chickens distributed in 31 Management Units in Asia were assessed. Phylogenetic analysis revealed there were 7 maternal origins of Asian indigenous chickens. Four subspecies of the red jungle fowls, G. g. spadiceus, G. g. gallus, G. g. jabouillei and G. g. murghi were involved in domestication of chicken in the 3 centers of origin, the Indian subcontinent (D clade), China (B, C, E and F clade) and Indonesia and/or surrounding areas (A clade). The domestication center for G clade remains unknown. The order of conservation priorities based on the genetic diversity, the genetic distinctiveness and distribution rate: Northwest Yunnan of China, Sri Lanka, Japan, Papua New Guinea, Shandong of China, India, Southwest and Southeast Yunnan of China, Indonesia and Henan China. The results from erosion model of commercial chicken revealed that the native chicken distributed in all areas were threatened by commercial chicken but to different extends.

Key Words: native chicken, genetic diversity, conservation priority

P3076 Genetic polymorphism of kappa-casein in Iraqi buffalo using polymerase chain reaction-restriction fragment length polymorphism Talib Ahmed Jaayid*, Muntaha Yakoub Yousief, Bashar Falih Zaqeer, and Jaafer Mohammed Owaid, *Animal Production Department, College of Agriculture, Basrah University, Basrah, Iraq.*

The aim of this study was to use the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) to detect the genetic variants of kappa-casein gene in buffalo using primer 1 (in this step) to amplify the gene segment followed by digestion by restriction enzyme (*Hin*dIII) for genotyping. DNA from 50 Iraqi buffaloes was extracted by phenol chloroform method, PCR was carried out in a final reaction volume of 50 µL, and the reaction mixture was subjected to a standard PCR protocol. The results of this work showed that among the examined 50 Iraqi buffalo were homozygous for the kappa-casein and genotyped as BB. Thus, PCR-RFLP using *Hin*dIII revealed all the samples to be monomorphic for this locus. For the first time completed research such specifications in Iraq, for the first time using molecular biology in genetic identification. Our objectives of this study have been to aid in understanding domestication, buffalo origin and their history and evolution, to identify genetically unique breeds, to provide an objective basis for conservation decisions and to aid the formulation of breeding plans. Financial support: Arab Science and Technology Foundation (ASTF), United Arab Emirates (contract number 2108).

Key Words: kappa-casein polymorphism, Iraqi

P4000–P4069

Genetic markers and selection

P4000 Validation of genomic breeding value predicted in a Duroc closed population. Aisaku Arakawa*[1], Masaaki Taniguchi[1], Naohiko Okumura[2], Toshimi Matsumoto[1], Kensuke Hirose[3], Kazuo Fukawa[3], Tetsuya Ito[3], Takeshi Hayashi[4], Hirohide Uenishi[1], and Satoshi Mikawa[1], *[1]National Institute of Agrobiological Sciences, Tukuba, Ibaraki, Japan, [2]JATAFF Institute, Tukuba, Ibaraki, Japan, [3]Central Research Institute for Feed and Livestock ZEN-NOH, Kamishihoro, Hokkaido, Japan, [4]National Agricultural Research Center, Tsukuba, Ibaraki, Japan.*

Genomic selection (GS) has been important tools for accelerating genetic improvement. In this study, we evaluated prospects for GS in pig breeding using the actual pig population. We maintained a closed population of Duroc breed, from which phenotypes of traits were recorded for > 2000 individuals in G1-G7 and 40K SNPs were genotyped for > 1000 individuals in G1-G6. The SNP effects were estimated using 665 individuals in G1-G3 as a training data set with BayesC method and evaluated the prediction accuracies using 394 individuals in G4-G6 as a test data set. Furthermore, putative QTL regions explaining > 1% genetic variations were selected based on the estimated SNP effects and the prediction models were refitted by incorporating those QTL regions, instead of SNPs, in the models as fixed effects. The prediction accuracy was defined as a correlation between breeding values predicted using genomic information and predicted breeding values with BLUP using pedigree information. The accuracies of the predicted genomic breeding values for backfat thickness (BF) were decreased from 0.79 for the animals in G4 to 0.20 for those in G6. When refitted model using the putative QTL regions and pedigree information were used, higher accuracies, e.g., 0.37 for BF, were obtained in G6. This result indicates that the SNP effects in GS would need to be re-estimated at early stage for an effective improvement.

Key Words: genomic selection, pig, QTL

P4001 Genetics of suri alpaca. B. R. Appleton*[1,2], *[1]Deakin University, Waurn Ponds, Victoria, Australia, [2]The University of Melbourne, Parkville, Victoria, Australia.*

Genomics research has become imperative for livestock industry development and improvement. In a short time the steadily growing alpaca industry has moved away from a world lacking any alpaca specific genetic resources to one on the brink of modern genomics capability. During this transition we have used microsatellites and SNPs to show strong association of a genomic region with the suri fleece trait. The 10x reference genome and our whole genome re-sequencing of 6 animals has allowed production of a new SNP array. The first information gleaned from these data will be discussed.

Key Words: genetic mapping, alpaca

P4002 Genomic regions associated with production and robustness traits in dairy cows. J. W. M. Bastiaansen*[1], D. P. Berry[2], S. Wijga[1], E. Wall[3], A. Lunden[4], R. F. Veerkamp[5], and H. Bovenhuis[1], *[1]Animal Breeding and Genomics Centre, Wageningen University, 6708 WD Wageningen, the Netherlands, [2]Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, Ireland, [3]Sustainable Livestock Systems Group, Scottish Agricultural College, EH25 9RG Midlothian, United Kingdom, [4]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden, [5]Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, 8200 AB Lelystad, the Netherlands.*

Dairy cows are selected for high yields of milk and its components. To efficiently produce large amounts of milk, dairy cows need to be robust. Genome wide association analyses have been performed in first lactation Holstein cows for a wide range of traits related to robustness, including reproduction, disease resistance and feed utilization, using the data from research herds across 4 European countries, which was combined in the RobustMilk project. Cows were genotyped with the bovine 50k SNP array. In the current analysis, SNPs were tested for associations with milk yield and yields of fat, protein and lactose in milk, as well as for fat/protein ratio in early lactation (FPRdev). FPRdev is an indicator of energy balance. Associations with FPRdev (false discovery rate <0.30) were found on chromosomes (BTA) 17, 20 and 23. The association on BTA23 was in a region associated with fat and protein corrected milk in one of the previous studies. Associations with milk, fat and lactose yield were found on BTA14, as expected near the DGAT1 gene. More interestingly, associations with lactose percentage were found on BTA6, near associations with protein percentage. Co-location of associations with yield and robustness traits were identified, for instance the beginning of BTA4 was found associated with reproduction and feed utilization as well as milk fat percentage.

Key Words: GWAS, milk production, robustness

P4003 Effect of transthyretin and calcium channel during muscle differentiation. Abdul R. Bhat*[1,3], Eun Ju Lee[1,3], Smritee Pokharel[2], and Inho Choi[1,3], *[1]School of Biotecnology, Yeungnam University, Gyeongsan, Republic of Korea, [2]Department of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea, [3]Bovine Genome Resources Bank, Gyeongsan, Republic of Korea.*

Muscle cell regeneration and differentiation process relies on satellite cells and is dependent on coordination of many factors and transporters. To understand mechanism involved in myogenic differentiation of skeletal muscle (MSC), role of transthytretin (TTR) and calcium channel was studied. TTR and calcium channels were upregulated during differentiation of MSCs. Voltage gated calcium channels were analyzed by patch clamp technique using C2C12 myoblast cells. Post differentiation, voltage gated calcium channels were analyzed in d 0, 2 and 5 cells. Only T- type channel was observed in control cells. However, both L- and T-type calcium channels were observed in d 2 and 5. T-type calcium channel opened faster than L-type calcium channel, but a study increase in L-type calcium channel was observed with aging. Knock-down of TTR gene performed using shRNA construct showed decreased expression of L-type channel (Cav1.1), but no effect was observed in T-type channel (Cav3.1). From these results, it would be nice to speculate a relationship between TTR and voltage gated L-type channels and their essential role in myogenesis, However further studies are needed in the direction for understanding the molecular mechanism underlying muscle MSCs differentiation. This interesting finding can further be utilized to enhance early muscle differentiation in bovine.

Key Words: transthyretin, calcium channel, muscle differentiation

P4004 Genetic polymorphism of some microsatellites related to reproductive traits and their association with calving interval in Kabinburi cows. Kalaya Boonyanuwat*[1] and Amnoy Puttaratanang[2], *[1]Biodiversity Research and Development Section, Bureou of Animal Husbandry and Genetic Improvement, Department of Livestock Development, Bangkok, 10400, Thailand, [2]Nongkwang Livestock Research and Breeding Center, Rachaburi Province, 70000, Thailand.*

This study aimed at detecting genetic polymorphisms in the reproductive of microsatellites, ESR, BL1071, and MM12E6 and established their association with calving interval (CI) as the fertility status during 2005 to 2010. Genomic DNA was extracted from whole blood of 96 Kabinburi cows at Nongkwang Livestock Research and Breeding Center, Department of Livestock Development. The PCR products of investigated microsatellites were run on electrophoresis to analyzed. The results showed that the ESR locus had 3 polymorphic bands with 107 (A, 15.63), 123 (B, 61.98%), and 136 (C, 22.39%) bps. The BL1071 locus had 4 polymorphic bands with 174 (A, 16.67), 186 (B, 42.71%), 199 (C, 9.38%) and 211 (D, 31.25%) bps. The MM12E6 locus had 3 polymorphic bands 117 (A, 30.73%), 124 (B, 37.50%) and 136 (C, 32.29%) bps. Correlations between the phenotypic data and the molecular results of microsatellites revealed that: a) BB genotypic of ESR locus recorded the lowest CI (515.00 ± 143.16 d, $P < 0.05$), b) AB genotypic of BL1071 locus recorded the lowest CI (432.00 ± 19.80 d, $P < 0.05$), and c) AC genotypic of MM12E6 locus recorded the lowest CI (514.25 ± 154.56 d, $P < 0.05$). The prospects are good because in this study the frequency of the ESR-BB, BL1071-AB, and MM12E6-AC genotype were the highest frequency in each locus in this population.

Key Words: fertility, Kabinburi, cow

P4005 DNA variants affecting fat distribution traits in cattle. A. Egarr[1,2], W. Pitchford[1,2], and C. Bottema*[1,2], *[1]Cooperative Research Centre for Beef Genetic Technologies, Australia, [2]School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia.*

The amount and distribution of adipose tissue is important in cattle production. Being able to re-partition fat to more valuable fat depots (eg marbling), while reducing fat in less valuable depots (eg intermuscular fat) would be advantageous. The best strategy to alter fat distribution is likely to be genetic selection. Thirty-two single nucleotide polymorphisms (SNPs) from 13 candidate genes were analyzed for association with fat deposition traits in a Jersey-Limousin double backcross herd. Twenty SNPs were associated with variation in fat traits, mostly with small effects. However, some candidate genes had sizeable effects, including tyrosine kinase, endothelial (14% and 28% of the phenotypic variation in channel and omental fat, respectively) and β,β-carotene 15, 15'-monooxygenase (20% in subcutaneous fat). Moreover, the combined effect of all SNPs affecting a single trait explained a large portion of the phenotypic variation (eg 23%, 25%, 26% and 38% of channel fat, seam fat, subcutaneous fat and omental fat, respectively). However, the estimates of size of effects are likely to be inflated and these results should be validated in other populations. Interestingly, although some genes were associated with variation in more than one fat trait, no single gene was associated with all fat traits or overall fatness, suggesting that selection for specific fat depots should be possible.

Key Words: fat depots, adipose, markers

P4006 Effects of calpain 1 gene interactions on beef tenderness. L. Chang[1,2], W. Pitchford[1,2], and C. Bottema*[1,2], *[1]Cooperative Research Centre for Beef Genetic Technologies, Australia, [2]School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia.*

Tenderness is regarded as one of the most important palatability attributes of beef and is commonly measured using Warner-Bratzler shear force. Calpain 1 (CAPN1) is a subunit of the proteolytic enzyme,

micro-calpain, involved in meat post-mortem tenderization, and variants of the CAPN1 gene are known to have a major effect on beef tenderness. However, studies with Limousin-Jersey backcross progeny herein showed that shear force is not only affected by 2 variants of CAPN1 (SNP316 and SNP530), but there are also significant epistatic interactions between CAPN1 and 5 other genes (SNIP1, FST, FSTL1, LOXL1 and IGF1). In many cases, the size of the effect of the interaction was larger (eg CAPN1-SNP316 and SNIP1-SNP3) than the effects of the individual CAPN1 variants. These epistatic effects of CAPN1 alleles were not necessarily additive. For instance, the CAPN1-SNP316/GG genotype in combination with the SNIP1-SNP3/TT genotype showed much higher shear force values than all other combinations of genotypes. The results were also not consistent across muscles. For example, the CAPN1-SNP316 had the opposite effect on muscles in that the G allele was dominant for the M. longissimus dorsi but recessive for the M. semitendinosus. Thus, epistasis and muscle-specific effects may lead to unintended consequences in breeding programs using marker-assisted selection of the CAPN1 variants for tenderness.

Key Words: cattle, epistasis, meat quality

P4007 Verification of gene variants associated with residual feed intake in cattle. F. E. Bowley*[1,2], C. D. K. Bottema[1], and W. S. Pitchford[1], *[1]Cooperative Research Centre for Beef Genetic Technologies, School of Animal and Veterinary Sciences, Roseworthy, SA, Australia, [2]AbacusBio Limited, Dunedin, Otago, New Zealand.*

Feed is the single largest input cost in beef production systems, and so making improvements in the efficiency with which cattle utilize feed is a prime target for increasing profitability. Residual feed intake (RFI) is a common measure of feed efficiency, but is expensive to measure. This study involved analyzing data from Beef CRC1 cattle to verify 12 single nucleotide polymorphisms (SNPs) found to be associated with RFI in the Davies Gene Mapping Herd. Four of these SNPs accounted for a total of 18% of the additive genetic variance in RFI, and more than half of this was attributed to a single SNP that was significant ($P < 0.05$). Based on the mean reduction in RFI for the favorable allele from each of these 4 SNPs, selection for all 8 favorable alleles would reduce RFI by 0.76 kg per day per animal. However, epistasis, variation in SNP effects between breeds, potential correlated effects on carcass traits and evidence of overdominance mean further validation studies are required before implementation of these SNPs in genomic selection of RFI.

Key Words: cattle, feed efficiency, variant

P4008 Identification of genetic markers associated with behavioral traits in cattle. J. Friedrich[1], B. Brand*[1], K. L. Graunke[2], J. Langbein[2], B. Brandt[1], S. Ponsuksili[1], and M. Schwerin[1], *[1]Research Group Functional Genome Analysis, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [2]Research Unit Behavioural Physiology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

The ability of cattle to cope with environmental stimuli is important for animal welfare. Animals with sufficient coping abilities are assumed to be less prone to stress and more adaptable to new management systems. Behavioral traits can be described as consistent action and reaction pattern to environmental stimuli. Stable behavioral traits like calmness, boldness, aggressiveness or fearfulness also influence ease of handling, productivity and health. Today little is known about the genetic background of behavior in cattle, and besides milking temperament there is a lack of phenotypes routinely monitored to be considered for sire evaluations. The objective of this study is to identify molecular markers that might help to discriminate behavioral traits on the molecular level and to gain further insights into the genetic background of cattle behavior. Therefore, female calves of a Charolais x German Holstein F2 resource population were genotyped (Illumina BovineSNP50 BeadChip) and subjected to 3 behavioral tests (open field, novel object and novel human test) at 90 d post natum. Latency, frequency and duration of behaviors like activity, exploration and grooming were monitored during each test. First results of a whole genome scan using these measurements as phenotype indicate that there are genomic regions affecting the predisposition to certain behavioral traits in cattle.

Key Words: cattle, behavior

P4009 Genomic selection for tick resistance in Braford and Hereford cattle using single-step methodology. F. F. Cardoso*[1,2], C. C. G. Gomes[1], M. M. Oliveira[1,3], V. M. Roso[4], M. L. Piccoli[4], F. V. Brito[4], R. H. Higa[5], S. R. Paiva[2,6], M. V. G. B. Silva[2,7], L. C. A. Regitano[2,8], M. J. Yokoo[1], A. R. Caetano[2,6], I. Misztal[9], and I. Aguilar[10], *[1]Embrapa Southern Region Animal Husbandry, Bage, RS, Brazil, [2]National Counsel of Technological and Scientific Development (CNPq), Brasilia, DF, Brazil, [3]Coordination for the Improvement of Higher Level Personnel (CAPES/PNPD), Brasilia, DF, Brazil, [4]Gensys Associated Consultants, Porto Alegre, RS, Brazil, [5]Embrapa Agriculture Informatics, Campinas, SP, Brazil, [6]Embrapa Genetic Resources & Biotechnology, Brasilia, DF, Brazil, [7]Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil, [8]Embrapa Southeastern Region Animal Husbandry, Sao Carlos, SP, Brazil, [9]University of Georgia, Athens, GA, USA, [10]National Agricultural Research Institute, Canelones, Uruguay.*

The *Rhipicephalus microplus* tick is one of the main sources of losses in tropical cattle production, causing decreased performance, hide devaluation, and increased costs with treatments and transmission of infectious agents. The aim of this work was to evaluate the utility of genomic evaluation of Braford and Hereford cattle for genetic resistance to ticks. Repeated tick counts were obtained in 2010 and 2011 from 3,114 Braford and Hereford cattle from 7 herds of the "Delta G Connection" breeding program, totalizing 8,004 records. A sample of 1898 Braford and 262 Hereford animals was genotyped using Illumina BovineSNP50 Beadchip. Averaged and log-transformed records were combined with pedigree and genotypes to carry out single step genomic evaluation using BLUPf90 programs. Heritability of tick counts was estimated to be 0.42 ± 0.05, with a 75% increase compared with the estimate based on traditional pedigree evaluation (0.24 ± 0.06). Finally, data was split into 8 subsets (7 Braford and 1 Hereford) for a cross-validation study. Correlation between genomic predictions from the cross-validation and from full data analyses were 0.61 ± 0.13 and 0.47 ± 0.05, respectively for Braford and Hereford animals. These results indicate that genomic selection could be used as a reliable tool to improve genetic progress for resistance to ticks in these breeds and to obtain resistant lines of cattle raised at South America.

Key Words: beef cattle, BovineSNP50 Beadchip, *Rhipicephalus microplus*

P4010 Genes involved in muscle lipid composition in 15 European *Bos taurus* breeds. S. Dunner*[1], N. Sevane[1], D. García[1], H. Levéziel[2,3], J. L. Williams[4], B. Mangin[5], and A. Valentini[6], *[1]Dpto Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain, [2]INRA Universitè de Limoges, Limoges, France, [3]Universitè de Limoges, Limoges, France, [4]Parco Tecnologico Padano, Polo Universitario, Lodi, Italy, [5]INRA Chemin de Borde-Rouge-Auzeville, Castanet-Tolosan, France, [6]Department for Innovation in Biological, Agro-Food and Forest Systems, Università della Tuscia, Viterbo, Italy.*

Consumers demand healthy and palatable meat, both factors being affected by the fat composition. It has been shown that polyunsaturated fatty acids (PUFA) have beneficial health effects; however red meat, especially beef, has relatively high concentration of saturated fatty acids (SFA) and low concentration of PUFA. Modifying the fat composition of meat may be achieved by changing the diets of the cattle or selecting animals predisposed to synthesizing particular fat types. In this paper an association study has been performed in which a large panel of candidate genes involved in adipogenesis, lipid metabolism and energy homeostasis has been tested for effects on fat composition in 15 European cattle breeds. Sixteen genes were found to have significant effects on different lipid traits, and among these CFL1 and MYOZ1 were found to have large effects on the ratio of 18:2/18:3, CRI1 on the amount of neutral adrenic acid (22:4 n-6), MMP1 on docosahexaenoic acid (22:6 n-3), and PLTP on the ratio of n-6:n-3. Several genes were also found to be associated with both lipid and organoleptic traits although with smaller effect. These included ALDH2, CHRNE, CRHR2, NEB, SOCS2, SUSP1, TCF12 and FOXO1. The results presented here help to understand the genetic and biochemical background underlying variations in fatty acid composition and flavor in beef.

Key Words: beef cattle, lipid, candidate genes

P4011 Genetic variation in porcine Zip4-like zinc transporter. G. Erhardt,* F. Siebert, and G. Lühken, *Department of Animal Breeding and Genetics, Justus-Liebig-University, Giessen, Hessen, Germany.*

Zinc (Zn) is an essential trace element which is involved in several metabolic pathways. On the other hand, Zn can cause environmental problems if its emission via manure is too high. The Zn transporter Zip4 gene is known to play a major role in intestinal Zn absorption. The aim of this study was to analyze in a first step the sequence variability of the porcine Zip4-like zinc transporter gene. For this purpose, cDNA samples were generated from intestinal mucosal tissue and used to amplify and sequence 4 fragments covering the complete coding region of the gene. The sequence analysis revealed the presence of 7 nucleotide substitutions. Six of the nucleotide substitutions were synonymous whereas a substitution of A with C in exon IX caused an amino acid exchange from glutamic acid to alanine. Genotyping results including DNA samples from Pietrain, Deutsche Landrasse, Deutsches Edelschwein, commercial crossings and wild boars suggest a breed-specific presence of the A allele in Pietrain for this amino acid substitution while wild boars were homozygous CC. Alignment of exon IX sequences of other species revealed that most species have alanine in this position of the Zip4 peptide chain. Association studies of identified sequence variants with apparent zinc absorption are in progress.

Key Words: zinc, transporter, absorption

P4012 Genomic selection and scan for major genes for a new lamb survival trait for the New Zealand sheep industry. B. Auvray[1], S. Vanderick[2], S.-A. Newman[1], and J. Everett-Hincks*[1], *[1]AgResearch Ltd., Mosgiel, Otago, New Zealand, [2]Gembloux Agro-Bio Tech. University of Liège, Gembloux, Belgium.*

Lambing percentage is one of the most significant factors affecting profitability on New Zealand sheep farms. Since the early 1990s, lambing percentage has increased at about 1% per year from a relatively stable level of approximately 100%, and top performing sheep

farms are now consistently achieving 150% or more. As lambing percentage increases, the proportion of ewes bearing twins and triplets increases accordingly. Lamb mortality rate in these multiples is higher than in singles, with triplets being particularly susceptible. Consequently, lamb survival has become increasingly important to the New Zealand sheep industry. Sheep Improvement Ltd. (SIL, New Zealand's national sheep genetic evaluation system owned by Beef + Lamb NZ) records lamb survival to weaning but genetic improvement has been limited due to the low heritability of the trait and the current method of recording. To address those issues, we have developed an improved survival to weaning trait for industry implementation, which is more accurate and more heritable than the current SIL trait. This poster will present results of applying genome-enabled prediction procedures to the new trait to obtain molecular breeding values. It will also describe results from a genome wide association study using the new trait.

Key Words: genomic selection, lamb survival, sheep

P4013 Mutations in the PLAG1 region are associated with height, weight, puberty, IGF1, and fat deposition in cattle. M. R. S. Fortes*[1,2], S. Sasazaki[1,3], K. E. Kemper[1,4], A. Reverter[1,5], J. Pryce[1,6], W. Barendse[1,5], R. Bunch[1,5], Y. D. Zhang[1,7], R. J. Hawken[1], M. E. Goddard[1,4], and S. A. Lehnert[1,5], *[1]Cooperative Research Centre for Beef Genetic Technologies, Armidale, NSW, Australia, [2]School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia., [3]Kobe University, Kobe 6578501, Japan, [4]Faculty of Land and Environment, University of Melbourne, Melbourne, Victoria, Australia, [5]CSIRO Livestock Industries, Queensland Bioscience Precinct, CSIRO LivestocBrisbane, QLD, Australia, [6]Biosciences Research Division, Department of Primary Industries Victoria, Biosciences Research Division, Department of Primary IndustBundoora, Victoria, Australia., [7]Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia.*

A previously identified and putatively functional SNP (rs109231213) located in the 3′ UTR of the PLAG1 gene at BTA14 was studied in beef cattle. Data from 8,336 animals (*B. indicus*, *B. taurus* and Tropical Composite) were used to test the association between rs109231213 and a range of growth and reproduction traits. Further, 23,496 SNP located on BTA14 were tested for association, both independently and fitted together with rs109231213. Allele frequency of rs109231213 differed between *B. indicus* (0.47), *B. taurus* (0.92) and Tropical Composite (0.71). In the PLAG1 region, rs109231213 and other SNP were associated ($P < 10^{-8}$) with IGF1, height, weight and fat deposition in *B. indicus* and Tropical Composites. There were associations with age at puberty in *B. indicus* ($P < 10^{-8}$) and Tropical Composites ($P < 10^{-5}$). Association results were less significant in *B. taurus* ($P < 10^{-2}$, for weight and height). When rs109231213 was fitted simultaneously as a fixed effect in the model, there was an overall reduction in the P-values observed for neighboring SNP. However, some SNP remained associated ($P < 10^{-4}$) with the studied traits. This could indicate that neighboring SNP and rs109231213 share a role in regulating height, weight, IGF1 and puberty. Further analysis is necessary to define the causative nature of mutations in this region and their pleiotropism.

Key Words: growth, fertility, PLAG1

P4014 A new parental assignment tool for complex mating and large number of animals. Laetitia Barbotte[1], Didier Boichard[1,2], Lucie Genestout*[1], Pierrick Haffray[3], Herve Chapuis[4], Celine Chantry Darmon[1], and Marie-Yvonne Boscher[1], *[1]LABOGENA, Jouy-en-Josas, France, [2]INRA UMR1313 GABI, Jouy-en-Josas, France, [3]SYSAAF Station SCRIBE INRA, Rennes, France, [4]SYSAAF Station de Recherches Avicoles INRA, Nouzilly, France.*

Recent evolution in genotyping technologies increases the opportunity of molecular parentage inference in animal breeding. For a genotyping laboratory, meeting the objectives of customers relies on the choice of an appropriate marker panel and on a highly performing assigning program. Our tool combines exclusion and likehood methods in a program that minimizes false exclusions due to null alleles, typing errors and mutations without increasing computational cost. It can rank putative couples of parents in case of polyassignment and discriminate the true parents in a sample with high relationship level. The most technically robust microsatellite markers were analyzed to estimate allele frequencies. These frequencies were used to simulate larger families with more relationships and different inbreeding levels. The resulting data were processed with the new assignment program to select the smallest efficient panel. Using 13 microsatellites, a 97% assignment rate was obtained in a rainbow trout *Oncorhynchus mykiss* population composed of 5192 offsprings and 233 putative parents (100 males and 133 females). This method was also applied with success in enlarged populations and complex mating schemes in pure species or interspecific hybrid using microsatellites or SNPs.

Key Words: parental assignment, microsatellites, SNP

P4015 Detection of three major chromosome regions involved in the arrival to puberty in bulls through genome-wide association. M. E. Fernandez[1], J. P. Liron[1], A. Prando[2], A. Rogberg-Muñoz[1], A. Baldo[2], and G. Giovambattista*[1], *[1]Instituto de Genética Veterinaria (IGEVET), CCT La Plata CONICET - Facultad de Ciencias Veterinarias, Universidad*

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In bovine, there are significant differences, among and within breeds, in time when bulls reach puberty. The objective of this work was to perform a genome-wide association study using a selective DNA approach for identification of genetic markers associated with age at puberty. Two hundred seventy-six Angus males were weighed and scrotal circumference measured every month. When first bull calves reached 26 cm of SC, sperm quality was added to the monthly measurements for the next 3 mo. Based on sperm quality, 2 groups, corresponding to the top and bottom 6.5% of the phenotypic distribution, were chosen. These samples were genotyping with the Illumina BovineHD Genotyping BeadChip. The number of associated SNP at 0.001 significance thresholds was 265, among which 6 were significant at 0.0001. These significant associated markers were located majorly in 5 chromosome regions: BTA3 (30.57% of associated SNPs), BTA24 (29.43%), and BTA1, 16 and 20 (23.77%). Fifteen additional chromosomes only had few associated SNPs, while the remaining 10 chromosomes did not exhibit significant associated markers. SNPs located into the BTA3, 20 and 24 associated regions were validated in the whole sample using pyrosequencing methods. The results obtained here will be useful for further identification of causative mutations and functional studies.

Key Words: puberty, bulls, GWAS

P4016 microRNAs in plasma: Candidate biomarkers for livestock? Sophie Dhorne-Pollet[1], Marie-Laure Endale Ahanda[1], Tatiana Zerjal[1], Marie-Helene Pinard-Van der Laan[1], Gerald Reiner[2], Hermann Willems[2], and Elisabetta Giuffra*[1], *[1]National Institute for Agronomical Research (INRA), Animal Genetics and Integrative Biology Unit, Teams: GIS and PSGEN, Jouy-en-Josas, France, [2]University of Giessen, Department of Veterinary Clinical Sciences, Swine Diseases, Giessen, Germany.*

MicroRNAs (miRNAs) are present in numerous body fluids, including serum, plasma, saliva, and amniotic fluid. In plasma, miRNAs circulate in high concentrations and are intensively explored as biomarkers for early disease detection in humans (cancer, cardiovascular disorders). We aimed to explore circulating miRNAs as biomarkers of biotic and abiotic stresses in livestock. The strategy was to sample different plasma fractions from selected chicken lines and a pig breed before and after challenge, and to profile by deep sequencing and RT-qPCR the miRNA content. The sample panel included: PHA+ chicken line (selected for cell mediated response induced by phyto-hemoagglutinine, PHA; challenge: intramuscular injection), R+ and R- chicken lines (divergently selected for residual feed consumption; challenge: 24h fasting followed by re-feeding) and Wiesenauer Miniature pigs (challenge: vaccination with an attenuated strain of Porcine Reproductive and Respiratory Syndrome virus, PRRSV). Bioanalyzer profiling, qPCR and immunoprecipitation confirmed findings in humans, i.e. plasma is enriched in miRNAs at least in part associated with Ago2 ribonucleo-protein complexes, and miRNAs can be amplified from different plasma fractions. A test small RNA library of chicken total plasma allowed mapping >18 millions reads to the chicken genome and identifying 75 circulating known chicken miRNAs (min: 5 reads coverage), as well as to establish conditions for ongoing deep sequencing of the sample panel.

Key Words: miRNA, biomarker, stress

P4017 Identifying genomic regions associated with production traits in Rambouillet sheep using a 50K SNP array. T. S. Hadfield*[1], C. Wu[1], D. Waldron[2], G. Moss[3], B. Alexander[3], D. L. Thomas[4], J. Kijas[5], M. Halling[1], B. Bellaccomo[1], and N. Cockett[1], *[1]Utah State University, Logan, Utah, 84322-4815 USA, [2]Texas AgriLife Research, San Angelo, TX 76901, USA, [3]University of Wyoming, Laramie, WY, 82071, USA, [4]University of Wisconsin-Madison, Madison, WI 53706, USA, [5]CSIRO Livestock Industries, Brisbane, 4067 Queensland, Australia.*

Results from genome-wide association studies (GWAS) may benefit sheep producers in the calculation of molecular breeding values (MBV) used to estimate an animal's genetic potential. In this study, a GWAS study was undertaken to identify genomic regions influencing several production traits in the Rambouillet breed. Genomic DNA was extracted from 259 animals in 35 flocks and genotyped with the Illumina Ovine SNP50 BeadChip. Phenotypes collected on the Rambouillet animals included both qualitative and quantitative measurements for wool, growth, reproduction, carcass, and conformation traits. Three "traits" that had been previously mapped to regions of the ovine genome were used in this analysis to demonstrate the capability of our approach. These traits included a classification of horns (horned/polled), the Booroola fecundity genotype, and the PRNP genotype at codon 171. Results using PLINK software showed consistency with genetic assignments reported for the horn gene (ovine chromosome 10 or OAR10), Booroola fecundity gene (OAR6), and the PRNP gene (OAR13). Additional phenotypes collected on the animals included eye pigmentation, color traits, striped hooves, cryptorchidism, scrotal circumference, fat depth and loin eye area measurements, bent leg,

weight measurements, average daily gain, and traits related to wool including wool variation and grade, face cover and belly wool, clean fleece weight and staple length. Initial analysis revealed significant SNPs (P < 4.06E-4) on OAR4, 6, 13, 15, and 21 for loin eye area, cryptorchidism, bent leg, striped hooves and face cover. Additional analyses of the SNPs will further the characterization of these genomic regions.

Key Words: ovine, GWAS, Rambouillet

P4018 Nador—A sustainable genetic approach to reduce boar taint. Pramod K. Mathur[1], Naomi Duijvesteijn[1], Franz-Josef Storck[2], Barbara Harlizius*[1], and Egbert F. Knol[1], *[1]IPG, Beuningen, The Netherlands, [2]Topigs SNW, Senden, Germany.*

Boar taint is one of the challenges of a pig production system in which male finisher pigs are not castrated. Meat from some non-castrated male pigs can produce an unpleasant odour when heated mainly caused by the 3 compounds androstenone, skatole and indole. Over 6,000 carcass fat samples were analyzed to determine the levels of these boar taint compounds. In addition, more than 27,000 fat samples from finisher boars have been scored by an expert test panel for boar taint human nose scores (HNS). Fat sample from each boar was scored by 3 experts independently. A dedicated SNP panel was selected from an association study for HNS and boar taint compounds with the porcine SNP60 Bead Chip. A genomic breeding value was estimated based on the genotypes and combined with several generations of pedigree. The breeding values were then combined into a boar taint index to rank elite boars and identify boars with lowest genetic levels of boar taint as Nador boars. The average proportion of boar taint is 4.6% in the current entire male pig population. The use of Nador boars can reduce this to 2.7% reducing the risk of boar taint by 40%. The Nador program is a sustainable approach to finish entire males with a very low risk of boar taint to stop castration and benefit from positive side effects of improved feed efficiency, increased meat percentage and improved animal welfare.

Key Words: boar taint, genomic selection, animal welfare

P4019 Residual methane production, a metric for finding low methane emitting ruminants. P. Moate[1], S. Williams[1], M. Deighton[1], B. Hayes*[2], and J. Jacobs[1], *[1]FFSR, DPI Victoria, Ellinbank, Victoria, Australia, [2]Biosciences Research Division, Melbourne, Victoria, Australia.*

Identification of genetically low methane emitting ruminants would allow selection for reduced greenhouse gas emissions from livestock. The most accurate method for measuring CH_4 emissions from ruminants uses open circuit respiration chambers for several days directly measuring the animals CH_4 emissions (g CH_4/animal/day). Over several experiments, CH4 emissions on a particular day per cow was 23.1 ± 2.4 times her dry matter intake (DMI) on that day, and DMI explained a significant proportion of CH_4 emissions.As DMI can be measured more readily than CH_4 emissions, the question is how much additional reduction in CH_4 emissions can be achieved over and above selecting for DMI? This will depend on heritability of residual methane (RM), that is CH_4 emissions adjusted for DMI. In lactating cows, bodyweight, stage of lactation and level of milk production must be taken into account. Feed variables such as fermentable carbohydrate, fat and plant secondary compounds substantially affect CH_4 emissions. Recent research suggests DMI on the previous day affects emissions. Given the large volumes of data needed to estimated genetic parameters, data will need to be collated across research centers. RM should be a convenient way to do this.

Key Words: methane, selection, genetic

P4020 The g+6723G>A myostatin mutation affects development of muscle in lambs. F. E. M. Haynes*[1,2], P. L. Greenwood[3], M. B. McDonagh[1,4], C. D. McMahon[5], and V. H. Oddy[2,3], *[1]CRC for Sheep Industry Innovation, Armidale, NSW, Australia, [2]University of New England, Armidale, NSW, Australia, [3]NSW Department of Primary Industries, Armidale, NSW, Australia, [4]Department of Primary Industries Victoria, Werribee, Vic, Australia, [5]AgResearch Ltd., Hamilton, New Zealand.*

Myostatin (MSTN) is a negative regulator of skeletal muscle development, and reduces myoblast proliferation in embryonic and postnatal skeletal muscle. The g+6723G>A mutation is thought to reduce translation of MSTN. We determined live muscle mass and body composition of lambs using computer tomography at 3 and 5 mo of age. Myostatin A/A lambs had greater lean (P = 0.002), less fat (P = 0.009) and lower organ (heart, liver, spleen and kidneys) mass, at 5 but not 3 mo of age. The number of A alleles (0 = G/G, 1 = A/G or 2 A/A) at the myostatin locus was proportional to the increase in muscle mass and reduction in fatness at 5 mo of age. At slaughter (5 mo) we found that A/A lambs had more muscle fibers (P = 0.02) in the m. longissimus dorsi than A/G and G/G lambs. This supports the idea that a reduction of functional MSTN protein is associated with an increase in the number of skeletal muscle myofibers. We are yet to confirm if there is a sustained allele specific reduction in MSTN protein in muscle or blood of lambs.

Key Words: myostatin, lambs, myofibres

P4021 Heritability estimates for hourly measures of methane emissions. J. C. McEwan, S. M. Hickey,*

E. Young, K. Dodds, S. MacLean, G. Molano, E. Sandoval, and C. Pinares-Patino, *AgResearch Limited.*

To select animals for reduced methane emissions, a rapid and low cost measurement method is essential. Our objective was to determine the genetic parameters of methane emissions across 1-h periods and the genetic correlations of these rapid measures with 24-h total emissions. An existing PGgRc trial measured methane emissions, at 5 min intervals, of 684 sheep placed in respiration chambers for 2 d, with repeat measurements several weeks later for another 2 d. Methane outputs at 1-h intervals were calculated for CH_4(g/day) and CH_4/kgDMI for each of 4 d. Single trait ASReml repeatability models were used to obtain estimates of heritability for each hourly period. Estimates for CH_4 averaged 0.20 ± 0.07 (range 0.12 ± 0.06 to 0.31 ± 0.08), and for CH_4/kgDMI averaged 0.08 ± 0.05 (range 0.02 ± 0.03 to 0.17 ± 0.06). The genetic correlations between each 1-h measure and the total 24-h period averaged 0.89 ± 0.07 for CH_4 and 0.76 ± 0.21 for CH_4/kgDMI. These results indicate there is genetic variation between animals for methane emission traits and that rapid measures are highly correlated genetically with 24-h period measures. Measurement error for shorter time intervals, however, led to genetic correlations that are less than 1. For use in industry, several short-term measurements spread over the first year of life are likely to be cheaper and more accurate than a single 24-h measurement.

Key Words: methane emission, sheep, heritability

P4022 Genome wide association study for fatty acid composition in Japanese Black cattle. A. Ishii*[1], K. Yamaji[1], Y. Uemoto[2], E. Kobayashi[2], N. Sasago[2], N. Kobayashi[3], T. Matsuhashi[3], S. Maruyama[3], H. Matsumoto[1], S. Sasazaki[1], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan, [2]National Livestock Breeding Center, Nishigo, Fukushima, Japan, [3]Gifu Prefectural Livestock Research office, Takayama, Gifu, Japan.*

Fatty acid composition is an important trait in the eating quality of meat in cattle. The objective of this study is to identify genomic regions associated with fatty acid composition. We selected 468 animals from a Japanese Black cattle population based on pedigree and phenotypic information and these animals were genotyped using 50K SNP array. A total of 40,657SNP 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 fatty acid composition (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 and C18:2). In the analysis, we also evaluated the effects of 2 previously reported SNP in fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) gene. Association analysis revealed that 30 significant SNP (13 for C14:0, 7 for C14:1, 5 for C16:1 and 5 for C18:1) were located in the BTA19: 44–52Mb region. FASN gene was mapped within this region but the effect of FASN was not observed. We also detected one significant SNP for C18:1 on BTA23 and 2 SNP for C16:0 on BTA25. The region around 17Mb on BTA26 harbored 2 significant SNP for C14:1 and SNP in SCD located in this region showed the strongest association with C14:1. These results suggested new candidate regions in BTA19, 23 and 25 for fatty acid composition.

Key Words: cattle, fatty acid composition, GWAS

P4023 QTL analysis for teat number in an F2 intercross between Landrace and Korean native pigs. Shil Jin*[1], Chae-Kyoung Yoo[2], Jae-Bong Lee[2], Hee-Bok Park[2,3], Hyun-Tae Lim[2,3], and Jun-Heon Lee[1], *[1]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea, 2BK21, Division of Applied Life Science, Gyeongsang National University, Jinju, Korea, [3]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Korea.*

A genome-wide linkage analysis was performed to identify the quantitative trait loci (QTL) that influence teat numbers in an F2 intercross between Landrace and Korean native pigs. The traits were measured in more than 960 F2 offspring. All experimental animals were genotyped using 173 microsatellite markers located throughout the pig genome. The GridQTL program (http://www.gridqtl.org.uk), based on the least square regression model, was used to conduct the QTL analysis. We identified 5 genome-wide significant QTL in 2 chromosomal regions (SSC1 and 7) and 8 suggestive QTL in 5 chromosomal regions (SSC1, 3, 8, 10 and 13). In SSC7, a QTL affecting total teat number was detected, having 5.6% of the phenotypic variance, which was the highest test statistics (F-ratio = 61.1 under the additive model, nominal P-value = 1.3×10^{-14}) observed in this study. The QTL identified in this study together with associated positional candidate genes could give important information for identifying the causative mutation(s) underlying the teat number variation in pigs.

Key Words: pig, quantitative trait locus, teat number

P4024 Genomically enhanced EBVs for beef cattle. D. Johnston,* B. Tier, and H-U. Graser, *University of New England, Armidale, NSW, Australia.*

Genomic predicted breeding values (GEBV) generated from high density bovine DNA SNP chips are becoming increasingly more accurate for a range of economically important traits in beef cattle. However to use them optimally in beef breeding requires the

GEBV to be incorporated into existing estimated breeding values (EBV) from BLUP based genetic evaluation systems such as BREEDPLAN. The usefulness of the GEBV depends on the accuracy of the GEBV as a predictor of the trait, that is, the amount of trait genetic variance explained by each GEBV. Currently the Beef CRC is developing genomic prediction equations for a range of growth, carcass, feed efficiency and reproduction traits for temperate and tropical beef breeds. To include GEBVs generated from these prediction equations into BREEDPLAN requires the estimation of the genetic correlations between the GEBVs and traits across a range of breeds. Importantly this needs to be done in animals not used in training and ideally animals should be related to the current population where the predictions will be used. To do this approximately 1300 industry sires from 9 key breeds have been genotyped with the Illumina 50K chip and their progeny records will be used in the estimation analyses. The magnitude of the correlations will determine the contribution of the GEBV to the genomically enhanced EBV in each breed. Incorporating GEBV will allow increased accuracies of EBVs at an earlier age, particularly for those traits that cannot be measured on young selection candidates.

Key Words: genetic evaluation, genomic breeding values, EBV accuracies

P4025 Genetic analysis of feral horse population from Brittany Triangle, British Columbia. E. G. Cothran[1], W. P. McCrory[2], and R. Juras*[1], *[1]Texas A&M University, College Station, TX, USA, [2]McCrory Wildlife Services, New Denver, British Columbia, Canada.*

There is widespread historic documentation that the common ancestral lineage of wild horses originally found in the Americas came from Spanish horses brought over from Spain in the early 1500s. It is assumed that their presence with First Nations in the Chilcotin area of British Columbia in the early 1800s was the result Spanish-derived horses brought along native trade routes from Plateau grassland areas to the south. Based on this evidence, it was decided to test the hypothesis that Chilcotin-Brittany Triangle horses came from Spanish bloodlines. Today wild horses in this remote wilderness enclave are considered the remotest surviving population left in mainland Canada. As this threatened population has been semi-isolated for several centuries by major river valleys, tests were also done for other genetic uniqueness. Genetic background was analyzed using a set of 15 microsatellite markers and mitochondrial DNA sequence variation. MtDNA sequencing (n = 32 random samples) revealed 3 unique haplotypes. Microsatellite data was used to assess levels of genetic diversity within the population and for phylogenetic analysis to compare with data from 85 other domestic and feral horse breeds. Preliminary results show closest resemblance to the Canadian Horse breed.

Key Words: horse, conservation, genetic variation

P4026 SNP-based parentage assignment in sheep: Application in Australian flocks. J. Kijas*[1], J. van der Werf[2,3], M. Ferdosi[2,3], A. Bell[1,3], S. Gill[3,4], K. Gore[3,5], F. Driver[4], and J. Maddox[6], *[1]CSIRO Livestock Industries, Brisbane, QLD, Australia, [2]University of New England, Armidale, NSW, Australia, [3]Sheep Cooperative Research Center, Armidale, NSW, Australia, [4]Meat Livestock Australia, Sydney, NSW, Australia, [5]Animal Genetics and Breeding Unit, University of New England, Armidale, NSW, Australia, [6]Port Melbourne, Melbourne, VIC Australia.*

The ability to correctly assign parentage is important to producers. The advantages of DNA based methods are well known, but industry uptake is conditional on cost effectiveness. We sought to develop and evaluate multiplexed SNP sets for their power to deliver parentage. 383 SNP were formatted into 6 multiplexes for Sequenom genotyping. We included SNP from the parentage panel developed by the International Sheep Genomics Consortium and markers for polled / horn. Blood cards were collected from 7 industry flocks (~2000 sheep). All animals were genotyped for each multiplex, before analysis sought to determine the minimum number of SNP sets capable of returning high accuracy assignment. A maximum likelihood based approach was applied and thresholds for assignment were defined using simulation within each flock. Under stringent thresholds (low type 1 error) the percentage of parent – offspring pairs correctly assigned using 3 multiplexes gave a high assignment rate. The results demonstrate that as few as 150 SNP can deliver accurate parentage within Australian flocks. Given the SNP have high minor allele frequency within international breeds, the results are likely to be replicated within other populations. The genomic location and identifier for each SNP is given to promote the uptake of a standardized test and encourage high volume and low unit cost genotyping.

Key Words: sheep, SNP, parentage

P4027 A real-time PCR-based method to detect and quantify bovine content in buffalo derived products. M. G. Drummond[1], B. S. A. F. Brasil[1], L. S. Dalsecco[1], R. S. A. F. Brasil[2], M. Y. Kuabara*[1], and D. A. A. Oliveira[1], *[1]Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]Escola de Engenharia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

Fraudulent species substitution in food products is a reality in many markets throughout the world. Molecular markers are useful tools to authenticate food content and prevent these occurrences, thus ensuring food safety. PCR based techniques fit the requirements of sensitivity and specificity and are currently widely used in forensic science. Real-time PCR is the method of choice when aiming to quantify contamination levels. We describe

a method for calculating bovine and buffalo content in food products using real-time PCR with sets of primers/probe designed to specifically amplify bovine or buffalo DNA. Amplification efficiencies showed satisfactory levels for both sets using either Taqman or SYBR Green systems in dairy or meat products. To correct potential deviations between real and obtained quantifications caused by biological differences among the involved species, we made use of a calibration curve, a set of points of controlled admixtures of bovine and buffalo material. The use of the proposed calibration curve for dairy samples always approximated the obtained to the expected quantification values. The technique was tested in commercial samples and presented efficacy and reliability appropriate for routine analysis of buffalo or bovine derived products. The method is subject of a patent document at Brazilian Patent Office and is protected under Brazilian specific regulations.

Key Words: food authentication, species substitution, buffalo

P4028 New SNPs in porcine MYF5 gene and associations with the muscle fiber characteristics and economic traits. E. A. Lee,* J. M. Kim, K. S. Lim, J. H. Kang, and K. C. Hong, *College of Life Sciences, Korea University, Seoul, South Korea.*

Muscle fiber characteristics are one of the candidate traits to improve both lean meat production ability and meat quality which are important porcine breeding goals. The MYF5 which belongs to the MyoD gene family can be considered a candidate gene responsible for the muscle fiber characteristics, due to key role in the initiation and development of skeletal muscle. The aim of this study was to find novel SNPs in porcine MYF5 gene by the association analysis with muscle fiber characteristics and related economic traits. By the direct sequencing for 4 breeds (Berkshire, Landrace, Duroc and Yorkshire), 19 SNPs were newly founded among the detecting 21 SNPs in the MYF5 gene. Two SNPs (c.-2089T>C and c.502–104G>C) were genotyped by the PCR-RFLP method using a total of 403 pigs of Berkshire pigs (n = 168, 75 castrated males and 93 females) and Yorkshire (n = 235, 83 castrated males and 152 females) pigs. While the c.-2089T>C had not any significant effects on measured traits, the c.502–104G>C was significantly associated with type I fiber area composition ($P = 0.0264$) and backfat thickness ($P = 0.0008$). Further studies about gene expression analysis will be performed to clarity the molecular function of the SNP.

Key Words: pig, MYF5, muscle fiber characteristics

P4029 Transthyretin and its effect on myogenic genes during myogenesis. Eun Ju Lee*[1,3], Abdul R. Bhat[1,3], Smritee Pokharel[2], and Inho Choi[1,3], *[1]School of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea, [2]Department of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea, [3]Bovine Genome Resources Bank, Gyeongsan, Republic of Korea.*

The capacity of skeletal muscle cell to proliferate and differentiate lies in muscle satellite cells (MSCs) present under the basal lamina. Several regulatory factors such as MyoD, myogenin, MRF4 etc. are known to be responsible for differentiation of MSCs into myotube-formed cell (MFC), but the complete molecular mechanism involved is yet to elucidate. Thus, we carried out microarray analysis in bovine MSCs and identified transthyretin gene (TTR) as one of the upregulated genes during myogenesis. TTR is a known transporter of thyroxine and retinol. Higher induction of TTR was observed on 6th day of cells culture in 2% FBS media. To confirm the role of TTR in myogenesis, knock-down of TTR gene was performed in C2C12 myoblast cell line using commercially available shRNA construct. When compared with the vector, approximately 60% reduction in myotube formation was observed in the transfected cells. Comparative gene studies showed decrease in myogenin and myosin light chain 2 (MYL2) expressions. Furthermore, the gene study of myogenin shRNA transfected cells revealed a decrease in MYL2 gene expression. However, no effect was found in TTR expression. In conclusion, the study on TTR is involvement during muscle differentiation imparts a new insight toward bovine muscle myogenesis.

Key Words: transthyretin, myogenin, muscle differentiation

P4030 Non-human DNA testing in the forensic sciences: Ensuring best practice. A. Linacre,* *Flinders University.*

The use of non-human DNA typing in forensic science investigations, and specifically that from animal DNA, is ever increasing. Typically there are 2 types of DNA testing performed. There is frequently a request to identify an unknown sample to species level; examples include the identification of endangered species. The second test performed is to link unknown material to a particular organism, population or geographical origin. Examples of this second type include the use of species-specific STR and SNP loci. There is currently a perceived lack of standardization of methodologies used in the forensic analysis of animal DNA or in reporting of results. There is little justifiable reason why standards in DNA typing of non-human DNA samples for the criminal justice system should be lower than when applying to human DNA. An International Society of Forensic Genetics Commission examined the use of non-human DNA in criminal investigations and made 14 recommendations with the aim to encourage high

quality work in the areas of non-human DNA testing. Following the NAS Report in the US, the Society of Wildlife Forensic Science was established along with a pioneering Certification process. This presentation will consider the ramification of the ISFG and NAQS reports and outline steps toward ensuring best practice.

Key Words: forensic, non-human DNA, best practice

P4031 Making your process, evidence, and reports bullet-proof. C. Lindquist,* *University of California at Davis, Veterinary Genetics Laboratory Forensic Unit, Davis, CA, USA.*

While laboratory accreditation provides a solid framework for ensuring that DNA test results will stand up in court, it is a process that takes a dedicated effort and a large commitment of time and resources. That level of oversight may be unrealistic for those laboratories that perform forensic testing infrequently. This presentation will cover practical steps that laboratories can take to strengthen their procedures and assure that their reports are accepted by the courts. The points covered will include documentation, evidence handling, security and reviews. In addition to providing sensible approaches to addressing these vulnerable points, resources that are becoming available through the work of the US-based SWGWILD (Scientific Working Group for Wildlife Forensic Science) will be discussed. This poster represents a visual presentation of the information presented in the Animal Forensic Genetics Workshop.

Key Words: forensic, accreditation, quality assurance

P4032 Optimization of a canine fecal extraction method. C. Lindquist,* T. Kun, and E. Wictum, *University of California at Davis, Veterinary Genetics Laboratory Forensic Unit, Davis, CA, USA.*

Many challenging sample types are encountered in the forensic laboratory. While human DNA crime laboratories may not encounter fecal samples often, they are a familiar sample type in non-human laboratories due to their prevalence in the environment. Fecal matter has many inhibitors, inorganic compounds, and other contaminants that interfere with amplification success as well as a relatively low number of nucleated cells from the defecator. Previous studies on extracting DNA from fecal matter have focused on isolating defecator DNA, obtaining DNA from the bacteria or pathogens present in the stool, or determining diet sources. Few studies have specifically addressed canine fecal matter and the challenge of obtaining high quality and high quantity nuclear DNA for individual profiling. Here we present our studies determining the optimum sampling and extraction methods for canine feces. After a comparison of our in-house fecal extraction, a modified version of that extraction, and commercially available kits, we established that the greatest yield of high quality DNA was obtained using our standard VGL-Fecal extraction protocol on 30–50 mg of fecal matter from the external surface of a stool that had been dried for 24 h. By optimizing our sampling, preservation and extraction procedure, we have achieved a consistent method of obtaining the maximum yield of high quality canine DNA from fecal samples.

Key Words: canine, feces, extraction

P4033 The association of FASN and SCD genes with fatty acid composition in broiler chicken. Dyah Maharani*[1,2], Cheorun Jo[1], and Jun-Heon Lee[1], *[1]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea, [2]Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia.*

Fatty acids (FAs) were considered in activating nuclear hormone receptors that play roles in the cellular lipid metabolism by the regulation of several genes. In this study, 2 FA related genes, fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) genes were used to identify the association with FA composition in broiler chicken. The initial study indicated one single nucleotide polymorphism (SNP) in FASN gene (SNP g.1222 A>G-intron 42) and 2 SNPs in SCD gene (SNP g.3728A>G-exon2; SNP g.12903G>A-exon 4) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The g.3728A>G SNP in SCD gene, was significantly associated with myristoleic acid (C14:1; $P < 0.05$), palmitic acid (C16:0; $P < 0.05$), palmitoleic acid (C16:1; $P < 0.05$) and saturated FA (SFA; $P < 0.05$). However, the SNP g.1222 A>G in FASN gene gave only suggestive association with higher arachidic acid (C20:0; $P = 0.08$). These first findings suggest that the SNP in exon 2 of SCD gene might useful marker to improve the breeding design to select birds having desirable FA composition in broiler.

Key Words: FASN, fatty acid composition, SCD

P4034 A candidate gene approach to investigate milk fatty acids and conjugated linoleic acid composition in Italian Brown Swiss cattle. F. Maretto*[1], C. Ribeca[1], A. Cecchinato[1], M. Mele[2], A. Rossoni[3], and G. Bittante[1], *[1]Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy, [2]Department of Agronomy and Agroecosystem Management, University of Pisa, Via S. Michele degli Scalzi 2, 56124 Pisa, Italy, [3]ANARB — Italian Brown Cattle Breeders' Association, Loc. Ferlina 204, 37012 Bussolengo (VR), Italy.*

The genetic variation in bovine milk fat composition has been investigated in several breeds. Despite their importance on milk quality, individual data on milk fat composition are not routinely collected in milk recording schemes due to their high phenotyping cost. Recent works indicated that short to medium chain (C4:0 to C16:0) fatty acids (FA), directly synthetized in the mammary gland, have a moderate to high hereditability while longer chain FA (C16 and higher), mainly originating from the diet and from endogenous lipids, have lower hereditability. The aim of this study was to investigate the association of 33 candidate genes, belonging to fat synthesis and metabolism pathways as well as genes involved in milk quality traits, with 47 individual FA traits. The analysis was performed on 1,271 Italian Brown Swiss cows for which individual milk fat composition was measured by gas chromatography including major and minor FA, such as saturated FA (C4:0 to C24:0), (cis9) monounsaturated FA (C10:1 to C18:1), polyunsaturated FA and conjugated linoleic acid (CLA). Results indicate that 27 genes were significantly associated with at least one of the traits analyzed. Among others the leptin (LEP), stearoyl-CoA desaturase (SCD), prolactin (PRL), prolactin receptor (PRLR), ATP-binding cassette (ABCG2) acetyl-CoA carboxylase α (ACACA), Lipin1 (LPIN) and the oxidized low density lipoprotein receptor 1 (OLR1) genes were significantly associated ($P < 0.05$) with one ore more of the aforementioned traits. Although further validation of the SNP is required, their association with FA traits could be exploited in gene assisted selection schemes to improve FA composition and quality in milk.

Key Words: milk, fatty acids, SNP

P4035 The SNP in the promoter region of the bovine ELOVL5 gene is effective on subcutaneous fat thickness. H. Matsumoto*[1], Y. Shimizu[1], A. Tanaka[1], T. Nogi[2], I. Tabuchi[2], K. Oyama[1], H. Mannen[1], and S. Sasazaki[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyougo, Japan, [2]Tottori Prefectural Agriculture and Forest Research Institute Livestock Research Center, Kotoura, Tottori, Japan.*

Fatty acid composition has become an important trait in the beef industry and genetic markers associated with fatty acid composition in beef are required. ELOVL genes encode for the enzymes that play an important role in elongation of long-chain fatty acids. In this study, we performed DNA polymorphism search in the CDS of the ELOVL genes. Furthermore, the promoter regions of ELOVL5 and 6 were analyzed, since the ELOVL5 and 6 contribute to the production of major fatty acids in cattle meat. As a result, 5 synonymous mutations were detected in the CDS regions of the ELOVL genes. Six polymorphisms were also identified in the promoter region of the ELOVL5. Two SNPs in the promoter region of the ELOVL5 were expected to alter the ELOVL5 expression and influence the economic traits, because of the high synteny of the region between cattle and mouse and the presence of the transcriptional factor SRE-1. The ELOVL5 g.-110T>C was effective on the plural economic traits including subcutaneous fat thickness ($P < 0.05$) in a Japanese Black population. Subcutaneous fat thickness of animals having T allele of the ELOVL5 g.-110T>C in the group (T/T type: 2.39 cm and C/T type: 2.35 cm) was thinner than that of the animals with C/C allele (2.68 cm). Our results suggest that the ELOVL5 g.-110T>C is a useful genetic marker for the breeding of beef cattle.

Key Words: ELOVL5, subcutaneous fat thickness, promoter

P4036 Genomic signatures of selection in the horse. Jessica L. Petersen, James R. Mickelson,* Stephanie J. Valberg, and Molly E. McCue, *University of Minnesota, St Paul MN USA.*

We have used genome-wide SNP data from more than 20 horses from 33 breeds to identify putative genomic regions under selection in the horse. Such loci were identified using the FST-based statistic (di) calculated in sliding 500 kb windows. This statistic detects locus specific deviation in allele frequencies for each breed relative to the genome-wide average of pair-wise FST summed across breeds. Numerous potential targets of selection were identified, and analysis of breeds fixed for the chestnut coat color mutation demonstrated the utility of this method. One striking feature of these genome scans was a 6 Mb region on ECA18 with a highly significant di value in the Quarter Horse. Further analysis of the region revealed a ~1 Mb conserved haplotype surrounding the MSTN gene that is present in 92.8% of QH and 50% of Thoroughbreds, but rare (<1%) in all other breeds. MSTN variants including a promoter SINE insertion and an intronic SNP are significantly associated with the conserved haplotype. Histological data from 79 horses shows a significant association of muscle fiber type proportions with the MSTN polymorphisms. Another striking result was that the gaited breeds, including the Standardbred, Icelandic, Peruvian Paso, and others, share a highly conserved haplotype on ECA23 under a strong signal of selection that contains a polymorphism demonstrated to be important in the ability to gait. Further, conserved haplotypes underlying signals of selection on ECA11 in the Belgian, Percheron, Shire, Clydesdale, and Miniature horse suggest the presence of a locus or loci important in the determination of size. Numerous other loci await a detailed evaluation. Mapping signatures of selection in the modern horse is the first step in the identification of genes important in the domestication and specialization of modern horse breeds.

Key Words: selection, equine, breeds

P4037 Frequency of severe combined immunodeficiency and cerebellar abiotrophy carriers in Sweden. Louise Hübinette and Sofia Mikko,* *Animal Breeding and Genetics, Uppsala, Sweden.*

Severe combined immunodeficiency (SCID), and cerebellar abiotrophy (CA) are 2 autosomal recessive lethal traits predominantly found in Arabian Horses and their crosses. Neither SCID nor CA carriers show any signs of the disease. SCID-affected foals lack a cell- and antibody mediated immune defense, and will die within a few months, whereas the CA-affected foals develop symptoms some weeks after birth. The SCID-mutation is a 5 bp deletion that produces a defect V(D) J-recombinase (Shin et al. 1997). In 2001 the frequency of carriers in Sweden were estimated to be about 0.02. In this study, we performed a new screening of the Swedish population of Arabian Horse foals born in 2009–2010. The frequency of carriers was estimated to be about 0.01–0.04. Thus, the frequency is still quite low but there has been no reduction in carrier frequency during this time. The signs of CA include neurological symptoms, resulting from degenerated Purkinje cells in the cerebellum. Penedo et al., recently published a possible causative mutation within the gene TOE1. In this study we screened the Swedish population of Arabian Horses for this CA SNP. The frequency of carriers was estimated to be about 0.18–0.25, similar to the situation in USA. Thus, if no DNA-testing were performed before breeding, about 0.01–0.02 affected foals would be expected to be born.

Key Words: SCID, CA, carrier frequency

P4038 Association of mastitis candidate genes with somatic cell counts in South African Holstein breeds. Z. E. L. Sikhosana[1], K. Kanyile[1], T. Dugmore[2], F. C. Muchadeyi*[3], and E. F. Dzomba[1], *[1]Discipline of Genetics, University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa, [2]Cedara College of Agriculture, Pietermaritzburg, KwaZulu-Natal, South Africa, [3]Biotechnology Platform, Agricultural Research Council, Pretoria, Gauteng, South Africa.*

Mastitis causes significant losses in milk yield and revenue to the dairy industry. Selection of cows genetically resistant to mastitis infection could be used for its control. The objective of this study was to investigate polymorphisms at the Toll-like receptor 2 (TLR2) and Lactoferrin (LTF) genes and their association with somatic cell score (SCS) is South African dairy Holstein cows. PCR-RFLP was used to detect genotypes of the TLR2 and LTF genes in a herd of 167 multiparous Holstein cows using ECORV and ECOR1 restriction enzymes respectively. Results showed that the T and G alleles for TLR2 were predominant. TRL2 allele frequencies were estimated as 74.1% T and 25.9% G. LTF alleles were observed at 76.5% A and 23.5% B, respectively. The Chi-squared test for goodness of fit between the observed and expected genotypic frequencies revealed that the population was not in Hardy-Weinberg equilibrium ($P > 0.05$) for both TLR2 and LTF genotypes. Genotypes of both genes significantly showed an association with SCS ($P > 0.05$). For the LTF gene, AB cows had the highest SCS while AA had the least. The TLR2 TT and TG genotype cows had significantly higher SCS than those of GG genotype ($P > 0.05$). Results present a scope to use LTF and TLR2 genes in marker assisted selection programs for mastitis resistance in South African Holstein cows.

Key Words: mastitis, candidate genes, marker assisted selection

P4039 Investigation of Holstein and Indigenous crossbreds performance in Iran. Jamshid Ehsani Nia*[1] and Mohammad Moradi Shahrbabak[2], *[1]University of Guilan, Rasht, Iran, [2]University of Tehran, Karaj, Iran.*

The objective of this study was to investigate performance of cattle crossbred considering productive traits using data collected between 1991 to 2003 by Animal Breeding Center. Crossbred animals were resulted of Holstein × Indigenous (H×I) crossbreeding systems in Iran. Milk and fat yield, fat percentage, lactation length were considered in this research. Genetic parameters were estimated using animal model (single traits and repeatability) and Derivative-Free Restricted Maximum Likelihood procedure for different traits. The estimated heritability and repeatability for milk and fat yield, fat percentage, lactation length were 0.261–0.3769 and 0.73, 0.36–0.725 and 0.72, 0.308–0. 486 and 0.48, 0.238–0.267 and 0.397, respectively for single and repeatability models. Esimates of breed, individual and maternal heterosis effects carried out with Weight Least Square method. These effects for were 20.9, 15.32, 5.55 kg for milk yield; 867, 651, 257 gr for fat yield; 0.025, 0.039, 0.002 for fat percentage; 1.79, 2.08, 0.105 days for lactation length respectively. The results showed that production potentials have been increased by crossbreeding, and crossbred animals with 50-87.5% of Holstein or Brown Swiss blood rations have showed higher performance.

Key Words: genetic parameter, crossbred cattle, heterosis

P4040 Microsatellite markers based population genetic analysis of commercial cattle breeds raised in Brazil. E. G. A. Coelho, B. S. A. F. Brasil, and D. A. A. Oliveira,* *Escola de Veterinária da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

Brazil holds the largest commercial cattle populations worldwide. The objective of this study was to

assess the levels of genetic diversity, phylogenetic relationships and patterns of taurine/zebuine admixture among 9 cattle breeds raised in Brazil. The cattle breeds analyzed were Brahma, Gyr, Girolando, Guzerat, Holstein, Jersey, Nellore, Senepol and Tabapuã. DNA polymorphism analysis was carried out with a total of 2,965 animals using a set of 11 microsatellite markers recommended by the International Society of Animal Genetics (ISAG). Despite the high genetic diversity detected, significant inbreeding was observed within some breeds and heterozygote excess was detected in others. Differences among breeds accounted for 14.72% of the total genetic variability. Most breeds clustered separately when the number of pre-defined populations was 9, with the exception of some closely related breeds that shared the same cluster and others that were admixed. Of note, Nellore cattle presented high levels of admixture, which is consistent with the history of frequent gene flow among zebuine populations during the establishment of this breed in the 20th century in Brazil. In conclusion, the genetic characterization of the 9 Brazilian commercial breeds analyzed and systematic use of molecular markers will facilitate the comprehensive management and aid in breeding programs of these populations. Financial Support: CNPq/Brazil (INCT 573899/2008-8) and FAPEMIG/Brazil (INCT APQ-0084/08).

Key Words: Brazilian cattle breeds, microsatellite markers, genetic diversity

P4041 GWAS for dagginess in New Zealand dual-purpose sheep. N. K. Pickering*[1,2], B. Auvray[1], H. T. Blair[2], R. E. Hickson[2], P. L. Johnson[1], and J. C. McEwan[1], *[1]AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand, [2]Institute of Veterinary and Biomedical Sciences, Massey University, Palmerston North, New Zealand.*

Dagginess or fecal soiling around the breech is a trait of interest for New Zealand sheep breeders/farmers due to removal costs and association with flystrike. To assess if there were any genomic regions associated with dagginess, a genome wide association study (GWAS) was performed. A dual-purpose New Zealand industry data set of 3.5M animals of which 8,639 animals (mainly sires) were genotyped with the Illumina OvineSNP50 BeadChip. De-regressed estimated breeding values (EBVs) with parent average removed for dags at 3 and 8 mo (LDAG, ADAG) were used as phenotypes. Only those EBVs with reliabilities greater than 0.8*h2 were used consisting of 2,800 animals with LDAG EBVs and 2,114 with ADAG EBVs. GWAS was then carried out using a 2-step procedure. First, a weighted BLUP animal model was fitted to the phenotypes, using a genomic relationship matrix calculated from the SNPs to structure the variance of the direct genetic random effect, and the first 6 principal components (PC) of this relationship matrix fitted as fixed effects to account for population structure. Second, the residuals from the BLUP animal model were analyzed using an ordinary weighted least square linear regression, fitting each SNP consecutively. The weighting factors used to weight each observation i for both steps are r2i / (1 – r2i). Significant SNPs ($P < 0.001$) were detected for LDAG and ADAG, on chromosome 6 and 15 respectively.

Key Words: dagginess, GWAS

P4042 A new DNA test for polled status in Australian beef cattle breeds. E. K. Piper*[1,2], J. M. Henshall[1,3], S. W. Corley[1,2], and B. Little[1,4], *[1]The CRC for Beef Genetic Technologies, Armidale, NSW, Australia, [2]The University of Queensland, Gatton, QLD, Australia, [3]CSIRO Livestock Industries, Armidale, NSW, Australia, [4]CSIRO Livestock Industries, Brisbane, QLD, Australia.*

Selecting for genetically polled cattle is an attractive alternative to dehorning, a practice that can have significant welfare implications for the animal. In a project undertaken by the CRC for Beef Genetic Technologies a microsatellite marker (CSAFG29) was discovered that in Brahman cattle was strongly associated with the horned/polled phenotype. The marker has now been tested across a range of *Bos indicus* and *Bos taurus* breeds totalling around 3,000 individuals. One allele at CSAFG29 (303 bp in size) accurately predicted poll status in approximately 85–90% of the Brahman, Santa-Gertrudis, Droughtmaster and Hereford animals tested. Other alleles observed at the CSAFG29 locus (35 in total) were associated with horns. However, Allele 303 was not present in a moderate to large proportion (30–50%) of polled Limousin, Simmental, Shorthorn and Charolais cattle. At least one copy of another allele (305 bp in size) was present in these individuals and consequently Allele 305 has associations with both polled and horned alleles. These results suggest that further development of the marker and / or incorporation of pedigree data are needed in Limousin, Simmental, Shorthorn and Charolais cattle for this test to be useful in breeding programs. The test is, however, very useful for Brahman, Santa-Gertrudis, Droughtmaster and Hereford where the frequency of Allele 305 is relatively low.

Key Words: polled, horned, microsatellite

P4043 Evaluation of bovine high-density genotyping platforms in Japanese Black cattle. L. R. Porto Neto*[1], E. K. Piper[1], J. F. Garcia[2], C. P. VanTassell[3], and T. S. Sonstegard[3], *[1]The University of Queensland, School of Veterinary Science, Animal Genetics Laboratory, Gatton QLD 4343, Australia, [2]Universidade Estadual Paulista (UNESP), Rua Clovis Pestana 793, Aracatuba, SP, Brazil, [3]United States Department of Agriculture, Agricultural Research Service, Bovine Functional Genomics Laboratory, Beltsville MD 20705, United States of America.*

Two platforms are available for high-density SNP genotyping on genome-wide scale in cattle, the Illumina 777K (Illm) and the Affymetrix BOS1 (Affy). We tested the performance of both platforms using Australian Japanese Black cattle. This breed has small effective population size, and has been intensively selected for many years, defining a challenging situation to any genotyping platform. Thirty animals were genotyped using the Illm and Affy, 28 animals in common. All samples had call rate > 0.95. First, SNP were filtered for a call rate ≥ 0.9, 99% of SNP were retained in both platforms (769,640 Illm and 616,325 Affy). SNP were then filtered for MAF > 0.01, retaining 379,023 SNP from Affy (61%) and 697,773 from Illm (90%). This defined a considerable difference between platforms for average gap between markers (AG) = 7.0 vs 3.8 Kbp, median gap (MG) = 3.7 vs 2.8Kbp, and max gap (MAX) = 2,732 vs 1,165 Kbp, respectively. Finally, linkage disequilibrium-based filters were applied to the data sets (r^2 > 0.5 and 0.9). This procedure removed less SNP from the Affy data set than from the Illm, and generally resulted in large but comparable AG and MG between platforms. MAX was still larger on Affy (4,032 vs 1,693 Kbp). In summary, both platforms produced high-quality genotypes, the Affy was very sensitive to MAF, and after LD pruning comparable sets of markers were produced.

Key Words: polymorphism, Wagyu, livestock

P4044 Bovine SNP50K bead chip for genome wide association study in South African beef cattle. S. O. Qwabe*[1,3], A. Maiwashe[1], F. C. Muchadeyi[2], and E. van Marle-Koster[3], *[1]Agricultural Research Council, Private Bag X2, Irene 0062, Pretoria, South Africa, [2]Biotechnology Platform, Agricultural Research Council, Private Bag X5, Onderstepoort, Pretoria, South Africa, [3]Department of Animal and Wildlife Sciences, University of Pretoria, Private Bag x20, Hatfield 0028, Pretoria, South Africa.*

Genome wide association studies (GWAS) in beef cattle are providing new ways of identifying genetic markers associated with traits of economic importance. These studies usually use Illumina Bovine SNP50K bead chip, which features 54 609 informative single nucleotide polymorphisms (SNPs) that uniformly span the entire bovine genome. However before genome wide association analyses are conducted, it is essential that data quality control is performed for both SNP markers and DNA samples for successful application of these studies. This allows identification and removal of DNA samples and SNP markers that could introduce bias in the analyses. Data quality control for SNP markers and DNA samples was conducted for 54 609 SNP genotypes on 51 animals (29 Nguni, 12 Angus and 10 Nguni x Angus cross) genotyped using the Illumina Bovine SNP50 bead chip and Illumina Infinium assay II. Quality control criteria was performed by identifying and removing SNPs and DNA samples with less than 95% call rate, samples with more than 10% missing genotypic data, SNPs having minor allele frequency less than 0.05 and SNPs not in Hardy Weinberg Equilibrium. Results of the data quality control are presented in this paper. SNPs considered in this study will form part of the GWAS aimed at identifying SNP markers associated with carcass and feedlot traits in South African beef cattle.

Key Words: quality control, SNP markers, GWAS

P4045 Exclusion of gene HEG1 as receptor locus for the *E. coli* F4ab/F4ac. A. Rampoldi*[1], H. U Bertschinger[1], E. Bürgi[2], P. Vögeli[1], C. B. Jørgensen[3], M. J. Jacobsen[3], and S. Neuenschwander[2], *[1]Institute of Animal Sciences, ETH Zurich, Zurich, Switzerland, [2]Department of Farm Animals, University of Zurich, Zurich, Switzerland, [3]Department of Basic Animal and Veterinary Sciences, University of Copenhagen, Frederiksberg, Denmark.*

Enterotoxigenic *E. coli* is the major cause of diarrhea among piglets. The bacteria adhere to the enterocytes by adhesive fimbriae. Fimbrial type F4 is one of the most prevalent in the world and shows 3 antigenic variants: F4ab, F4ac and F4ad. In pigs, the resistant or susceptible phenotype for fimbriae F4ac is inherited as a monogenetic trait, with the susceptible allele being dominant over the resistant one. The receptor for F4ac or a distinct receptor (F4abR) at a closely linked locus binds F4ab. Current results in sequencing resistant and susceptible pigs suggest that the F4ab/F4ac receptor (F4bcR) locus maps to the HEG1-MUC13-ITGB region (~0.4 Mb). MUC13 is a strong candidate for the F4bcR locus and it has provided genetic markers for selection of F4ac resistant pigs. Recently, we found a pig which showed a recombination in HEG1. The animal was susceptible in the adhesion test, but showed a homozygote genotype associated with resistance for F4ac in SNPs in high linkage disequilibrium with F4bcR. This pig recombined between gene MUC4, located 0.85 Mb proximal of HEG1, and microsatellite KVL1293 located in intron 12 of HEG1. Subsequently, the region between KVL1293 and the 3′ end of HEG1 was sequenced in homozygote susceptible and resistant pigs and no SNPs were found to be in linkage disequilibrium with F4bcR. Our results suggest that the locus for F4bcR is outside HEG1, but closely located to MUC13.

Key Words: F4ac, pig, HEG1

P4046 Association of KCNJ11 gene variants with tenderness in Nelore breed. Polyana C. Tizioto[1], Marcela M. de Souza[1], Mauricio de A. Mudadu[2], Patrícia Tholon[2], Sarah L. C. Meirelles[3], Rymer R. Tullio[2], Renata T. Nassu[2], Antônio do N. Rosa[4], Sérgio

R. de Medeiros[4], Fabiane Siqueira[4], Gelson L. D. Feijó[4], and Luciana C. de A. Regitano*[2], *[1]PPGGEv/UFSCar, São Carlos, São Paulo, Brazil, [2]Embrapa CPPSE, São Carlos, São Paulo, Brazil, [3]Departamento de Zootecnia/ UFLa, Lavras, Minas Gerais, Brazil, [4]Embrapa CNPGC, Campo Grande, Mato Grosso do Sul, Brazil.*

The KCNJ11 (potassium inwardly-rectifying channel, subfamily J, member 11) gene is located in BTA15, near a quantitative trait loci for meat tenderness. In this study, single nucleotide polymorphisms (SNPs) were described in KCNJ11 and associated with Warner – Bratzler shear force (WBSF) at different aging times: 1 d after slaughter (WBSF0), after 7 d (WBSF7) and 14 d (WBSF14) of aging. Fourteen steers of Nelore breed, characterized as extreme for the distribution of WBSF0 in a half-sib population of 500 progenies from 32 sires, were selected for sequencing. Twenty-two SNPs were found and the disequilibrium pattern indicated the SNP 2126C>T as a principal TagSNP. Allele frequency difference between extremes of WBSF0 (Fisher test, $P \leq 0.05$) was found for the SNP 2942T>C and both SNPs were genotyped in the whole population. A mixed model was used to investigate association with WBSF. Associations were found between the SNP 2126C>T and WBSF7 ($P \leq 0.0458$) and for the SNP 2942T>C with WBSF0 and WBSF7 ($P \leq 0.0487$ and $P \leq 0.0356$, respectively). Allele substitution effects of 0.92 g on WBSF0 ($P \leq 0.0487$) and of 0.88 g on WBSF7 ($P \leq 0.0356$) were found for the SNP 2942T>C, with the T allele associated with reduced WBSF. The KCNJ11 gene appears to have an additive genetic effect on meat tenderness and may be useful for marker assisted selection in this breed.

Key Words: cattle, SNP, meat

P4047 Tissue-specific expression of porcine TFAM. Andrej Rencelj* and Peter Dovc, *Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Domzale, Slovenia.*

Mitochondria play a leading role in energy metabolism of mammalian cells. The number of mitochondria in the cell can change in a short time because it depends on energy requirements of the cell. The biogenesis of mitochondria is very complex process and it involves several regulatory proteins; for example, NRF-1 and NRF-2, PGC-1, TFAM, POLG, POLRMT. Irregular expression and mutations the genes coding these proteins can cause abnormal function of mitochondria, leading to the vast range of cell and organ pathologies. The purpose of this study is to develop the gene network regulating mitochondrial biogenesis and to establish their expression profiles in different tissues. Our previous experiments suggested the presence of two different splicing forms for the mitochondrial transcription factor TFAM. Because the expression of TFAM plays an important role in muscle type determination and oxidative potential of different tissues, we developed qPCR assay for quantification of both splice forms in different tissues. Our results confirm presence of the long (whole length transcript) and short (missing exon 4) splice forms in all analyzed tissues (eye nerve, eye muscle, brain, muscle, liver, kidney, spleen). The proportion of the short form in all tissues was very low, except for the m. semispinalis capitis, where the short form represents about 20% of transcripts and eye muscle, where the short form is predominant.

Key Words: TFAM, splicing, mitochondria

P4048 First results on genomic selection in French show-jumping horses. A. Ricard*[1], S. Danvy[2], and A. Legarra[3], *[1]Institut National de la Recherche Agronomique, UMR1313, Jouy-En-Josas, France, [2]Institut Français du cheval et de l'Equitation, Exmes, France, [3]Institut National de la Recherche Agronomique, UR 631, Castanet-Tolosan, France.*

Genomic selection could be highly interesting for horse breeding because it would reduce the currently high generation interval, at a low cost compared with the value of an animal. The aim of this study was to estimate the observed accuracies of genomic estimated breeding values. A sample of 908 stallions specialized in show jumping (71% Selle français (SF), 17% Foreign sport horses (FH), 13% Anglo Arab(AA)) were genotyped. Genotyping was performed using Illumina Equine SNP50 BeadChip and after quality tests, 44444 SNP were retained. From whole population BLUP-based estimated breeding values and their reliability, a specific procedure was developed to obtain de-regressed proofs combining own performances and performances of relatives outside the genotyped sample. Two methods were used for genomic evaluation: GBLUP and Bayes CΠ, and 6 validation data sets were compared, chosen according to breeds SF+FH+AA or SF+FH, family structure (more than 3 half sibs), reliability of sires (>0.97) or sons (>0.72). Results showed low advantage of genomic evaluation. On the validation sample SF+FH+AA, the correlation between deregressed proofs and GBLUP or BayesCΠ predictions was: 0.39, 0.37, 0.51 according to the different validation data sets compared with 0.36, 0.33, 0.53 obtained with BLUP predictions. GWAS analysis would be performed on the same data.

Key Words: horse, genomic selection, jumping

P4049 Association of the single nucleotide polymorphisms in cholecystokinin type A receptor gene with growth traits in Japanese Hinai-dori crossbred chickens. Kazuhiro Rikimaru*[1,2], Megumi Komatsu[1], Daiki Takahashi[1], Keiichi Suzuki[2], Yoshinobu Uemoto[3], Hisato Takeda[4], and Hideaki Takahashi[4], *[1]Akita Livestock Experiment Station, Daisen, Akita,*

Japan, [2]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [3]National Livestock Breeding Center, Nishigo, Hukushima, Japan, [4]National Institute of Livestock ang Grassland Science, Tsukuba, Ibaraki, Japan.

The Hinai-dori is a slow-growing breed of chicken native to Japan. We previously identified QTL for body weight (BW) and average daily gain (ADG) in a common region between MCW0240 (chr 4: 69.9 Mb) and ABR0622 (chr 4: 86.3 Mb) on GGA4 in an F2 resource population produced by crossing low- and high-growth lines of the Hinai-dori breed. We focused our investigation on the cholecystokinin type A receptor (CCKAR) gene as a positional candidate. The association study on the growth traits and CCKAR haplotypes showed that a haplotype was superior to other haplotypes in BW at 10 and 14 weeks of age, ADG between 4 and 10 weeks, 10 and 14 weeks, and 0 and 14 weeks of age. We assumed that a SNP (A/C) at YY1 binding site in the 5′-UTR of CCKAR affect its gene expression and resultantly affect growth traits. In this study, we genotyped the SNP using a mismatch amplification mutation assay and investigated its association with growth traits in a Hinai-dori F2 intercross population. The data showed that A was superior to C in BW at 10 and 14 weeks of age, ADG between 4 and 10 weeks, 10 and 14 weeks, and 0 and 14 weeks of age. Thus, we conclude that the SNP in the 5′-UTR of CCKAR is a useful marker of growth traits and could be used to develop strategies for improving growth traits in the Hinai-dori breed.

Key Words: Hinai-dori, CCKAR, growth traits

P4050 Evaluation of STR set for bovine traceability in the context of Chinese Beef Imports and Argentine-Chinese beef trade. A. Rogberg-Muñoz*[1], S. Wei[2], M. V. Ripoli[1], B. L. Guo[2], D. E. Goszczynski[1], M. H. Carino[1], N. S. Castillo[1], L. Melucci[3], E. Villarreal[3], J. P. Liron[1], J. A. Crespi[1], Y. M. Wei[2], and G. Giovambattista[1], *[1]Instituto de Genética Veterinaria (IGEVET), CCT La Plata – CONICET - Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plta, Provincia de Buenos Aires, Argentina, [2]Key Laboratory of Agro-Products Processing and Quality Control, Ministry of Agriculture, Institute of Agro-Products Processing Science and Technology, Chinese Academy of Agricultural Sciences, China, [3]Unidad Integrada Balcarce (Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria / Facultad Ciencias Agrarias, Universidad Nacional de Mar del Plata), Balcarce, Provincia de Buenos Aires, Argentina.*

Genetic traceability allows individual, breed or species identification. Furthermore, it has a proved use to detect frauds and, to protect and valorise local productions. The objective of the present work was to evaluate 22 STRs in 357 animals corresponding to 4 Chinese populations, and 9 *Bos taurus* and 2 *B. indicus* breeds raised in Argentina, and commonly raised in the whole world. PCA showed that the first PC accounted for 22% of the total variance and differentiate Zebuine from Taurine breeds, and admixtured were intermediate located. The second PC (16% of the variance) distinguished the European from Asiatic Taurine breeds. FST showed significant differences across the populations (FST = 0.12). AMOVA differences among and within populations account for 11.42% and 88.58% of genetic variance. When breeds were grouped according to their origin, AMOVA showed differences among groups of 2.16%, while among populations within groups was 9.82%. Variance within individuals explained 88.02%. For K = 13 Structure clustered all Argentine breeds independently, but Brangus assigned with Angus. Part of Chinese populations shared a common cluster, while the other was wrong allocated as Limousin. The results evidence that it would be possible to differentiate many of the most commonly raised beef breeds from those typically produced in China.

Key Words: beef, china, traceability

P4051 Genome wide association study for residual feed intake in the pig. S. K. Onteru, D. M. Gorbach, J. M. Young, D. J. Garrick, J. C. M. Dekkers, and M. F. Rothschild,* *Department of Animal Science and Center for Integrated Animal Genomics, Iowa State University, Ames, Iowa, USA.*

Residual feed intake (RFI) is the difference between the observed feed intake and the feed requirement for an animal's growth and maintenance, with efficient pigs having negative RFI. A genome-wide association study for RFI using Bayesian variable selection methods had previously been performed using genotypes from the Porcine SNP60 BeadChip on 716 animals from generations 0, 4, 5 and 6 from the ISU low RFI selection and control lines. That previous study reported genome regions on SSC2 and SSC3 having large effects on RFI. In this validation study, data from 312 additional animals from generations 4 and 7 of the ISU-RFI lines were combined with the earlier data, giving a total of 1,042 animals. With the same model as used in the earlier analyses, regions on SSC2 and 3, which had smaller effects in the previous analyses, showed the largest effects in the combined data. Regions on SSC4 and 9 now appear to be associated with RFI and, QTL regions on SSC3 and 4 overlapped between the 2 data sets. The difference between the 2 analyses might be due to increased power from the additional animals. Excluding line from the model did not affect results for the important genomic regions on SSC2, 3, 4, and 9 but identified additional regions on SSC14, 15 and 17. Functional

annotation of these regions revealed genes involved in food intake. Further statistical analyses with additional animals may confirm these results.

Key Words: RFI, pigs, GWAS

P4052 Molecular genetic analysis of sexual diversities in Tenrecs. Martina Safrova,* Barbora Blahova, Marketa Dajbychova, Katerina Saskova, and Katerina Stampachova, *Genomia s.r.o., Pilsen, Czech Republic.*

Tenrecs are extraordinary placental mammals having cloaca with origin in Madagascar and south of Africa. Tenrecs are bred in many zoos around the world. To manage breeding in captivity successfully, it is necessary therefore to know the gender. However, animals do not show sexual dimorphism and it is not possible to distinguish the gender on secondary sexual characteristics. Differentiation is possible only post mortem. In cooperation with Pilsen Zoological and Botanical Garden located in Pilsen, Czech Republic, we made a try to find a PCR based method for sex determination. We analyzed part of genes ZFX-ZFY using P1-5EZ and P2-3EZ primers that were successfully used for sexing of many species. We used samples of *Echinops telfairi*, *Hemicentetes semispinosus*, *Setifer setosus* and *Tenrec ecaudatus*, with post mortem sex determination as control set. We collected unpublished ZFX-ZFY sequences for all four species and compare them with each other and with other species as well. We confirmed that examined parts of ZFX-ZFY genes are conserved in lots of species; however we have not found single nucleotide polymorphism distinguishing ZFX and ZFY in Tenrecs. We found few differences in part of ZFX-ZFY genes in Tenrec eucadatus compared to other members of Tenrecinae subfamily. We have worked on another approach on analysis Y-specific sequence SRY. We used generally available sequences of related species with highly conserved blocks to select suitable primers. These primers positioned to SRY sequences concluded our work. Detailed results on control set will be presented at the ISAG 2012 conference.

Key Words: Tenrecs, sexing

P4053 The SNPs in the ACACA gene are effective on fatty acid composition in Holstein milk. T. Sakamoto*[1], H. Matsumoto[1], K. Sasaki[1], T. Bessho[1], E. Kobayashi[2], T. Abe[2], S. Sasazaki[1], K. Oyama[1], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan, [2]National Livestock Breeding Center, Nishigo, Fukushima, Japan.*

Fatty acid composition is an important economic trait for both dairy and beef cattle and controlled by genetic factors. Acetyl-CoA carboxylase is the flux-determining enzyme in the regulation of fatty acid synthesis in animal tissues. One of 2 isozymes of this enzyme, acetyl-CoA carboxylase-α (ACACA), catalyzes the first committed step of fatty acid synthesis in mammalian cytosol, leading to the biosynthesis of long-chain fatty acids. In the current study, the sequence comparison of the coding sequence (CDS) and 2 promoter regions (PIA and PIII) in bovine ACACA gene was performed between Japanese Black and Holstein cattle to detect nucleotide polymorphisms influencing fatty acid composition in milk and beef. Five single nucleotide polymorphisms (SNPs) were identified in the CDS region, 28 SNPs in the PIA region and 3 SNPs in the PIII region. Association study revealed that the SNPs in the PIII region were effective on fatty acid composition, C14:0 and C16:0, in the milk of the Holstein cattle. The same SNPs were also influenced C18:2 in the meat of the Japanese Black. In addition, the SNP in the CDS region was effective on C18:0 in Holstein milk. Since PIII is the promoter specific to mammary gland during lactation, the altered expression of ACACA gene owing to the SNPs in the PIII region may influence the fatty acid composition in the milk.

Key Words: ACACA, fatty acid composition, cattle

P4054 UTS2R gene polymorphisms are associated with fatty acid composition in Japanese beef cattle. S. Sasazaki*[1], K. Akiyama[1], T. Narukami[1], H. Matsumoto[1], K. Oyama[2], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan, [2]Food Resources Education & Research Center, Kobe University, Kasai, Hyogo, Japan.*

Fatty acid composition of beef adipose tissue is one of important traits because high proportion of monounsaturated fatty acid is related to favorable beef flavor and tenderness. In this study, we searched polymorphisms in full length CDS of urotensin 2 receptor (UTS2R) and investigated the effects on fatty acid composition (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, MUFA, SFA). Eight SNP were identified by sequence comparison among 8 animals including 5 Japanese Black and 3 Holstein cattle. One of these SNP (866C>T) was predicted to cause amino acid substitutions (P289L) and the other 7 synonymous SNP, including c.267C>T, were in linkage disequilibrium. Therefore we selected 2 SNP (267C>T and 866C>T) for further analysis. We investigated associations between these genotypes and fatty acid composition in 3 Japanese Black populations (n = 438, 245 and 287) and Holstein population (n = 202). Tukey-Kramer′s honestly significant difference test revealed that C/C genotype in 267C>T indicated lower C14:0 and higher C18:1 than the other genotypes in Japanese Black cattle and C/C genotype in 866C>T showed lower C16:1 than C/T genotype in Holstein

cattle ($P < 0.05$). These results suggested that UTS2R genotypes would contribute to production of high-grade meat as selection markers in beef cattle.

Key Words: urotensin 2 receptor, fatty acid composition, cattle

P4055 Goat improvement in Africa: Genomic tool development and application in a Feed the Future project. B. L. Sayre*[1], T. S. Sonstegard[2], J. Silverstein[2], H. J. Huson[2], J. Woodward-Greene[2,9], M. Rothschild[3,8], V. Nene[4], D. F. Mujibi[4], S. Kemp[4], C. W. Masiga[5], S. Mubiru[5], J. F. Garcia[6], J. Sölkner[7], and C. P. Van Tassell[2], *[1]Virginia State University, Petersburg, VA, USA, [2]United States Department of Agriculture, Agriculture Research Station, Beltsville, MD, USA, [3]United States Agency for International Development, Washington, DC, USA, [4]International Livestock Research Institute, Nairobi, KENYA, [5]Association for Strengthening Agricultural Research in Eastern and Central Africa, Entebbe, UGANDA, [6]São Paulo State University-UNESP, Araçatuba, BRAZIL, [7]University of Natural Resources and Life Sciences, Vienna, AUSTRIA, [8]Iowa State University, Ames, IA, USA, 9George Mason University, Manassas, VA, USA.*

In Africa, ruminants are essential sources of milk, meat, income and are increasingly becoming a major source of fuel from manure. These animals are main sources of livelihoods for poor people in arid, semi-arid and marginal areas as scavengers where crops cannot be grown. The majority of cattle, sheep and goats found in developing countries have undergone many generations of adaptation and genetic isolation or bottlenecks, which have led to great phenotypic variation between breeds. Identifying genes associated with disease susceptibility and resistance in locally adapted breeds is a "starting point" for initiating genetic improvement programs. These genes are potentially critical for sustainable improvement in production of locally adapted goats. Genomic and genetic studies to find these survival genes have been limited in goats. Of particular interest are the genes involved in resistance to internal parasites and resilience to climate differences. A key aim of this project is to catalyze an international cooperative effort to apply genomic tools to aid characterization of the structure of caprine genomes in locally adapted, native breeds throughout sub-Saharan Africa and identify top crossbreeds for specific environments. This information will aid plans to implement small ruminant improvement programs in Africa, with the goal of improving the livelihoods of livestock keepers and the rural poor. This project is a joint effort initiated by USAID and USDA, with key contributions from ILRI, ASARECA and Farm Africa.

Key Words: goat, genome, adaption

P4056 Comparison of effects of six selection strategies in oval cocoons silkworm pure lines of 110. Alireza Seidavi*[1], Mani Ghanipoor[2], Seyed Ziaeddinmirhosseini Mirhosseini[3], Alireza Bizhannia[2], and Moeinoddin Mavvajpour[2], *[1]Animal Science Department, Rasht Branch, Islamic Azad University, Rasht, Iran, [2]Iran Silkworm Research Center, Rasht, Iran, [3]Animal Science Department, University of Guilan, Rasht, Iran.*

To determination of the most appropriate selection strategy in pure line 110 silkworm, 450 cocoons (225 male and 225 female) were recorded to formation of the base population and then the male and female moths were mated randomly. In next generation, obtained silkworm eggs randomly divided to 6 categories, each category included 12 family groups. Selections were conducted based on (1) random matings (control group), (2) selection based on phenotypical characteristics of cocoon, (3) individual selection based on cocoon weight, (4) individual selection based on shell cocoon weight, (5) the base index (total product economic factor in the corrected phenotype of each trait), and (6) the normal selection index (based on individual records for the 3 traits of cocoon weight, cocoon shell weight and cocoon shell percentage), respectively. The highest average of cocoon weight belonged to strategy 3 (1.446 g) and lowest average belonged to strategy 5 (1.387 g) was ($P < 0.05$). The highest average of cocoon shell weight belonged to strategy 3 (0.296 g) and the lowest average belonged to strategy 1 (0.283 g), the highest average of cocoon shell percentage belonged to strategy 4 (20.67%) and lowest average belonged to strategy 6 (20.34%) ($P < 0.05$).

Key Words: silkworm, selection strategy, cocoon

P4057 Discrimination of Korean native chicken lines using fifteen microsatellite markers. Dong-Won Seo*[1], Hee-Bok Park[2,3], M. D. Rashedul Hoque[1], Nu-Ri Choi[1], Hyun-Tae Lim[2,3], Cheorun Jo[1], and Jun-Heon Lee[1], *[1]Department of Animal Science and Biotechnology, Chungnam National University, Deajeon, Korea, [2]Department of Animal Science, Gyeongsang National University, Jinju, Korea, [3]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Korea.*

There are 5 Korean native chicken lines available in Korea, mainly classified by the feather colors. These 5 chicken lines have been used for the development of synthetic lines by crossing with broilers. The genotyping of 150 microsatellite markers among the native chicken lines was investigated and 15 highly polymorphic microsatellite markers were selected. The expected heterozygosity (Hexp) and polymorphic information content (PIC) values of the selected markers were higher than 0.7 and the number of alleles of the selected numbers

are more than 5. As the results, the expected probability of identity values among genotypes of random individuals (PI), random half sibs (PIhalf-sibs) and random sibs (PIsibs) were estimated as 5.42×10^{-27}, 3.37×10^{-19}, and 1.88×10^{-08}, respectively. These selected markers show high discriminate powers indicating the possible use of the markers for traceability system in these chicken lines.

Key Words: heterozygosity, Korean native chicken, microsatellite marker

P4058 Association of genes involved in carcass and meat quality traits in fifty European bovine breeds. S. Dunner[1], N. Sevane*[1], D. García[1], A. Valentini[2], JL Williams[3], B. Mangin[4], and H. Levéziel[5,6], *[1]Dpto Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain, [2]Dipartimento di Produzioni Animali, Universitá della Tuscia, Viterbo, Italy, [3]Parco Tecnologico Padano, Polo Universitario, Lodi, Italy, [4]INRA Chemin de Borde-Rouge-Auzeville, Castanet-Tolosan, France, [5]INRA, Limoges, France, [6]Universitè de Limoges, UMR, Limoges, France.*

To identify new molecular markers for meat quality, an association study was performed in 15 breeds of cattle using 389 single nucleotide polymorphisms belonging to 206 candidate genes known to be involved in muscle development, metabolism and structure. Fifty-four SNPs belonging to 20 different genes were found associated with different live, carcass and meat quality traits. Some of them were novel associations and other were validations of known associations. Among the former, the gene-network associated with the calpain/calpastatin system was shown to be associated with meat texture, although small effects are found for the examined polymorphisms. Novel associations also included SNP in AANAT which was associated with collagen, CAST with fatty acid muscle composition, CYP1A1 with juiciness, DGAT2 with physical traits and lipid content in muscle, MADH3 with the myofibrilar fragmentation index, NEB with weight, PCSK1 with juiciness, PLOD3 with carcass performance and fatty acids, and PGAM2 and VIM with post-mortem maturation. These data provide a starting point to investigate the complex gene-networks underlying economically important traits which are of importance to the beef industry for the improvement of production efficiency and meat quality.

Key Words: *Bos taurus*, candidate genes, meat quality

P4059 Molecular BVs for dam traits—Do we genotype progeny tested sires or the dams themselves? G. H. Shackell* and B. Auvray, *AgResearch Invermay, Mosgiel, New Zealand.*

Breeding values for most dam traits are predicted from other traits measured on the animal. Molecular Breeding Values (mBVs) can increase accuracy of selection index predictions, but need the support of reliable phenotype records. The accuracy of mBVs is partly a function of number of animals genotyped and reliability of the phenotype used. De-regressed BVs with parent average removed are a typical phenotype used in genomic selection. Using standard empirical formulae we examined the relationship between number of genotypes and mBV accuracy. The data generated by the model were used to determine the best way of utilizing resources within phenotyping or genotyping cost constraints. Given a finite number of samples to be genotyped, low phenotyping cost and moderate to high heritability, there was very little difference in total cost when genotyping the same number of either progeny tested sires or recorded dams. When trait heritability was low, mBV accuracy was lowest when only dams were genotyped, improved by genotyping a combination of progeny tested sires and recorded dams and highest when only sires with good numbers of recorded progeny were genotyped. However, when phenotyping cost was high, the increased accuracy gained by genotyping progeny tested sires was eroded by the number of phenotype records required, making it more economical to genotype only dams.

Key Words: mBVs, genotyping, phenotyping

P4060 LD: The power of two. E. Lipkin[1], M. Dolezal[2], A. Bagnato[2], N. O'Sullivan[3], E. Santus[4], J. Fulton[3], and M. Soller*[1], *[1]Dept. Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]VSA, University of Milan, Milan, Italy, [3]HyLine International, Dallas Center, Iowa, USA, [4]ANARB Associazione Nazionale Allevatori Razza Bruna, Bussolengo, Italy.*

LD based on r2 was studied in Italian Brown Swiss cattle and a commercial Brown-egg layer, using 60K and 36K Illumina SNP arrays, respectively. In both populations, an upper LD limit was set by the separation distance (dS) and minor allele frequency difference (dMAF) between the markers. Yet within these upper limits, LD ranged widely. The explanation lies in the fact that LD = 1.0 is found when alleles at the 2 markers distribute identically among the haplotypes of the block. This is necessarily the case when there are only 2 haplotypes in the block. With 3 or more haplotypes, marker haplotype distribution can differ, and LD can range widely; depending on the number of haplotypes, as determined by the balance between drift causing loss of haplotypes, and recombination generating new haplotypes. The smaller dS, the less recombination, and the greater the tendency of drift to reduce the number of haplotypes. dMAF deterministically sets an upper limit to LD. Within this limit, LD can vary widely depending on allele frequencies at the 2 markers. Thus,

even for very dense arrays an appreciable proportion of marker pairs will present LD < 1.0. The implication is that even such arrays will not deliver LD = 1.0 between markers and all QTL. (This research supported by the EC-funded FP7 Project 'Quantomics'; ANARB, Associazione Nazionale Allevatori Razza Bruna; and HyLine International Corp.).

Key Words: linkage disequilibrium, Brown Swiss cattle, brown egg layer chicken

P4061 Production of chickens with high thigh meat yield using DNA microsatellite marker-assisted selection. Ken Tatsuda* and Emi Nishiyama, *Hyogo Prefectural Institute of Agriculture, Forestry and Fisheries, Kasai, Hyogo, Japan.*

Generally, breast meat is more popular than thigh meat because it is healthier and easier to cook than other parts of the chicken. In contrast, most Japanese people prefer thigh meat to breast meat. We have developed a special prefectural chicken called "Hyogo-Ajidori", obtained from a 3-way cross between a White Plymouth Rock and a Hyogo, which is a 2-way cross between a Satsumadori male and a Nagoya female. We developed a resource population with 420 F2 birds from 2 pairs of Satsumadori males and Nagoya females to detect QTLs affecting thigh meat weight ratio to live body weight (%, TMR), relating breast meat weight ratio to live body weight (%, BMR), and other carcass traits. As a result of association tests between marker alleles and the carcass traits, alleles of 2 markers were significantly associated with TMR or BMR. In marker ADL0019 (122 cM of Ch.1) and LEI0068 (145 cM of Ch.1), the A allele was associated with higher TMR. Four Hyogo males with A allele in ADL0019 and LEI0068 were crossed with 8 White Plymouth Rock females with A allele in the 2 markers. Sixty Hyogo-Ajidori chickens were produced and their TMRs were measured at 16 weeks of age. The average TMR was 24.8 ± 0.9% and was significantly greater than the original TMR (20%). We plan to utilize this information to improve the TMR of Hyogo-Ajidori chickens.

Key Words: chicken, MAS, TMR

P4062 A genome wide association study for withers height in German Warmblood Horses. J. Tetens*[1], P. Widmann[2], C. Kühn[2], and G. Thaller[1], *[1]Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Kiel, Germany, [2]Res. Unit Molecular Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

Although withers height is a striking stature phenotype, which is easy to measure, there is only limited data concerning the genetic background of this trait in horses. Within the current study, a genome-wide association study for withers height was performed in 782 German Warmblood stallions belonging to 4 major German breeding associations. Withers height was measured at the time of licensing and all animals were genotyped using the Illumina EquineSNP50 BeadChip comprising a total of 54,602 evenly spaced SNP-markers. After standard filtering, 42,642 SNPs remained for the analyses. The association study was carried out using the R-package GeneABEL and applying a principal-component approach to correct for population stratification. Furthermore, age was included as a covariate in the model. Thereby, a single QTL showing genome-wide significance was identified on horse chromosome 3. The best associated SNP had an additive effect of 0.963 ± 0.15 cm and explains more than 6 percent of residual variance. The QTL region contains the LCORL/NCAPG locus, which has repeatedly been found to significantly affect adult height in humans. Furthermore, the NCAPG gene has recently been shown to have a major influence on growth and stature traits in cattle and thus is a strong candidate gene for withers height in horses.

Key Words: withers height, horse, GWAS

P4063 Evidence for similar location of QTL for guard hair length and thickness in mink (*Neovison vison*). J. P. Thirstrup*[1], B. Guldbrandtsen[1], R. S. Labouriau[1], R. M. Anistoroaei[2], K. Christensen[2], M. Fredholm[2], and V. H. Nielsen[1], *[1]Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Aarhus, Denmark, [2]Department of Basic Animal and Veterinary Sciences, Faculty of Life Science, Copenhagen University, Copenhagen, Denmark.*

Mapping of quantitative trait loci (QTL) with effect on guard hair length, guard hair thickness and density of wool in mink was performed in a 3-generation population (F2-design). The parental generation consisted of Nordic wild mink crossed reciprocally to American short nap mink. For this F2-design, a population of 1083 mink was established, representing 21 Nordic wild type mink, 25 short nap mink, 103 mink in the F1-generation and 934 mink in the F2-generation. Genotyping was performed using 104 microsatellites covering all 14 autosomal chromosomes. The marker spacing was approximately 11 cM. Recordings of fur quality traits were made on all genotyped mink by Kopenhagen Fur. The QTL analyses were performed by least square regression developed for crosses between outbreed lines and implemented in the software GridQTL. Evidence was found for QTL for the fur quality traits on eight autosomal chromosomes (LOD score > 3.0). QTL were detected for guard hair thickness on chromosomes 1, 2, 3, 4, 6, 11 and 13, for guard hair length on chromosomes 2, 3 and 6 and for wool density on chromosome 6, 7 and 13. Similar locations of QTL for guard hair length and guard hair thickness on chromosomes 2, 3 and 6 were

detected. The QTL also had the same mode of gene-action. This suggests that these traits are in part under the influence of the same genes.

Key Words: F2-design, microsatellites, fur quality

P4064 Expanding the flexibility of qPCR design in animal genotyping assays. Mario Van Poucke,* Alex Van Zeveren, and Luc J. Peelman, *Laboratory for Animal Genetics, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Real-time quantitative PCR (qPCR) has evolved into a flexible, application-made method for the quantification and identification of nucleic acids, due to the availability of a large number of fluorescent nucleic acid binding dyes (e.g., SYBR green, SYTO-dyes) and labels (e.g., FAM, Texas Red) used for fluorescence resonance energy transfer (FRET)-based nucleic acid detection methods (e.g., TaqMan assays). The current flexibility can be expanded by considering the spectra of the dyes, the factors that influence the produced/detected fluorescence (e.g., temperature, quenching molecules, consumables), the melting point of the amplicon and the advantages of certain polymerase mixes (e.g., direct blood PCR mixes). Here we performed some animal genotyping assays demonstrating the possibility to (1) perform TaqMan assays in SYBR green qPCR mixes, useful during optimization of the assay and for the detection of possible null alleles, (2) differentiate between FAM and SYBR green signals in a single tube genotyping assay, despite the fact that their spectra are overlapping, and (3) perform direct blood TaqMan assays.

Key Words: qPCR, genotyping assay, design

P4065 Genetic selection for racing durability in Thoroughbreds. B. D. Velie,* N. A. Hamilton, and C. M. Wade, *The University of Sydney, Sydney, NSW, Australia.*

Concern for racehorse welfare is receiving increasing global attention. Racehorse attrition, or wastage, has become a contentious topic, with many believing the amount of wastage in horseracing is unacceptably high. Genetic influences on the racing durability of thoroughbreds are yet to be fully explored. Our objective is to help improve the welfare of racing thoroughbreds through the creation of a selection index that emphasizes racing durability. Complete pedigrees and performance records were acquired for all thoroughbreds racing in Australia and Hong Kong between August 1, 2000 and February 22, 2011. The sample includes 2,882,773 individual records and 169,049 horses. Ten thousand randomly selected horses were used for preliminary analyses. A model including sex and color as fixed effects yielded heritabilities for career length (0.19 ± 0.03), career starts (0.17 ± 0.03), career earnings (0.10 ± 0.02), spells per 10 starts (0.09 ± 0.02), and spells per year (0.14 ± 0.03). Starts per month does not appear to be heritable (0.02 ± 0.02). Estimation of heritabilities and other genetic parameters using the entire sample based on an expanded model are currently underway. The assessment of genetic parameters for these traits is the first step in the construction of a selection index emphasizing racing durability and will aid in identifying horses suited to genetic mapping studies for specific issues relating to durability.

Key Words: horse, welfare, wastage

P4066 Association of ETH10 microsatellite to estimated breeding values of Angus sires in Australia. L. R. Porto Neto[1], K. L. DeAtley[2], D. R. Waine*[1], H. Duong[1], S. W. Corley[1], K. Lyons[1], R. Millewski[1], A. Blyth[1], S. N. Buttsworth[1], W. K. Armstrong[1], and E. K. Piper[1], *[1]The University of Queensland, Gatton, QLD, Australia, [2]New Mexico State University, Las Crues, NM, USA.*

The microsatellite ETH10 is part of the marker panel recommended by ISAG for parentage verification in cattle. It is located on BTA5 within the promoter region of the gene STAT6, which plays a role in several physiological pathways and has been associated to production traits in cattle. To further investigate these findings, alleles and genotypes of ETH10 were tested for association to estimated breeding values (EBVs) for 17 traits and 4 indexes published by the Angus Society of Australia. Among the 802 evaluated sires, there were 5 alleles varying from 215 to 223bp in size, defining 17 genotypes. Low frequency alleles and genotypes were excluded from the association analyses. There were consistent significant ($P < 0.005$) associations between alleles and genotypes to 3 measurements of growth (weight at 200, 400 and 600 d), and 2 carcass traits (fat depth at the P8 rump and at the 12/13th rib sites). Alleles 215 and 221 were associated ($P < 0.005$) to those traits with opposing effects, the 215 was positive for growth and negative for carcass and 221 the opposite. These alleles and genotypes explained ~2% of the residual variance of each trait and could be used to assist animal breeding; however, the definition of breeding objectives would be important as the same allele is often not favorable for all traits.

Key Words: estimated breeding value, growth, microsatellite

P4067 Estimation of genetic parameters from industrial crops. P. J. Whatmore* and W. R. Knibb, *University of the Sunshine Coast.*

Genetic parameters including trait heritabilities and genetic corrections are required information to build commercial selection indexes which in turn are used to select animals for optimal economic return. Until recently, genetic parameters typically were estimated using

dedicated experiments involving large-scale controlled experimental designs in dedicated experimental rearing facilities. Accordingly, the cost and logistics of running genetic experiments could be prohibitatively large and complex. We have used DNA tags and samples of animals from routine production runs to estimate genetic parameters for a range of traits including weight, colour, fat content, disease incidence in prawns and kingfish. Specifically, transcriptome and genome DNA sequencing was used to identify 100s of candidate DNA microsatellite loci for use as molecular tags. Candidate loci were screened and culled to provide a minimum of 10 very high grade loci per species. Samples of industrial production crops were taken, measured for various traits and assigned to family groups using the DNA markers, even when parental information was absent. This pedigree information was analysed using ASREML to provide genetic parameter estimates. The applicability of this experience to other species, and its cost effectiveness relative to more traditional approaches, will be discussed.

Key Words: aquaculture, heritabilities, optimal trait selection

P4068 Identification of quantitative trait loci affecting economic traits based on divergently selected regions between Japanese Black and Holstein cattle. K. Yamaji*[1], D. Hosokawa[1], A. Ishii[1], S. Sasazaki[1], K. Oyama[2], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Nada, Kobe, Japan, [2]Food Resources Education & Research Center, Kobe University, Kasai, Japan.*

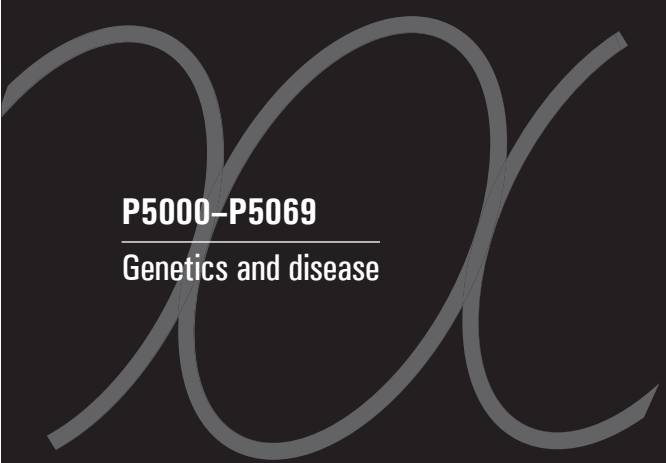
Domesticated cattle have been under strong artificial selection for various economic traits. In our previous study, we examined divergently selected regions between Japanese Black (JB) and Holstein cattle (JH) based on 50k SNP array and sliding window approach. As a result, we identified 11 genomic regions. The aim of present study was to investigate the association between these regions and economic traits, including 7 carcass and 5 milk production traits. For this purpose, representative SNP markers were selected from each 11 region and used for estimating the effects on the traits in JB (n = 478) and JH (n = 198). Association analysis revealed that 5 SNP showed significant effect on carcass traits; SNP2–1 (SNP from region 1 on chromosome 2) with fatty acid composition, SNP3–1 with beef marbling, SNP5–2 with yield estimate, SNP13–2 with carcass weight and fatty acid composition, SNP21–1 with rib-eye area in JB and 5 SNP showed significant association with milk traits; SNP3–1 with milk yield, fat yield and protein yield, SNP12–1 with protein percentage, SNP13–1 with fat yield, SNP21–1 with fat yield, SNP21–2 with milk yield, fat yield and protein yield in JH ($P < 0.05$). These results suggested that the regions might include causative genes and polymorphisms for these traits.

Key Words: QTL, SNP array, artificial selection

P4069 Looking for breed differentiating SNP loci and for a SNP set for parentage testing in Mangalica pig. Attila Zsolnai*, Gábor Tóth, János Molnár, Viktor Stéger, Ferenc Marincs, Anna Jánosi, Gabriella Ujhelyi, Erika Koppányné Szabó, Anita Mohr, István Anton, Réka Szántó-Egész, István Egerszegi, Klára Dallmann, Péter Tóth, Adrienn Micsinai, Klaus-Peter Brüssow, and József Rátky, *University of Kaposvár, Kaposvár, Hungary.*

The whole genome of Mangalica pigs was screened on the Illumina porcine chip giving the possibility (1) to replace the previously applied 10 microsatellite markers by 9 SNP loci to classify the Blond, Swallow-Belly, and Red Mangalica individuals into 3 different breed groups ($P > 0.95$) and (2) to propose 54 SNP loci for parentage testing in Mangalica pigs where the exclusion probability of identity is 1.54×10^{-23}.

Key Words: markers, genotyping, porcine



P5000–P5069

Genetics and disease

P5000 Fine-mapping of chicken genomic aberrations by identifying CNVs associated with *Campylobacter jejuni* colonization. J. Abernathy*[1], X. Li[2], X. Jia[3], and H. Zhou[1], *[1]University of California Davis, Davis, CA 95616, [2]Shandong Agricultural University, Taian, China, [3]China Agricultural University, Beijing, China.*

Identifying QTLs affecting disease resistance in chickens is important to potentially reduce the spread of pathogens by genomic selection. Certain breeds, such as Fayoumi and Leghorn, display different susceptibility to diseases like avian influenza, coccidiosis, and leukosis. Two distinct commercial broiler lines, line A and line B, have also been studied for their differences in intestinal colonization of *Campylobacter jejuni*. We used the Agilent 244K chicken Comparative Genomic Hybridization array (aCGH) on 64 individuals from inbred Fayoumi and Leghorn lines, and line A (resistant) and line B (susceptible) broilers to identify CNVs in these 4 genetically distinct chicken lines. A combined total of 692 genomic regions with significant chromosomal aberrations among them were identified. In the present study, our goal was to fine-map these regions in line A and line B birds to help identify potential QTLs for C. jejuni colonization. A custom 4X44K Agilent aCGH was built to saturate the 692 variable regions. DNA from 10 line A and 10 line B individuals, and Red Jungle fowl (reference) was used for the hybridization. After processing and analysis with R-bioconductor and Agilent Genomics Workbench, 426 and 420 CNVR were found in line A and line B, respectively. Differential aberrations between the lines were then examined for significant enrichment (P-value < 0.05). In line A, 25 amplifications with average length of 1,410 bp and 57 deletions with average length of 4,581 bp were found. In line B, 29 amplifications with an average length of 5,630 bp and 85 deletions with an average length of 2,359 bp were found. Various regions were selected for quantitation by digital PCR and will be validated in independent broiler populations. Combined mapping with gene expression data and SNP genotype for QTL candidates is underway.

Key Words: chicken, CNV, *Campylobacter jejuni*

P5001 Association mapping of the chicken L alloantigen. Chris M. Ashwell* and Mary P. Bulfin, *North Carolina State University, Raleigh, NC, USA.*

Susceptibility to disease remains a significant concern within the commercial poultry industry due to evolving viruses and resistance to therapeutic interventions such as anticoccidials. Improving the chicken's inherit immunological response is a potential method for addressing these issues. The chicken erythrocyte alloantigen system L has 2 haplotypes, L1 and L2, and is associated with multiple factors involved in immunological response. A resource population to identify the causative variant responsible for the L system was produced by mating one heterozygous male with 4 heterozygous females, and the resulting progeny characterized for their L phenotype. DNA pools were prepared representing each L haplotype from each family for SNP typing. The 4 parents and pools from each of the 4 families were genotyped using a 42,000 SNP assay on a custom Illumina Bead Array panel. A SNP-association analysis was performed using JMP genomics as a case/control population and P values were adjusted for multiple testing using false discovery rate (FDR). Investigation of the L alloantigen locus reveals multiple candidate genes present whose protein products are localized on the cell surface. Further sequence analysis is required to determine the causative variation responsible for the L alloantigen system.

Key Words: immune response, association mapping, poultry

P5002 Association of bovine leukemia virus (BLV) Tax function and various host responses by gene expression microarray. Yoko Aida*[1,2], Mariluz Arainga[1,2], Eri Takeda[1], Kazunori Yamada[1,2], Mayuko Jimba[1,2], and Shin-nosuke Takeshima[1,2], *[1]RIKEN, Viral Infectious Diseases Unit, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan, [2]The University of Tokyo, Laboratory of Viral Infectious Diseases, Department of Medical Genome Sciences, Graduate School of Frontier Science, The University of Tokyo, Wako, Saitama 351-0198, Japan.*

Tax protein of BLV is a transcriptional activator of viral replication and a key contributor to oncogenic potential. We previously identified interesting mutant forms of Tax with elevated (TaxD247G) or reduced (TaxS240P) transactivation effects on BLV replication and propagation. To identify the effects of these mutations on functions other than transcriptional activation, we used microarray containing approximately 18,400 human mRNA transcripts. We found several alterations after the expression of Tax proteins in genes involved in many cellular functions such as transcription, signal transduction, cell growth, apoptosis, stress response, and immune response, indicating that Tax has multiple biological effects on various cellular environments. We also found that TaxD247G strongly regulated more genes involved in transcription, signal transduction, and cell growth functions, contrary to TaxS240P, which regulated fewer genes. In addition, the expression of genes related to stress response significantly increased in the presence of TaxS240P as compared with wild-type Tax and TaxD247G. By contrast, the largest group of down-regulated genes was related to immune response, and the majority of these genes belonged to the interferon family. Finally, the expression of important cellular factors obtained from the human microarray results were

validated at the RNA and protein levels. A comparative analysis of wild-type and mutant Tax proteins indicates that Tax protein exerts a significant impact on cellular functions as diverse as transcription, signal transduction, cell growth, stress response and immune response. Importantly, our study is the first report that shows the extent to which BLV Tax regulates the innate immune response.

Key Words: bovine leukemia virus, gene expression microarray, mutant Tax

P5003 Identification of bovine leukocyte antigen (BoLA) class II haplotypes that regulate bovine leukemia virus (BLV) proviral load in Japanese Black cattle. Yoko Aida*[1,2], Taku Miyasaka[1,3], Mayuko Jimba[1,2], Naohiko Kobayashi[4], Tamako Matsuhashi[4], Hiroshi Sentui[3], and Shin-nosuke Takeshima[1,2], *[1]Viral Infectious Diseases unit, RIKEN, Wako, Saitama, Japan, [2]Laboratory of Viral Infectious Diseases,The University of Tokyo, Wako, Saitama, Japan, [3]Nihon University, Fujisawa, Kanagawa, Japan, [4]Gifu Prefectural Livestock Research Institute, Takayama, Gifu, Japan.*

BLV is the etiological agent of enzootic bovine leukosis, which characterized by B-cell leukemia/lymphoma. Bovine leukocyte antigen (BoLA) is strongly involved in the subclinical progression of BLV infectious. Especially, it previously appeared that BoLA-DRB3 gene may play a direct role in controlling the number of BLV-infected peripheral B lymphocytes in vivo in Holstein cattle. However, it still remains to identify the specific BoLA class II allele and DRB3-DQA1haplotypes determining the BLV proviral load in Japanese Black cattle. In this study, we focused on the association of BLV proviral load and polymorphism of BoLA class II in Japanese Black cattle. We genotyped a 186 BLV infected but clinical normal cattle for BoLA-DRB3 and BoLA-DQA1 by polymerase chain reaction-sequence-based typing method. BoLA-DRB3*0902 and BoLA-DRB3*1101 influence resistance to proviral load, and BoLA-DRB3*1601 influence susceptibility. Furthermore, BoLA-DQA1*0204 was related with a lower proviral load but BoLA-DQA1*10012 was related with a higher proviral load. Moreover, we confirmed the correlation between the DRB3-DQA1 haplotype and BLV proviral load. Two haplotypes, namely, 0902B or C (DRB3*0902-DQA1*0204) and 1101A (DRB3*1101-DQA1*10011) associated with the low level of BLV proviral load, while one haplotype 1601B (DRB3*1601-DQA1*10012) associated with the high level of BLV proviral load. Finally, we indicated that resistant is dominant trait and susceptible is recessive trait, and, in addition, resistant is common between Japanese Black and Holstein cattle and susceptible was differed. Collectively, this result is first report to define DRB3-DQA1 haplotype that play a central role in controlling of BLV proviral load even at the aleukemic cattle.

Key Words: BoLA class II haplotype, bovine leukemia virus, Japanese Black cattle

P5004 Genetics of carrier status for equine arteritis virus: Association with in vitro virus infectivity assay and a haplotype on ECA11. E. Bailey,* Y. Y. Go, P. J. Timoney, and U. B. R. Balasuriya, *MH Gluck Equine Research Center, University of Kentucky, Lexington, KY USA.*

Equine arteritis virus (EAV) is the causal agent of equine viral arteritis, a respiratory and reproductive disease of horses. Following EAV infection, a variable proportion of stallions (30–70%) can become persistently infected carriers and continuously shed the virus in their semen. Studies in our laboratory have shown that an in vitro assay based on dual color flow cytometry analysis of CD3+ T cells could be used to divide the horses into susceptible and resistant groups and that this phenotype is associated with a haplotype on ECA11. In this study, peripheral blood mononuclear cells (PBMCs) were collected from carrier (n = 7) and non-carrier (n = 7) stallions and subjected to in vitro infection with EAV. The susceptible or resistant CD3+ T cell phenotype of each animal was defined by dual color flow cytometric analysis. The CD3+ T cells from all 7 carrier stallions exhibited the susceptibility phenotype while all 7 non-carriers exhibited the resistant phenotype. The in vitro CD3+ T cell susceptibility of 5 carrier stallions correlated with a haplotype on ECA11 and in 1 of 3 non-carriers that were tested. In conclusion, development of the carrier state was associated with the in vitro susceptibility assay, which in turn, is associated with a haplotype on ECA11.

Key Words: horse, virus, immunity

P5005 Identification of SNPs associated to Red Maasai × Dorper resistance to gastrointestinal parasite infections. M. V. Benavides*[1,2], T. Sonstegard[2], S. Kemp[3], and C. Van Tassell[2], *[1]Embrapa LabEx USA, Beltsville, MD, USA, [2]USDA ARS, Bovine Functional Genomics Laboratory, ANRI, Beltsville, MD, USA, [3]International Livestock Research Institute (ILRI), Nairobi, Kenya.*

Gastrointestinal (GI) parasitic infection is a main health constraint that affects small ruminant production. Anthelmintic drugs are the sole control method, however their long-term use has led to selection pressure on parasites. New alternative control methods are needed. The aim of this study is to identify polymorphisms strongly associated with sheep host resistance against GI parasite infections. A Red Maasai × Dorper flock from ILRI was genotyped with the OvineSNP50KBeadChip where average fecal egg counts (AVFEC), packed cell

volume (AVPCV), and liveweight (AVLWT) were analyzed. Association analyses indicated significant SNPs with -Log10 *P*-values > = 3 were observed on 15, 23 and 15 chromosomes for AVFEC, AVPCV and AVLWT, respectively. Three individual SNPs on chromosomes 7, 15 and 26 had significant estimate effects on AVPCV and AVLWT and other 4 individual SNPs on chromosomes 13, 14, 15 and 17 had concomitant estimate effects on AVPCV and AVFEC. Revised significance levels are being calculated using permutation tests. It is expected the results generated here will enable the identification of a subset of SNP to potentially allow selection for sheep resistance to GI parasites based on a reduced density SNP panel.

Key Words: parasite resistance, sheep, SNPs

P5006 Pigmentary chorioretinopathy: A novel disease in Chinese Crested Dogs. T. F. Bergström*[1], M. Shrestha[1], M. Kierczak[1], G. Andersson[1], L. Andersson[1,2], and K. Narfström[3], *[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, [2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [3]Department of Ophthalmology, Mason Eye Institute, University of Missouri-Columbia, Columbia, MO, USA.*

Pigmentary chorioretinopathy is a recently discovered presumed inherited eye disease in the Chinese Crested Dog (CCD) observed in a large number of CCDs mainly in Scandinavia. Clinically, circular pigmented lesions were first observed in 4–6 year-old dogs with lesions that increased in number and spread more centrally with time. The morphological characteristics included degenerative changes in the choroidal structures and in the retinal pigment epithelium (RPE), followed by progressive degeneration of rods and cones, leading to severe visual impairment or blindness. The mode of inheritance has, however, been difficult to resolve because of the multiple inbreeding loops in the available CCD pedigrees. Nineteen affected CCD's and 21 normal dogs were genotyped using Canine HD 170K SNP chip (Illumina). The analysis was performed using the GenABEL computer package. The results from the genome wide association study (GWAS) show significant association to a region on chromosome 8 that is currently being further investigated. The results from the ongoing analyses will be presented.

Key Words: pigmentary, chorioretinopathy, canine

P5007 Assessment of the human cutis laxa genotype in chronic progressive lymphedema affected draught horses. K. De Keyser, A. Stinckens, and N. Buys,* *KU Leuven, Leuven, Belgium.*

The elastin gene (ELN) codes for a precursor tropoelastin, which is assembled and cross-linked into elastin. This extracellular matrix protein constitutes the main part of an elastic fiber. A deletion of an adenine residue in exon 32 of the ELN gene was shown to produce aberrant elastic fibers in humans with cutis laxa. This rare condition is partially characterized by a loose, hypoelastic skin, producing redundant skinfolds. These histopathological findings closely resemble those of draft horses susceptible to chronic progressive lymphedema (CPL). This disorder is associated with the formation of multiple skinfolds, mainly at the lower limbs. Comparative alignment shows that exon 32 of the human ELN is similar to a region in the 3′UTR of ELN in horses. As it was suggested that the 3′UTR contains regulatory properties and a mutation in this region could therefore be functional, the described corresponding region was sequenced in 3 CPL-affected draft horses and compared with a human and equine reference sequence to search for possible polymorphisms. It was shown that the consensus sequence of 3 CPL affected draft horses is identical to the equine reference sequence and shows 67% homology to the human reference sequence. The mutation in exon 32 that causes cutis laxa in humans could however not be detected in the draft horses.

Key Words: CPL, horse, cutis laxa

P5008 A lipid nanoemulsion (LDE) resembling LDL allows efficient and specific cholesterol-siRNA delivery to cancer cells. J. L. M. Ruiz[1], D. Levy[1], C. Oliveira[2], R. C. Maranhão[3], and S. P. Bydloswki*[1], *[1]Laboratory of Genetics & Molecular Hematology-LIM-31, University of São Paulo Medical School, São Paulo, SP, Brazil, [2]Experimental Physics Department, Institute of Physics, University of São Paulo, São Paulo, SP, Brazil, [3]Lipid Metabolism Laboratory of the Heart Institute (InCor), University of São Paulo Medical School, São Paulo, SP, Brazil.*

Conjugation of siRNA with lipophilic molecules enhances cell uptake with efficient gene silencing. However, siRNA is not stable in serum and lacks cell specificity delivery. Here we describe the physical characterization and biological evaluation of a novel lipid nanoparticle (LDE) with functional characteristics of low density lipoprotein (LDL). LDL is able to transport lipophilic siRNAs, improving cell internalization through LDL receptor. The LDE sizes and interaction with cholesterol-siRNA (C-siRNA) were characterized by SAXS, dynamic light scattering, TEM and fluorescence binding kinetics. It was shown that LDE can bind to C-siRNA maintaining its capacity to bind to LDL receptor. In vitro studies were done in uterine sarcoma MES-SA/DX5 cells which express the multidrug resistance gene. C-(mdr1-siRNA)-LDE complex was internalized in cells through LDL receptors and inhibited mdr1 gene expression. The physical properties of the

complex were characterized. The complex was nontoxic and increased C-siRNA uptake by cell through LDL receptors. A specific mdr1-siRNA bound to LDE was able to knockdown mdr1 gene expression and Pgp activity. In conclusion, we described a novel C-siRNA lipid system (LDE) that is able to specifically and effectively target cancer cells. This complex could potentially be used for gene therapy purposes.

Key Words: gene therapy, nanoemulsion, siRNA

P5009 From brachyspina to a genotype-driven screen for embryonic lethals compromising fertility in cattle. Carole Charlier*[1], Wanbo Li[1], Latifa Karim[1,2], Wouter Coppieters[1,2], and Michel Georges[1], *[1]Unit of Animal Genomics, Interdisciplinary Institute of Applied Genomics & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, [2]GIGA-Genomics Core Facility, University of Liège, Liège, Belgium.*

Brachyspina syndrome (BS) is a very rare, severe congenital defect described in Holstein cattle. We have recently identified its causative mutation: a large deletion in the FANCI gene. To our surprise, 7.5% of Holstein-Friesian animals appeared to be carrier. Therefore, many more newborn animals should be affected with BS. Using field fertility data we estimated that at least halve of the homozygous mutant embryos/fetuses die in utero. Thus, what appeared to be a very rare genetic defect turns out to be a relatively common cause of fertility failure. We resonated that other embryonic lethals (EL) must segregate in specialized cattle breeds but would have remained completely unnoticed because all homozygous mutant embryos/fetuses would have die early during gestation. To hunt for, we have set up a genotype-driven screen in which the entire exome of □100 elite sires/breed will be resequenced. Sequence data will be mined bioinformatically to identify candidate EL. The effect on fertility of candidate EL mutations will subsequently be tested by genotyping a large data set and searching for (i) the absence of homozygous mutants among healthy animals, (ii) an effect of carrier status on measurements of fertility. Latest results will be presented.

Key Words: brachyspina, embryonic lethal, fertility

P5010 Effect of TLR4 and Lactoferrin polymorphisms over somatic cells in Chilean dairy cattle. A. Carvajal,* P. Huircan, and A. Lepori, *Chilean Institute for Agricultural Research.*

Mastitis is the most frequent and costly disease in Chilean dairy herds. It is characterized by a mammary gland inflammatory response caused mainly by contagious or environmental pathogens that invade the mammary gland. It is common to observe an increased number of somatic cells and many alterations in milk during inflammation of the gland. Mastitis resistance or susceptibility is a complex trait and several functional candidate genes are receiving attention with aim to improve herd health through animal selection. The objective of this study is to determine the effect of molecular markers of 2 host genes related to somatic cell score (SCS) and the presence of specific bacteria in milk. Four single nucleotide polymorphisms (SNPs) were genotyped in Toll-like receptor 4 (TLR4) and Lactoferrin (Lf) genes in several breeds of dairy cattle presents in southern Chile obtaining allelic and genotypic frequencies. The SNPs analyzed showed to be in Hardy-Weinberg equilibrium. Preliminary results have shown a statistical significance of TLR4 markers over SCS but no milk production.

Key Words: mastitis, immune genes, SNP

P5011 Nonketotic hyperglycinaemia: Identification of mutations in the GLDC gene in cataract captive-bred Vervet monkeys. C. Chauke*[1], J. Sharma[2], Z. Magwebu[1], Z. Arieff[2], and J. Seier[1], *[1]Medical Research Council, Primate Unit, Tygerberg, South Africa, [2]University of the Western Cape, Department of Biotechnology, Belville, South Africa.*

Nonketotic hyperglycinaemia (NKH) also known as glycine encephalopathy is an inborn error of glycine metabolism characterized by accumulation of glycine in body fluids and various neurological symptoms. NKH is caused by deficiency of the glycine cleavage system (GCS) with 3 specific components encoded by GLDC, AMT and GCSH. NKH is inherited in an autosomal recessive pattern and 80% of the disease is caused by mutations in the GLDC gene. Captive-bred Vervet monkeys (*Chlorocebus aethiops*) that are maintained at the MRC Primate Unit and suffering from cataract have been reported to have high levels of glycine in their plasma and cerebrospinal fluid (CSF). To elucidate the background of this underlying genetic defect, mutation analysis of the complete coding sequence of the GLDC was performed in 11 cataract monkeys. So far, 7 of the 27 exons have been screened and 12 gene alterations have been identified, confirming the large molecular heterogeneity of the GLDC gene. Four alterations were clearly disease-causing (c.474_475insT, c.2230delT, c.2467delT, c.3062delT). Additionally, 3 missense mutations (I91M, L101S, T642M) and 5 silent mutations (I91I, T269T, N380N, G618G, H760H) were identified. Although many changes have not been proven to be deleterious based on these preliminary results, it is clear that an interaction or association between cataract and NKH exists at a molecular level.

Key Words: nonketotic hyperglycinemia (NKH), GLDC gene, cataract

P5012 Increased serum cholesterol and testosterone in male Plin1 null mouse do not affect testis lipids accumulation and fertility. Min Chen,* Qingyong Meng, and Ning Li, *State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China.*

Plin1 plays an important role in lipolysis. Previous investigations have demonstrated that Plin1 mutation disturbed lipids metabolism. Adipose tissue was reduced in the plin1-null mice as a result of enhanced basal lipolysis and fatty acid oxidation. The adipose tissue may affect reproduction via hormones and endocrine. Paradoxically, some works showed there is little effect of Plin1 knockout on fertility. As Plin1 has been shown to be expressed in tests, it is desirable to study the role of Plin1 in reproduction in a more detailed manner. Here we found that Cyp51, a critical gene associated with cholesterol synthesis, was upregulated in both mRNA and protein level. Serum cholesterol level in 5month and 9month old male mice increased 31% and 21% respectively. Serum testosterone level rose significantly in 5month old plin1-null male mice. However, the lipid content in testis was comparable to the wide type mice. The spermatozoa number and activity as well as the litter size were almost unaffected. The data here uncover the relationship between plin1, cholesterol level and testosterone level of the mice. We suppose that the increased cholesterol and testosterone is a compensation of plin1 deficiency, which promotes lipid storage in the testis and rescues the fertility.

Key Words: plin1, cholesterol, testis

P5013 QTL analysis for porcine blood serum traits in an F2 intercross population. In-Cheol Cho*[1], Chae-Kyung Yoo[2], Jae-Bong Lee[2], Eun-Ji Jung[2], Hyun-Tae Lim[3], Sang-Hyun Han[1], Sung-Soo Lee[1], Moon-Suck Ko[1], Taeyoung Kang[4], Joon-Ho Hwang[5], Yong Sang Park[1,4], and Hee-Bok Park[2,3], *[1]Subtropical Animal Experiment Station, National Institute of Animal Science, RDA, Jeju, Jeju-do, South Korea, [2]Animal Science Major, Division of Applied Life Science, Gyeongsang National University, Jinju, Gyeognam, South Korea, [3]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Gyeognam, South Korea, [4]College of Veterinary Medicine, Jeju National University, Jeju, Jeju-do, South Korea, [5]Biotechnology Regional Innovation Center, Jeju National University, Jeju,Jeju-do, South Korea.*

Blood serum traits are essential when examining the health status of individuals. The aim of this study was to identify quantitative trait loci (QTL) and the associated positional candidate genes affecting serum traits in a reciprocal F2 intercross between Landrace and Korean native pigs. Twenty-five serum phenotypes related to serum traits (e.g., hepatic function parameters, renal function parameters, electrolyte, lipids) were measured in >970 F2 progeny. All experimental samples were subjected to genotyping analysis using 173 microsatellite markers located across the genome. We identified 11 genome-wide significant QTLs in 6 chromosomal regions (SSC 2, 7, 8, 13, 14, and 15) and 60 suggestive QTLs in 18 chromosomal regions (SSC 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, and X). Genome-wide analysis revealed a significant evidence for an imprinted QTL on SSC4 affecting serum amylase levels. The imprinted QTL was found to be paternally expressed. In conclusion, our study detected both novel and previously reported QTLs influencing serum traits in pigs. The identified QTLs together with the positional candidate genes identified here could play an important role in elucidating the genetic structure of serum phenotype variation in humans and swine.

Key Words: serum traits, quantitative trait locus, Landrace and Korean native pigs

P5014 Identification of genomic regions associated with piglet survival and mortality. Yun-Jeong Choi*[1], Sang-Wook Kim[1], Pengxia Niu[1], Chankyu Park[2], and Kwan-Suk Kim[1], *[1]Chungbuk National Unversity, Cheongju, Chungbuk, Korea, [2]Konkuk University, Seoul, Korea.*

Mortality of young pigs involves many management factors providing stressful environments, but the survival capacity under low quality management and disease outbreak associated with high mortality may be largely resulted in the genetic component of individual piglets. The objective of present study was to identify genetic factor(s) responsible for piglet survival and mortality under commercial field condition with PCVD. We investigated survival and mortality from birth to 14 weeks in a cohort of 55 litters (n = 485) generated by F2 crosses between Korean native pig and Yorkshire breeds from a single farm. The animals were genotyped for 262 SNP markers spanning 18 autosomes. Differences of each allelic frequency were analyzed between mortality variables (268 dead vs. 215 alive vs. 485 total pigs). In a Chi-squared test, 19 SNPs were significantly different in their allelic frequencies between alive and dead pigs. Of particular interests, the FTSJD2 locus was most significantly and consistently associated with survival capacity (allele freq: 0.357 dead vs. 0.478 alive) and located close to pig MHC region on SSC7. This preliminary study suggests that MHC might play an important genomic region for early piglet immunity which is associated with survival capacity under PCVD. Further work is underway to validate the relationship between MHC locus and PCVD development.

Key Words: piglet mortality, genetics, disease

P5015 Genome-wide identification of endogenous retroviruses from a crocodilian genome. A. Y. Chong*[1], S. Isberg[1,2], L. Melville[3], D. A. Ray[4], T. C. Glenn[5,6], D. G. Peterson[7], X. Shan[4], and J. Gongora[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [2]Porosus Pty Ltd., Palmerston, NT, Australia, [3]OIC Berrimah Veterinary Laboratories, Department of Resources, Darwin, NT, Australia, [4]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, USA, [5]Department of Environmental Health Science, University of Georgia, Athens, GA, USA, [6]Georgia Genomics Facility, University of Georgia, Athens, GA, USA, [7]Institute for Genomics, Biocomputing & Biotechnology, Mississippi State, MS, USA.*

The saltwater crocodile (*Crocodylus porosus*) is one of 2 species of crocodile found in Australia and the only one that is commercially farmed. It has been proposed that endogenous retroviruses (ERVs) may play a role in the high incidence of runtism seen in farmed saltwater crocodile hatchlings. Here we will be discussing the detection of full length ERVs from a saltwater crocodile genomic library and subsequent explorative studies into the ERV complement of crocodilians. Several potentially functional fragments have been isolated from the saltwater crocodile, and are the focus of our selective screening of the genomic library. These are of specific interest due to their potential links with disease. However, to obtain an overall idea of the scope of ERV integration in crocodilians, a more thorough approach is to utilize available genomic resources to screen for common ERV motifs. Thus we are also exploring the use of bioinformatic resources to identify ERV sequences from available crocodile genome sequences.

Key Words: ERV, *Crocodylus porosus*

P5016 Over-expression of TLR-2 in transgenic goats accelerated pathogen clearance through up-regulating infiltration of inflammatory cells. Shoulong Deng*[1,2], Kun Yu[1,2], Baolu Zhang[1], and Zhengxing Lian Lian[1,2], *[1]Laboratory of Animal Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, China Agricultural University, Beijing, P.R.China, [2]National key Lab of AgroBiotechnology, Beijing, P.R.China.*

TLR-2 (toll-like receptor 2) is the important receptor for host to recognize the invaded gram-positive microbes, which caused serious mastitis, anthrax, tetanus and so on. Transgenic goats constitutively overexpressing TLR2 in all tissues will be the best model to uncover the role of TLR-2 on bacteria clearance. *Capra hircus* TLR-2 overexpression vector (p3S-TLR2-Loxp) was used to generate transgenic goat by egg microinjection and in total 3 transgenic goats were identified by Southern blotting, the integration efficiency of foreign gene by micro-injection reached 8.57% (3/35). Transgenic goats (Tg) showed higher levels of mRNA expression and protein secretion of TLR2. Monocytes-macrophages from circulation of transgenic goats were stimulated with Pam3CSK4 in vitro, cytokines (IFN-γ, IL-10, IL-6) and the content of nitric oxide (NO), malondialdehyde (MDA), lysozyme (LZM) were measured. Ear tissue samples, which were stimulated with 1 mg of Pam3CSK4 by hypodermic injection, were collected and stained with H&E to evaluate the immune response histologically. It was concluded that overexpression of TLR-2 decreased radical damage to host cells through low level production of NO and MDA, promoted clearance of invaded bacteria by upregulating lysozyme secretion and filtration of inflammatory cells to infected site.

Key Words: goat, TLR2, over-expression

P5017 Over-expression of TLR4 improved the clearance killing ability of immuno-cells of against gram-negative bacteria in transgenic sheep. Shoulong Deng*[1] and Zhengxing Lian[1,2], *[1]College of Animal Science and Technology, China Agricultural University, Beijing, P.R.China, [2]National key Lab of AgroBiotechnology, Beijing, P.R. China.*

Generally, livestock are infected easily by Gram-negtive pathogenic bacteria in animal industry. Therefore, it is meaningful and urgent to prevent animals from infections. TLR4 is one of the important toll-like receptors (TLRs), which is critical in the immune response against gram-negative bacteria. In this study, inflammation response and oxidative damage of transgenic sheep overexpressing TLR4 were detected in vivo and in vitro. Monocyte/macrophage isolated from peripheral blood was stimulated by LPS. The secretion of TNF-α, IL-10, IFN-γ and il-6 by monocyte/macrophage increased significantly. The secretion of NO also increased. GSH was in a relatively high level. MDA content were significantly different at 0.5h ($P < 0.05$), and then recovered to normal level. Monocyte/macrophage of transgenic sheep had extremely higher phagocytosis index than non-transgenic sheep ($P < 0.05$). In vivo, the observation of tissue sections and infiltration of neutrophile showed that transgenic individuals can initiate inflammation response immediately. Overexpression of TLR4 in transgenic sheep enhanced the clearance of invaded microbe through secretion of cytokines, activation of Macrophage, oxidation damage of RNI and infiltration of neutrophile.

Key Words: toll-like receptor 4, over-expression, sheep

P5018 Molecular mechanisms involved in equine osteochondrosis. C. Desjardin*[1], A. Vaiman[1], T. Balliau[1], J. Rivière[1], R. Legendre[1], X. Mata[1], M. Zivy[2],

E-P. Cribiu[1], and L. Schibler[1], *[1]INRA, Jouy-en-Josas, France, [2]CNRS.*

Equine osteochondrosis (OC) is a juvenile osteoarticular pathology characterized by a local failure of cartilage maturation, leading to dissection of an articular flap or to subchondral cysts development in joints. With an incidence of 10 to 30%, OC is a major concern in terms of animal health care and economy. Many factors have been suggested including nutrition, trauma and genetics. The aim of our study is to bring new insight into molecular mechanisms and biological process involved in OC susceptibility. We performed a comparative study focused on histology, proteome and microtranscriptome of normal cartilage and subchondral bone from healthy and OC affected foals. Proteomic analyses of cartilage have long been impaired because of technical challenges related to their biochemical properties. We have developed an efficient method to characterize the proteome of cartilage and bone and compare healthy and OC foals. Our study highlighted several modulated proteins involved in extracellular matrix structure and dynamics, chondrocyte metabolism as well as bone mineralization. In addition, a SOLIDTM RNA-Seq strategy was followed to establish the cartilage and bone miRNA catalogs and define a set of modulated miRNAs. Taken together, our findings point out the heterogeneous nature of OC and suggest that both cartilage and bone defects may be involved in the physiopathology.

Key Words: equine osteochondrosis, proteomic, microtranscriptome

P5019 Genome-wide association study for immunity traits in French Large White pigs. J. Estelle*[1], L. Flori[1], Y. Gao[1,2], M. P. Sanchez[1], J. P. Bidanel[1], and C. Rogel-Gaillard[1], *[1]INRA, Laboratory of Animal Genetics and Integrative Biology, Jouy-en-Josas, France, [2]University of Wisconsin-Madison, Department of Nutritional Sciences, Madison, USA.*

Improving robustness and resistance to pathogens is a high priority in most livestock species, particularly in pigs. We study immunocompetence in French Large White pigs and have previously shown that the levels of a wide range of both innate and adaptive immunity traits (ITs) are heritable. Our aim is now to dissect the genetic architecture of these ITs' by performing a genome-wide association study (GWAS) on 325 animals using the Illumina PorcineSNP60 beadchip. Significant associations have been found for a large number of various traits, as white blood cells subpopulations, specific antibody levels after vaccination or phagocytosis and in vitro cytokine production. The functional annotation provided by GWAS results will allow us to identify putative relevant candidate genes to be further studied. Since many animals have also been analyzed for blood transcriptome, we will combine genetic variations and gene expression profiles to go deeper in the detection of candidate genes underlying the studied ITs. A further step will be to relate heritable traits that qualify immunocompetence to disease resistance/susceptibility. In conclusion, our overall results provide new data on the genetic control of ITs and should contribute to evaluate the feasibility of including health traits in future selection schemes.

Key Words: pig, GWAS, immune response

P5020 Whole genome re-sequencing of the MeLiM porcine model for cutaneous melanoma. J. Estelle*[1,2], D. Esquerre[3,4], O. Bouchez[3,4], S. Vincent-Naulleau[1,2], P. Wahlberg[1,2], and E. Bourneuf[1,2], *[1]INRA, UMR1313 GABI, Jouy-en-Josas, France, [2]CEA, DSV/iRCM/SREIT/LREG, Jouy-en-Josas, France, [3]INRA, UMR444 LGC, Castanet-Tolosan, France, [4]GeT-PlaGe, Genotoul, INRA, Castanet-Tolosan, France.*

The Melanoblastoma-bearing Libechov minipig (MeLiM) has been selected for spontaneous melanoma occurrence and provides an excellent model to study these cutaneous tumors. QTL and genome-wide association analyses have already evidenced genomic regions potentially harboring causal polymorphisms. To help fine-mapping these regions, we have resequenced the whole genome of 2 MeLiM individuals and 2 pools of healthy and sick animals. Globally, over 700 Gb of paired-end sequences were generated on a HiSeq2000 instrument. QC-filtered reads were aligned to the reference genome (Sscrofa v10.2) and provided a read depth > 30X for each of the samples. Variant calling on the 2 MeLiM individuals identified more than 12 million variants (~10 million SNPs) between MeLiM sequences and the reference genome (Duroc). Finally, a Fisher's exact test was performed to compare the reads supporting the reference or an alternative (MeLiM allele) event in the 2 pooled groups; the frequency of the reference allele should be lower in the sick pool than in the healthy individuals' pool. Results identified over one hundred thousand MeLiM variants with significant allele frequency differences on the 2 pools and provide a short-list of potential causal variants for porcine cutaneous melanoma.

Key Words: melanoma, pig, whole genome sequencing

P5021 Case-control study for Salmonellosis confirms genetic resistance in commercial chickens. M. S. Fife,* N. S. Salmon, and K. Billington, *Institute for Animal Health, Compton, Berkshire, UK.*

Systemic Salmonellosis in chickens causes a typhoid-like infection and destruction of the spleen and liver of infected animals. Resistance to this disease is a genetically determined complex trait. We previously identified, and recently refined a novel resistance QTL

for systemic Salmonellosis on chicken chromosome 5 (SAL1). Gga5: 54–54.8 MB spans 14 genes, including a microRNA and 2 very striking functional candidate genes: CD27-binding protein (Siva) and the RAC-α serine/threonine protein kinase, AKT1 (protein kinase B, PKB). Identification of genes contributing to disease resistance in inbred poultry lines is of great scientific interest. However, the question remains as to whether these observations are relevant in commercial poultry flocks. In a recent devastating outbreak of *Salmonella* Gallinarum on a UK poultry farm, it was observed that, despite all birds being exposed to the pathogen, a small percentage of birds appeared to show resistance. Analysis of the SAL1 locus in this population enabled replication of the original association and confirmation that the resistance locus is relevant in commercial poultry. In this present study, significant effects for resistance to Salmonellosis were confirmed in commercial layer birds at the refined SAL1 locus. The presence of the protective allele in this commercial population is significant, as genetic selection of lines with resistant SAL1 haplotypes will increase Salmonella resistance in commercial poultry flocks, and therefore mitigate outbreaks of disease and avoid the severe economic consequences.

Key Words: Salmonella, genetics, AKT

P5022 Whole genome association study of Type 2 polysaccharide storage myopathy (PSSM) in Quarter Horses. K. L. Fritz*[1], S. J. Valberg[2], A. K. Rendahl[2,3], M. A. Lucio[2], J. R. Mickelson[1], and M. E. McCue[2], *[1]Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA, [2]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA, [3]School of Statistics, University of Minnesota, Minneapolis, MN, USA.*

We previously identified a mutation within the GYS1 gene in horses with Type 1 polysaccharide storage myopathy (PSSM), which results in an accumulation of glycogen and amylase-resistant abnormal polysaccharide in skeletal muscle fibers; however, a proportion of horses diagnosed with PSSM do not have the GYS1 mutation. The high prevalence of this form of PSSM, termed Type 2 PSSM, in breeds such as the Quarter Horse, suggests that Type 2 PSSM may have a genetic basis. The purpose of this study was to utilize a genome-wide association mapping strategy to identify positional candidate genes for Type 2 PSSM in Quarter Horses. The genotypes of 50,856 SNPs were analyzed among 124 Quarter Horse controls and 104 Quarter Horse PSSM Type 2 cases in a logistic regression test with 10,000 case/control label-swapping permutations. The results revealed suggestive associations of a single SNP on ECA3 and 6 SNPs within a haplotype on ECA18 (most significant P-value = 2.0×10^{-6}). The strength of the association on ECA18 persisted when the data was analyzed to account for population stratification and cryptic relatedness, suggesting that the association was real and not spurious. In the future, a functional mutation for Type 2 PSSM may be revealed by sequencing genes in this region of ECA18.

Key Words: GWAS, equine SNP chip, tying up

P5023 Rapid point of care diagnostic for PRRS detection in swine. Advaita Ganguly,* Raimar Loebenberg, and Hoon Sunwoo, *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.*

Recurrent porcine reproductive and respiratory diseases results in huge economic loss to the pork industry to the tune of millions of dollars. Surveillance of PRRS necessitates the use of diagnostic assays to develop management strategies beneficial to the industry. There is an urgent need for new and fast technology for viral diagnosis overcoming the drawbacks of present detection methods like cell culture, viral isolation, PCR, etc. Loop mediated Isothermal Amplification Assay to distinguish the PRRS infected population can be used for development and reduction of economic burden of the pork industry. The system can run thousands of samples to identify viral antigen without either a lab set up or skilled personnel based on the use of multi-primer loop amplification technology. This system includes PRRS RNA/DNA sample with 4 primers designed for the specific gene and Isothermal Device at 65°C for the amplification of product. The technology has the potential to replace traditional PCR based detection pursuant to its simplicity, rapidity, specificity and cost. The overall objective is to implement and demonstrate the concept toward a multiple primer based diagnostic that can be deployed at point-of-care in farms. It is our goal to prove and disseminate this technology which should allow for fast, inexpensive and accurate detection of PRRS providing quality of life for pigs and producing safer meat products

Key Words: rapid, LAMP

P5024 Identification of functional genetic variation in porcine cerebroside-sulfatid metabolism. Tiphanie Goetstouwers*[1], Mario Van Poucke[1], Annelies Coddens[2], Ut Nguyen Van[2], Vesna Melkebeek[2], Eric Cox[2], and Luc J. Peelman[1], *[1]Ghent University, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Merelbeke, Belgium, [2]Ghent University, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Merelbeke, Belgium.*

Glycosphingolipids can function as specific receptors for microbes and microbial products, for example the binding of Shiga toxin 1 to globotriaosylceramide, *Pseudomonas aeruginosa* and *Candida albicans* to

gangliotetrasylceramide, and porcine rotavirus (OSU strain) to GM3 ganglioside. This interaction can be a critical determinant for establishing host cell infections. The aim of this study is to identify functional variation in genes involved in the biosynthesis of the carbohydrate structure in glycosphingolipids of the cerebroside-sulfatid metabolism in pig. The 10 genes investigated (ARSA, B4GALT6, GAL3ST1, GALC, GBA, GLA, GLB1, NEU1, UGCG, UGT8) were found in the pig genome annotation of Ensembl and National Center for Biotechnology Information (NCBI). By cDNA sequencing, we verified the complete coding sequence and expression of these genes in the jejunum of 8 crossbred pigs (Belgian Landrace, Large White and Piétrain).The sequences of the investigated transcripts were identical to those annotated in the databases. Mutation analysis revealed 58 mutations: 41 silent mutations and 17 missense mutations. Ongoing studies will provide better insight into how these missense mutations may play a role in the adhesion of microorganisms to the jejunum.

Key Words: glycosphingolipids, pig, variation

P5025 Heritability of epistaxis associated with exercise-induced pulmonary haemorrhage in Australian racehorses. N. A. Hamilton*[1], G. Chaudhuri[1], P. T. Thomson[1], and P. K. Knight[2], *[1]Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia, [2]Discipline of Biomedical Science, Sydney Medical School, Lidcombe, NSW, Australia.*

The pathogenesis of exercise induced pulmonary hemorrhage (EIPH) is highly disputed, with inconclusive results suggesting the onset of the disease is multifactorial. Previous research into the South African Thoroughbred racing population demonstrated that EIPH-related epistaxis has a strong genetic basis. We aimed to estimate the heritability of EIPH-related epistaxis in Australian racehorses to investigate whether genetics plays a significant role in the occurrence of this disease. Pedigree information was supplied by the Australian Studbook, and records of all horses banned from racing due to epistaxis between 1999 and 2009 were supplied by Racing Industry Services Australia. Using a sire model, heritability for incurring one episode of epistaxis associated with EIPH was estimated at 0.51 ± 0.01; and at 0.14 ± 0.03 for a recurrent episode. This was similar to the heritability reported in the previous study. Sex and age were also significant predictors of the disease; which was positively associated with geldings and older horses. Estimated breeding values for sires ranged from –1.12 to 2.10, indicating a 25–fold difference in the odds of the disease. The heritability using an animal model is currently being calculated. These results suggest there is a genetic basis to the disease in Australian Thoroughbreds.

Key Words: horse, epistaxis, heritability

P5026 Development and application of TaqMan probed real-time quantitative reverse transcription PCR to differentiate the transcriptions of duplicated chicken MHC BF or BLB genes. Cai-Xia Gao[1], Ling-Xia Han[2], and Jian-Lin Han*[1,3], *[1]CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100193, China, [2]Laboratory Animal Center, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Harbin 150001, China, [3]International Livestock Research Institute (ILRI), PO Box 30709, Nairobi 00100, Kenya.*

The BF and BLB genes in chicken MHC are believed to associate with genetic resistance or susceptibility of MHC haplotypes. It is difficult to accurately and efficiently quantify the transcriptions of duplicated BF and BLB genes which have a homology above 92% in their CDS both within and among haplotypes and most polymorphisms present only in exons 2 and 3 of BF genes and in exon 2 of BLB genes. We developed specific TaqMan probed real-time quantitative reverse transcription PCR (TaqMan qRT-PCR) methods according to the diagnostic SNPs present in duplicated BF or BLB genes. The results showed very similar amplification efficiency and no cross-reaction between duplicated BF or BLB genes of the same haplotype. Spleen mRNA samples of Marek's disease virus infected and uninfected B2 and B19 chickens were used to validate the methods. We observed that the transcriptions of both BF genes were downregulated but the BF2 gene was dominantly transcribed in terms of BF2:BF1 ratio in all infected B2 and B19 chickens. The transcripts of both BLB genes were significantly increased in infected B2 birds but reduced in B19 birds. The BLB2:BLB1 ratio remained no change in infected B2 chickens while it was decreased in infected B19 birds. Our findings verified that the principles adopted to establish these specific TaqMan qRT-PCR methods in this study can be applied to differentiate the transcripts of duplicated BF or BLB genes of other MHC-B haplotypes and the diversified promoter sequences determine the function of duplicated BF or BLB genes in chicken.

Key Words: TaqMan qRT-PCR, MHC BF and BLB, chicken

P5027 Estalishment of heterozygous knock-out PKD1 pigs using zinc finger nuclease. J. He,* L. Zhang, X. Hu, and N. Li, *State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China.*

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most commonly inherited kidney diseases, characterized by the formation of fluid-filled cysts in bilateral kidneys, which afflicts 1/400–1/1,000

people worldwide. Mutations in the polycystic kidney disease 1 (PKD1) gene account for ~85% of the cases in ADPKD. Currently, there is no available effective treatment for ADPKD. To provide new perspectives of the molecular mechanisms and potential therapeutic methods for ADPKD, we report the establishment of PKD1 heterozygous KO pigs using ZFN technique. The KO efficiency in our experiment is 16.7% (12 mutant fibroblast colonies out of 72). After somatic cell nuclear transfer, we obtained 20 cloned pigs in total, 13 pigs of which were heterozygous KO for PKD1 alleles. These PKD1+/− pigs can be separated into 2 categories: TGCT insertion and T insertion, respectively. RNA extracted from pig ear biopsies were subjected to quantitative RT-PCR for detecting the gene expression of PKD1. Our results showed that TGCT group had a 40% reduction, whereas T group had a 30% reduction in comparison to their WT controls. Kidneys from 2 TGCT pigs and one WT pig were analyzed by H&E. No obvious morphological changes were observed at present, whereas the protein expression levels determined by immunobloting decreased significantly in PKD1+/− pigs.

Key Words: ADPKD, PKD1, ZFN

P5028 New options for assessing disease susceptibility traits. P. W. Hunt*[1], N. Andronicos[1], S. Dominik[1], J. McNally[1], M. Menzies[2], G. Wijffels[2], A. Ingham[2], D. Niemeyer[1], I. Purvis[1], and J. Kijas[2], *[1]Commonwealth Scientific and Industrial Research Organisation, Armidale, NSW, Australia, [2]Commonwealth Scientific and Industrial Research Organisation, St Lucia, QLD, Australia.*

Improved options for measuring an animal's susceptibility to disease are needed. A refined definition of disease susceptibility allows measured traits to be aligned with biological processes which are under genetic control. Our study species is the sheep (Ovis aries) and the gastro-intestinal nematode parasites which infect it. These parasites cause significant pathology world wide, are able to persist in the environment and the host and so are both of practical and scientific importance. The trait normally measured for breeding sheep resistant to these parasites is the number of nematode eggs detected in fecal samples (FWEC). This measurement can be influenced by a range of genetic and environmental factors, and we propose that multiple biological processes underlie the gross FWEC measurement. We show data supporting some new techniques for measuring components of FWEC, including the measurement of metabolites and peptides in plasma and white blood cells and dissection of FWEC into components for each nematode species. In addition we propose some new approaches including a cell culture system for assessing innate and acquired immune responses directed against nematode parasites and an automated assessment platform for measuring the behavioral components of parasite susceptibility.

Key Words: sheep, disease susceptibility, nematode parasites

P5029 Mapping of the blue-eyed white phenotype in alpacas: Genetic links between pigmentation and deafness. F. Jackling*[1], W. Johnson[2], and B. Appleton[1], *[1]Department of Genetics, The University of Melbourne, Melbourne, Victoria, Australia, [2]Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Maryland, USA.*

The blue-eyed white (BEW) phenotype is present in a variety of animals including cats, dogs, rabbits, horses and alpacas. In alpacas, the phenotype is characterized by 2 blue eyes, white hair over the whole body and often deafness. Breeders hypothesize that the BEW phenotype in alpacas is caused by the combination of the gene causing gray fleece and a white-spotting gene. Genetic markers within KIT showed a strong association with the blue-eyed white phenotype ($P < 0.0001$). Two microsatellite alleles were associated with the BEW phenotype, both being required for the display of the phenotype. Based on observations of alleles in animals of various colors the following are proposed: (1) An allele named bew1 is abundant in gray individuals, (2) homozygosity for an allele named bew2 results in a completely white coat and (3) these 2 alleles (bew1 and bew2) when co-inherited result in the BEW phenotype. Whole genome sequencing of 6 alpacas (3 BEW, 3 non-BEW) using the SOLiD4 platform was performed. We pinpoint genomic alterations which may affect KIT function in BEW animals. Among the DNA variants discovered we highlight potentially functional SNPs and structural variants. An intriguing inheritance pattern of the BEW phenotype is reported in alpacas, and we provide diagnostic opportunities and practical guidelines for breeders.

Key Words: alpacas, pigment, deafness

P5030 MHC class I diversity in saltwater crocodiles and its association with lymphoid proliferation, vasculitis and encephalitis syndrome. Weerachai Jaratlerdsiri*[1], Sally Isberg[1], Damien Higgins[1], Lorna Melville[2], and Jaime Gongora[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, New South Wales Australia, [2]OIC Berrimah Veterinary Laboratories, Department of Resources, Darwin, Northern Territory Australia.*

Lymphoid proliferation, vasculitis and encephalitis (LVE) syndrome, with a presumed viral etiology, has recently caused high mortalities of farmed saltwater crocodiles (*Crocodylus porosus*). Published studies suggest that in some instances, susceptibility to viral

infection in individuals can be associated with diversity of the major histocompatibility complex (MHC) class I genes or their supertypes, which are MHC gene variants grouped together based on similarities between putative structure and function in the peptide binding regions (PBR). Here we explore this hypothesis by studying the diversity of MHC class I gene exons 2 and 3, known to code for PBR, using deep sequencing of amplicons from diseased (n = 30, apparently affected by LVE) and healthy (n = 47) saltwater crocodiles. A total of 96 and 12 variants for exons 2 and 3, respectively, were identified using strict frequency criteria of the least abundant consensus sequences between 2 replicates within an individual. Hierarchical clustering analyses grouped these variants into 13 and 4 super-types, respectively. Interestingly, an exon 3 variant and the super-type it represents appear to be associated with the diseased animals. Interpretation of the significance of this finding is underway.

Key Words: lymphoid proliferation, vasculitis and encephalitis (LVE) syndrome, MHC class I, saltwater crocodile

P5031 Genome-wide association study of body weight at 140 days in an F2 intercross between Landrace and Korean native pigs. Eun Ji Jung*[1], Jae Bong Lee[1,2], Chae Kyoung Yoo[1], Beom Mo Kim[1], Hye In Kim[1], Hee Bok Park[1,2], In Cheol Cho[3], and Hyun Tae Lim[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Gyeongnam, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam, Korea, [3]National Institute of Animal Science, Rural Development Administration, Jeju, Jeju, Korea.*

A genome-wide association study (GWAS) was performed to identify single-nucleotide polymorphism (SNP) markers associated with body weight at 140 d. To detect SNP markers related to the trait at a genome-wide level, an F2 intercross between Landrace and Korean native pigs (n = 1105) was analyzed using the porcine SNP 60K chip from Illumina (USA) and a mixed-effects model approach accounting for familial relationships between individuals. After implementation of quality control performance criteria, 42,773 SNP markers were left for GWAS. When the genome-wide significant threshold level for the quantitative trait locus (QTL) of body weight at 140 d was set using the Bonferroni method ($P = 1.17 \times 10^{-6}$), significant SNP markers in chromosomes such as SSC5 ($P = 1.65 \times 10^{-9}$), SSC8 ($P = 5.52 \times 10^{-7}$), and SSC12 ($P = 1.74 \times 10^{-11}$) were detected under additive model.

Key Words: GWAS, body weight at 140 days, SNP marker

P5032 A comparative study on reproductive performance of zebu and taurus genotypes. M. A. S. Khan* and M. E. Uddin, *Dept. of Dairy Science, Bangladesh Agricultural University, Mymensingh, Dhaka, Bangladesh.*

Poor reproductive performance due to inadequate nutrition of crossbred cattle in Bangladesh is a major problem to develop dairy industry. But, there is no specific study related to reproductive performance of zebu and taurus genotype in our tropical environment. So, the present study has been taken into consideration to investigate the suitable genotype in tropical environment having better reproductive performance. Total of 80 crossbred cows was selected of which 40 cows were Holstein cross (HC) (local zebu × Holstein) and 40 cows were Sahiwal cross (SC) (local zebu × Sahiwal). From each genotype 20 cows were supplemented with Urea Molasses Block and 20 cows remain as control. The animals were < 2nd lactation and average body-weight was 286 kg. All animals were fed rice straw ad libitum and limited amount of seasonal cut and carry grass (3 kg/d). All animals were free access to drinking water. Reproductive intervals were reduced by supplementation in both genotypes. Reduction of calving to 1st Progesterone rise was 14 and 39 d in HC and SC cows respectively which differs significantly ($P > 0.05$). The interval from calving to conception was reduced by 54 and 81 d in HC and SC cows ($P > 0.05$). The result suggest that between the 2 genetic group of cows, SC cows performed better related to reproductive parameters than that of HC cows.

Key Words: reproductive performance, urea molasses block, genotypes

P5033 Equine spermatogenesis; Meiotic chromosome behavior and recombination frequency. A. I. Al-Jaru[1,2], W. Goodwin[2], J. Skidmore[3], and K. A. Khazanehdari*[1], *[1]Molecular Biology and Genetics, Central Veterinary Research Laboratory, Dubai, UAE, [2]Department of Forensic and Investigative Science, School of Science and Technology, University of Central Lancashire, Preston, UK, [3]Camel Reproduction Centre, Dubai, UAE.*

One of many factors known to influence stallion's fertility is the outcome of spermatogenesis. This is the first detailed study of meiotic process in horse that provides a better understanding of the genetic basis of horse spermatogenesis. Testicular sample from 14 stallions were studied with specific focus on the meiotic process. All 32 chromosomes formed 16 bivalents at metaphase I stage. The average frequency of autosomal chiasmata was 49.45 ± 2.07 and the majority of bivalents had 1 or 2 chiasmata. FISH was used to identify individual bivalents 8 of which were scored for chiasma frequency and distribution. Prophase I nuclei were examined for

frequency and distribution of MLH1 foci on synaptonemal complexes. The distribution of MLH1 foci (mean of 50.11 ± 2.35) was closely correspond to that of chisamata on metaphase I bivalents. Sperm variability was determined by Chicago sky blue staining for viability of head and tails and FITC-PSA and MitoTracer green were used successfully to assess the functional acrosome and the mitochondrial. Simultaneous assessment of functional sperm parameters as well as investigating the synapses and recombination frequency and distribution during meiosis would increase the accuracy of stallion fertility prediction and should greatly reduce the risk of abortion in mares.

Key Words: spermatogenesis, recombination, horse

P5034 Genomics of heat stress in poultry. S. J. Lamont*[1], M. G. Kaiser[1], M. F. Rothschild[1], M. E. Persia[1], C. M. Ashwell[2], and C. J. Schmidt[3], *[1]Iowa State University, Ames, Iowa, USA, [2]North Carolina State University, Raleigh, North Carolina, USA, [3]University of Delaware, Newark, Delaware, USA.*

Heat stress negatively impacts animal welfare and animal productivity. Understanding the genetic control of resistance to heat stress will facilitate genomic selection for animals with improved welfare and productivity under hot conditions. We have initiated a major project to elucidate the genomics of resistance to heat in poultry. Over 2 generations, nearly 2,000 chickens were studied in experimental designs that allow investigation of the effects of breed, thermal conditioning of embryos, cyclical heat exposure of chicks, and interaction of heat stress with response to inflammatory agents. An advanced intercross line (F18-F19) was included to facilitate the fine mapping of QTLs. Analyses of livebird blood biomarkers in Generation One demonstrated significant effects of chick heat stress (but not egg thermal conditioning) on blood CO_2 partial pressure, pH, saturated oxygen, hemoglobin, and hematocrit; and many 2-way interactions of thermal treatment (embryo or chick) with genetic line. These initial data validate the biological model of heat stress and the feasibility of identifying genetic markers controlling important phenotypes in the response to heat. The animal trials have provided extensive physiologic phenotypes and biological samples that will serve as the foundation of continued studies to elucidate the genes, networks and epigenetics of control of heat stress in poultry.

Key Words: heat stress, genomics, poultry

P5035 Novel SNP identification and association with bovine TB in African buffalo. N. le Roex*[1], H. Noyes[2], A. Brass[3], S. Kay[2], P. D. van Helden[1], and E. G. Hoal[1], *[1]Stellenbosch University, [2]University of Liverpool, [3]University of Manchester.*

Mycobacterium bovis infection causes bovine tuberculosis (BTB) in domestic animals, wildlife species and humans. The African buffalo, Syncerus caffer, acts as maintenance hosts for *M. bovis* in the game parks of South Africa due to aspects of its population dynamics and ecology, and this can have major economic, ecological and public health impacts. Two pools of African buffalo DNA were run on the ABI SOLiD next-generation sequencing platform and mapped to the *Bos taurus* reference genome. This enabled the identification of single nucleotide polymorphisms (SNPs) found between the buffalo and the cow, as well as SNPs located within the buffalo population. Approximately 6.5 million novel SNPs were identified, of which nearly 2 million were within genic regions. Bta4 (*Bos taurus*) gene annotation was added to all SNPs identified within whole gene regions. Validation was performed on 174 SNPs by fluorescent genotyping. The SNPs were selected for their location in particular genes or pathways of interest for further investigation of BTB susceptibility in African buffalo. The validated SNPs were genotyped in a total sample set of 848 individuals, comprised of both BTB test positive cases and test negative controls, to investigate association with BTB.

Key Words: buffalo, sequencing, bovine tuberculosis

P5036 Genome-wide association study of serum amylase levels in an F2 intercross between Landrace and Korean native pigs. Jae-Bong Lee*[1,2], Eun-Ji Jung[1], Chae-Kyoung Yoo[1], Beom-Mo Kim[1], Hye-In Kim[1], Hee-Bok Park[1,2], In-Cheol Cho[3], and Hyun-Tae Lim[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, inju, Gyeongnam 660-701, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea, [3]National Institute of Animal Science, Rural Development Administration, Jeju 690-150, Korea.*

Amylase is a digestive enzyme and its low concentration in blood is known to be associated with metabolic syndrome and diabetes. The amylase activity in serum and urine is used as the only indicator for acute and chronic pancreatitis. This study was conducted to search for single-nucleotide polymorphism (SNP) markers associated with the serum amylase levels by using a genome-wide association study (GWAS). To select SNP markers associated with serum amylase levels at a genome-wide level, an F2 intercross between Landrace and Korean native pigs (n = 1105) was analyzed using the porcine SNP 60K chip from Illumina (USA) and a mixed-effects model approach accounting for familial relationships between individuals. After implementation of quality control performance criteria, 42,773 SNP markers were left for GWAS. When the genome-wide significant threshold was determined using the Bonferroni method ($P = 1.17 \times 10^{-6}$), 162 SNP markers in SSC4 were founded to be significantly

associated with serum amylase levels. Of these, *P*-value of the most significant SNP marker was 1.42×10^{-52} under additive model.

Key Words: GWAS, amylase, Bonferroni method

P5037 Genetic analysis of hereditary nasal parakeratosis (HNPK) in Labrador Retrievers. Vidhya Jagannathan[1,2], Cord Drögemüller[1,2], Marta Owczarek-Lipska[1,2], Regula Hauswirth[1,2], Hannes Lohi[3,4], Monika Welle[2,5], Monika Linek[6], Silvia Rüfenach[2,7], Manon Paradis[8], Petra Roosje[2,9], and Tosso Leeb*[1,2], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]DermFocus, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]Department of Veterinary Biosciences, Research Programs Unit, Molecular Medicine, University of Helsinki, Helsinki, Finland, [4]Folkhälsan Research Center, Helsinki, Finland, [5]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [6]Tierärztliche Spezialisten, Hamburg, Germany, [7]Dermavet, Tierklinik Aarau-West, Oberentfelden, Switzerland, [8]Department of Clinical Sciences, Faculté de Médecine Vétérinaire, University of Montreal, St-Hyacinthe, Québec, Canada, [9]Division of Clinical Dermatology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Hereditary nasal parakeratosis (HNPK) is a genodermatosis of Labrador Retrievers with a monogenic autosomal recessive inheritance. HNPK-affected dogs develop crusts and fissuring of the nasal planum at a young age but are otherwise healthy. Histopathological changes consist of parakeratotic hyperkeratosis and an accumulation of proteinacious fluid (′serum lakes′) within the stratum corneum and the superficial stratum spinosum. HNPK currently cannot be cured, but the symptoms can be alleviated with symptomatic therapy. We mapped the locus for HNPK by a genome-wide association study (GWAS) using 13 cases and 23 controls, which were genotyped on the illumina canine_HD SNP chip. Homozygosity mapping in the 13 cases delimited a shared interval of 1.6 Mb, predicted to harbor the causative mutation. We sequenced the genome of an affected Labrador Retriever at 38x coverage using an illumina HiSeq2000 instrument. We identified a total of 1357 SNPs and 621 indels in the critical interval with respect to the Boxer reference genome sequence. Three of these are non-synonymous variants. We will present an updated analysis of our efforts to identify the causative mutation for HNPK during the conference.

Key Words: dog, whole genome sequencing, causative mutation

P5038 Variations in miR-155 are associated with haematological parameters in pig and mouse. Congcong Li,* Xinyun Li, Jianhua Cao, Dan Huang, and Shuhong Zhao, *Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, Hubei, PR China.*

MiR-155 is one of the well known miRNAs in host immune response, which is involved in hematopoiesis, inflammation and immune responses. We found miR-155 played positive regulatory roles in TLR3/TLR4 signaling pathways. One T/C SNP of miR-155 was significantly associated with basophil percentage (BA%), absolute eosinophili value (EO) and the distribution width of the least squares means of CD3—CD4—CD8+ T cells (DWT) in pigs. In this study, a hyplotype of miR-155 from KunMing mice were found, this hyplotype containing 4 mutant bases within 256 bp fragment pre-miRNA sequence. One is located in the ring of the miRNA secondary structure, another is 8 bp from the 3′ end of precursor. We predicted that the 2 bases are involved in the maturation of the miR-155 by bioinformatics analysis of the RNA secondary structure. We also found that the mutations of miR-155 were associated with hematological parameters in mice via association analysis performed in 120 mice. There were significant associations between different hyplotypes and the hematological parameters of WBC (white blood cell count), RDW (red cell distribution width), MCV (mean cell volume), MPV (mean platelet volume), PDW (platelet distribution width). Further function analysis of the different hyplotypes is ongoing. Our study offered new evidence on the function of miR-155.

Key Words: miR-155, hyplotype, association

P5039 Genome-wide association study for serum gamma-glutamyl transpeptidase levels in pigs. Hyun-Tae Lim*[1,2], Jae-Bong Lee[1,2], Eun-Ji Jung[1], Chae-Kyoung Yoo[1], Beom-Mo Kim[1], Hye-In Kim[1], In-Cheol Cho[3], Jun-Heon Lee[4], and Hee-Bok Park[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, inju, Gyeongnam 660-701, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea, [3]National Institute of Animal Science, Rural Development Administration, Jeju 690-150, Korea, [4]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea.*

Because symptoms of liver disease are generally non-specific before the end stage of the diseases, analysis of hepatic enzymes, such as gamma-glutamyl transpeptidase (GGT), are required for the diagnosis of liver diseases. The aim of this study was to identify quantitative trait loci (QTL) affecting baseline serum GGT levels in pigs. A genome-wide association study (GWAS) was performed in an F2 intercross population between

Landrace and Korean native pigs using the 60K porcine single nucleotide polymorphism (SNP) beadchip and a mixed-effects model approach accounting for familial relationships between individuals. Data used in this study included 1105 F2 offspring. After implementation of quality control performance criteria, 42,773 SNP markers were left for GWAS. In SSC14, SNP markers with associations surpassing a Bonferroni threshold of 1.17 × 10–6 were detected under additive model.

Key Words: GWAS, gamma-glutamyl transpeptidase, Korean native pigs

P5040 Investigating the genetic basis of a Canine Motor-Sensory Neuropathy by HD Canine 170000 SNPs array. Michele Polli[1], Stefano P. Marelli[1], Cristina Di Palma[2], Alessandra Gessi[3], Alessandra Mezzelani[4], Jacopo Riva[1,2], and Maria Longeri*[1], *[1]Università degli Studi di Milano; Dipartimento di Scienze Animali - Sez. Zootecnica Veterinaria, Milan, Italy, [2]Clinica Veterinaria C.so Concordia, Milan, Italy, [3]VEtoGene srl, spin-off Università degli Studi di Milano, Milan, Italy, [4]Institute for Biomedical Technologies - National Research Council of Italy, Segrate (MI), Italy.*

The aims of the present research is to investigate the genetic basis of a Canine Motor-Sensory Neuropathy (CMS) clinically very similar to human Charcot-Marie-Tooth (CMT) inherited disease. Recently some cases of degenerative neuropathy in Rhodesian Ridgeback (RR) dogs have been clinically studied. RR can be considered a valuable animal model for CMT and for inherited developmental disorders of Peripheral Nervous System. The 9 generations family pedigree (79 records) of 3 dogs suspected of CMS was drawn suggesting an autosomal recessive inheritance of the disease. Parentage was verified comparing the genetic profiles to ensure genealogical accuracy. The affected dogs showed fasciculations and tremors, atrophy, limb muscles degeneration plus demyelination and myelin outfolding. Clinical and histological features resulted in a description reporting signs very similar to human CMT subtypes: CMT4B-1, CMT4B-2 and CMT4H (all with autosomal recessive inheritance). MTMR2, MTMR13 and FGD4 are considered to be the causative genes for these forms of the human disease respectively. MTMR2, MTMR13 and FGD4 human genes were identified on dog genome nucleotide sequence assembly and homology and conservation were evaluated in silico. Out of the pedigree, genomic DNA of 3 healthy and 3 affected dogs were extracted and a Genome-Wide Association (GWA) was performed using high density SNP arrays (~170,000 SNPs - Illumina). By Homozigosity Mapping 17 candidate regions were significantly associated.

Key Words: Rhodesian dog, Canine Motor-Sensory Neuropathy, high density SNP array

P5041 Pedigree of Abyssinian cats with amyloidosis and polymorphism of SAA gene in several feline breeds. Maria Longeri*[1], Stefano P. Marelli[1], Paolo Valiati[1], Andrea Mapelli[1], Jacopo Riva[1,2], and Michele Polli[1], *[1]Università degli Studi di Milano; Dipartimento di Scienze Animali - Sez. Zootecnica Veterinaria, Milan, Italy, [2]VEtoGene srl, spin-off Università degli Studi di Milano, Milan, Italy.*

Amyloidosis is a rare disorders occurring in many species including, humans, chickens, and mainly domestic and wild felids. It is characterized, and the diagnosis is only possible so far, by post-mortem evidence of huge amyloid deposits in single organs or systemic. In Abyssinian/Somali and Siamese/Oriental cats a juvenile form with storage of apolipoprotein aposerum amyloid (apo-SAA) occurs most frequently than in other breeds and has been repeatedly suggested as familial. In the past SAA aminoacidic and coding sequence variations and amiloidogenic variants have been recorded on small cohorts of Abyssinians, Siameses and domestic shorthair cats. Additional amyloid associated SAA genes and predisposing factors (such as infections and inflammatory process) involved in the disease onset and development have also been suggested. The present work mainly aims to present a family pedigree (73 records) of Abyssinian cats with more than 20 subjects recording an anamnesis of death due to Amyloidosis. Moreover SAA coding region has been sequenced in the 31 available samples (both affected and healthy) out of the pedigree, in a group of 50 cats belonging to the following breeds: Siamese, Oriental, domestic shorthair cat, Bengal, Devon Rex, Chartreuse, Siberian, Thai, Ragdoll, Scottish Fold, Persian, Exotic, Birman, Norwegian Forest cat, Sphynx and in 9 samples of Panthera tigris. The pedigree/phenotype data seem to reconfirm familial predisposition and the SAA sequences present the previously suggested amiloidogenic motives. The importance of understanding the mechanisms of amyloidogenesis also for human health, encourage the constitution of an "Amyloid network" and a wide genome analysis.

Key Words: Abyssinian cat, amyloidosis, SAA polymorphism

P5042 Genetic control of swine responses to PRRSV infection: Progress of the PRRS Host Genetics Consortium. J. K. Lunney*[1], I. Choi[1], C. J. Souza[1], K. P. C. Araujo[1], S. M. Abrams[1], J. P. Steibel[2,3], M. Arceo[2], C. W. Ernst[2], J. M. Reecy[4], E. Fritz[4], J. C. M. Dekkers[4], N. J. Boddicker[4], E. H. Waide[4], X. Zhao[4], M. F. Rothschild[4], G. S. Plastow[5], R. A. Kemp[6], J. C. S. Harding[7], M. Kerrigan[8], B. Trible[8], and R. R. R. Rowland[8], *[1]USDA, ARS, BARC, APDL, Beltsville, MD, USA, [2]Dept. Animal Science, Michigan State Univ.,*

East Lansing, MI, USA, [3]Dept. Fisheries and Wildlife, Michigan State Univ., East Lansing, MI, USA, [4]Dept. Animal Science, Iowa State Univ., Ames, IA, USA, [5]Dept. Agricultural, Food and Nutritional Science, Univ. of Alberta, Edmonton, AB, Canada, [6]PigGen Canada, Guelph, ON, Canada, [7]Western College of Veterinary Medicine, Univ. of Saskatchewan, Saskatoon, SK, Canada, [8]Dept. Diagnostic Medicine and Pathobiology, Kansas State Univ., Manhattan, KS, USA.

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important infectious disease threatening pig production worldwide. The PRRS Host Genetics Consortium (PHGC) was established to probe the role of host genetics in resistance to PRRSV infection and related growth effects. Using a nursery pig model, 11 groups of 200 commercial crossbred pigs were infected with PRRSV and followed for 42 d post infection (dpi). Blood serum and Tempus (RNA) samples were collected at 9 time points and weekly weights recorded. Genomic DNA was genotyped with the Porcine SNP60 SNPchip for genome wide association studies. All pigs were viremic, peaking at 4–14 dpi. Using Bayes-B analyses for 5 groups, 8 1-Mb regions accounted for 35.7% of the genetic variance for viral load from 0 to 21 dpi with an SSC4 region explaining 17.7%. For weight gain 10 regions accounted for 31.2% of the genetic variance with SSC4 accounting for 14.3%. Sera and RNA are now being analyzed using the Pigoligoarray, RNA-seq, QPCR and multiplex immunoassays to elucidate factors involved in viral replication, recovery from infection, including speed and levels of immune cytokine expression. These analyses and SSC4 region sequencing should identify markers that distinguish PRRS resistant/maximal growth pigs from PRRS susceptible/reduced growth pigs. PHGC funding: US National Pork Board, USDA ARS and NIFA, NRSP8 Swine Genome and Bioinformatics Coordinators, Genome Alberta/ALMA, Genome Canada, and private companies.

Key Words: virus resistance, GWAS, immune cytokines

P5043 Three Burmese cat specific diseases identified by genome-wide association studies. B. Gandolfi[1], R. Malik[2], T. Gruffyd-Jones[3], C. Helps[4], C. Rusbridge[5], A. Cortes[1], and L. A. Lyons*[1], *[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2]Centre for Veterinary Education, University of Sydney, AUstralia, The University of Sydney NSW 2006 Australia, [3]Feline Centre, University of Bristol, Bristol, University of Bristol, Langford, UK, [4]Molecular Diagnostic Unit, University of Bristol, Bristol, University of Bristol, Langford, UK, [5]Stone Lion Veterinary Centre, Wimbledon, Wimbledon, UK.*

Burmese is an old and popular cat breed, however several diseases are prevalent and endangering the general health of the population. Three genome-wide association studies using the Illumina Infinium Feline 63K iSelect DNA array led to the chromosomal localization of Burmese-specific conditions, including hypokalemia, a craniofacial defect and orofacial pain. Hypokalemia is characterized by low serum potassium concentration with a skeletal muscle weakness presentation. A genome-wide case-control study resulted in the identification of a locus on chromosome E1 associated with hypokalemia. Within ≈1.2 Mb of the highest associated SNP a viable candidate gene was identified and sequencing revealed a mutation within WNK4 causing a pre-mature stop codon. All cases were homozygous for the mutation. A second case-control study was performed using craniofacial affected cases. The disease is a remarkable outcome of developmental failure of the facial prominences surrounding the mouth. A strong association was identified on chromosome B4 and a main haplotype spanning over 7 Mb was identified in all the Burmese cats. Within the haplotype a SNP with an Odd Ratio of 210 suggested a region of ≈500 Kb were all the cases had a unique haplotype. A candidate gene was identified and a 12 bp deletion is being confirmed as a causative mutation. A third GWA was performed on a set of orofacial pain syndrome cases. The syndrome shows similarities with human trigeminal neuralgia and characterized by severe pain in the distribution of the trigeminal nerve causing the cats to self-mutilate. Preliminary data suggest an associations on chromosomes B1 and C1, requiring further investigation. Genetic tests to screen and remove the defects within the active breeding population are in development to help improve the general health of the breed.

Key Words: hypokalemia, craniofacial defect, orofacial pain

P5044 Genetic parameters for tarsocrural osteochondrosis (OC) and palmar/plantar first phalanx osteochondral fragments (POF) in Standardbred trotters. S. Lykkjen*[1], H. F. Olsen[2], N. I. Dolvik[1], K. H. Røed[1], A. M. Grøndahl[3], and G. Klemetsdal[2], *[1]The Norwegian School of Veterinary Science, Oslo, Norway, [2]The Norwegian University of Life Sciences, Ås, Norway, [3]Norwegian Veterinary Institute, Oslo, Norway.*

Osteochondrosis and POF represent common developmental orthopedic joint diseases that often influence performance in the athletic horse. In Standardbreds, we have recently reported the prevalence of tarsocrural OC and POF to be 19.3% and 23.1%, and the phenotypes not associated. Earlier studies have documented heritability estimates over a wide range, but no significant genetic correlation. Here, the prevalence results for tarsocrural OC and POF in 2 radiographic studies (#1217 horses) were combined and sire pedigree information included. Heritability of OC at the distal intermediate

ridge of tibia and/or lateral trochlear ridge of talus was estimated to 0.29 ± 0.15. However, the heritability estimate of OC at the distal intermediate ridge of tibia alone was as large as 0.40 ± 0.17. Heritability was estimated to be 0.23 ± 0.13 for POF in general, 0.26 ± 0.13 for metatarsophalangeal POF alone and 0.31 ± 0.14 for medial metatarsophalangeal POF. The most specific lesions resulted in the highest heritability, emphasizing that recording by anatomic location is required for efficient breeding against the diseases. Estimates of genetic correlation between OC and POF were between 0.71 ± 0.26 and 0.76 ± 0.27, suggesting the traits have shared genes and etiologic similarities.

Key Words: osteochondrosis, Standardbreds, heritability

P5045 Genetic selection for high immune response. B. A. Mallard*[1], K. Thompson-Crispi[1], M. Paibomesai[1], L. Wagter-Lesperance[1], and B. C. Hine[1,2], *[1]Pathobiology, University of Guelph, Guelph, Ontario, Canada, [2]CSIRO, Animal Production, Armidale, NSW, Australia.*

Identifying cattle with superior breeding values for immune response (IR) reduces disease, increases farm profit, improves milk quality and increases animal well-being. In Canada, it can cost the dairy producer up to $320/mastitis treatment, and 1 out of every 5 quarters is infected with a mastitis pathogen at any given time. Therefore in keeping with the European Unions proactive thinking that prevention is better than cure, genetic methods to identify animals at lower disease risk are being sought. An appealing option is to take advantage of the animals own genetic potential by selecting animals with the most robust immune system. To this end, we have identified dairy cows, calves and bulls as high, average or low immune responders using a Guelph patented protocol. Individuals with both higher and more optimally balanced antibody (AMIR) and cell-mediated immune responses (CMIR), are referred to as High Immune Responders, and this method is known as the High Immune Response (HIR) technology. Heritability for AMIR and CMIR is ~25% allowing for improvement via genetic selection. Noted benefits included less mastitis, and other diseases. Also improved response to vaccines and colostrum quality. DNA collected from high, average and low immune responder cows in Canada and the US, tested on the Illumina Chip platform, show specific SNP patterns associated with these diverse immune response phenotypes.

Key Words: disease, selection, immunity

P5046 A simple method for pre-implantation genetic gender diagnosis of biopsied equine embryos. Carolina Herrera[1], M. Cecilia Ratti[2], Cristian Sporleder[1], and M. Marcela Martinez*[2], *[1]Genetec S.A., Ruta 28 km 6.5, Pilar, Buenos Aires, Argentina, [2]Laboratorio de Genética Aplicada, SRA., Juncal 4431 20, Buenos Aires, Argentina.*

Polo Argentino is a worldwide famous equine breed dedicated to Polo sport. In terms of the game, mares are preferred over stallions due both to their agility and ease for training. During the polo season, mares are not available for breeding. However, embryo production from valuable mares overcomes this drawback. Gender development of transferred embryos is monitored by ultrasound and male pregnancies are usually interrupted by dam hormonal treatment. To avoid this, a preimplantation sex diagnosis has been developed. Using a microinjection pipette (I.D. 9um), 1 biopsy sample of 3–10 cells each was obtained from 32 in vivo produced blastocysts before being transferred to recipient mares. Gender testing was carried out by double PCR of AME and SRY markers. Single PCR previously showed not enough sensibility for this testing due to low DNA amount. Therefore, a pre-amplification technique is required like whole genome amplification or a pre-PCR followed by a second round. We preferred the last one due to lower costs and simplicity. Forty percent (40%) of biopsied embryos could be tested and gender compared with the results obtained by ultrasound. Pregnancy rates did not differ between biopsied and intact embryos. Since rate success grows with the number of cells in the biopsy, our next goal will be to develop a biopsy procedure that allows high cell recovery without loosing embryo viability.

P5047 Diversity of *Escherichia coli* virulence genes for colibacillosis in piglets. R. P. Mohlatlole*[1], E. Madoroba[2], F. C. Muchadeyi[3], A. T. Kanengoni[4], M. Chimonyo[1], and E. F. Dzomba[1], *[1]University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa, [2]Microbiology Section, Agricultural Research Council, Onderstepoort, Pretoria, Gauteng, South Africa, [3]Biotechnology Platform, Agricultural Research Council, Onderstepoort, Pretoria, Gauteng, South Africa, [4]Animal Production Institute, Agricultural Research Council, Irene, Pretoria, Gauteng, South Africa.*

Escherichia (*E. coli*) infections in growing pigs result in colibacillosis with the most common symptoms being diarrhea and edema. Enterotoxigenic (ETEC), shigatoxin producing (STEC) and enteroaggregative (EAEC) *E. coli* are major causes of colibacillosis. The objective of this study was to investigate the prevalence of enterotoxin (LT, STa, STb), shiga-toxin (Stx1, Stx2, Stx2e), enteroaggregative heat stable *E. coli* (EAST-1), associated fimbriae and adhesin genes in South African pigs. E. coli strains were isolated from 263 piglets of either the Landrace, Large White, Duroc or Indigenous breed. PCR analysis showed the presence of virulence genes (VGs) in 40.30% of the isolates which

were classified as ETEC (18.63%), STEC (0.38%) and EAEC (17.49%) with 3.82% harbouring genes associated with either ETEC and EAEC or STEC concurrently. Individual VGs were found in the following proportions: EAST-1 (20.53%), STb (19.77%), LT (0.38%), STa (3.42%), and St2xe (1.14%) toxins. No fimbrial genes were detected in the isolates positive for toxin genes. Instead, afimbrial adhesin factors EAE (1.4%), AIDA-1(5.7%) and PAA (7.22%) were detected suggesting their use as the colonisation factors. Overall, 25 pathotypes were identified from the isolates carrying VGs. Pathotypes EAST-1 (12.17%), STb (5.32%) and STb/AIDA-1 (4.18%) were the most prevalent.

Key Words: diarrhoea, *Escherichia coli*, virulence genes

P5048 Low MHC diversity in the Tasmanian devil pre-dates European settlement. K. Morris*[1], J. Austin[2], and K. Belov[1], *[1]Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia, [2]Australian Centre for Ancient DNA, The University of Adelaide, Adelaide, SA, Australia.*

The Tasmanian devil (*Sarcophilus harrisii*) was once widespread on mainland Australia but today is restricted to the island of Tasmania. The devil is currently at risk of extinction due to the emergence of a contagious cancer known as Devil Facial Tumour Disease (DFTD). The emergence and spread of this disease has been linked to a lack of histocompatibility barriers in the devil due to low diversity in the major histocompatibility complex (MHC). The aim of this project was to determine whether loss of MHC diversity was caused by human impacts or whether it predates European settlement in Australia. MHC class I alleles were cloned and sequenced from 9 museum samples spanning the last 200 years since European settlement, a single Tasmanian sample from pre-European colonisation and 4 mainland devil samples which are at least 3000 years old. The 3000 year old MHC alleles are the oldest ever sequenced. Our results reveal no additional diversity in the Tasmania samples. The mainland devils had common modern alleles as well as novel alleles which are not present in modern Tasmania. However, these novel alleles are highly similar to existing alleles. We conclude that low MHC diversity has been a feature of devil populations for over 3000 years and that a history of population crashes is consistent with a paucity of genetic diversity in key immune response genes.

Key Words: Tasmanian devil, ancient, MHC

P5049 The double deletion diplotype showed low levels of prion protein at two indel loci of PRNP in the medulla oblongata of Japanese Brown cattle. George Msalya*[4,1], Takeshi Shimogiri[2], Shin Okamoto[2], Kotaro Kawabe[3], and Yoshizane Maeda[2], *[1]United Graduate School of Agriculture, Kagoshima University, Kagoshima 890 0065, Japan, [2]Faculty of Agriculture, Kagoshima University, Kagoshima 890 0065, Japan, [3]Frontier Science Research Centre, Kagoshima University, Kagoshima 890 0065, Japan, [4]Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro, Tanzania.*

Transmissible spongiform encephalopathies (TSEs) are a class of fatal neurodegenerative diseases caused by abnormally folded prion proteins (PrP). The PrP is necessary for the transmission and propagation of TSE diseases. In this study, PrP was quantified in the medulla oblongata of 39 Japanese Brown (JBr) animals that were genotyped for 2 indels in the PRNP gene - a 23 bp deletion in the promoter region and a 12-bp deletion in the first intron. The mean level of PrP was greater in the ++/++ diplotype than in Ã¢â‚¬â diplotypes, although the differences were not significant. These results suggest that the amount of PrP in the medulla oblongata of animals is related to these indels. However, given that there have been no reported cases of BSE in Japanese Brown animals, the relationship of the indels and PrP levels with the incidence of BSE is unclear.

Key Words: double deletion diplotype, prion protein, Japanese Brown cattle

P5050 A de novo germline mutation in MYH7 causes a progressive dominant myopathy in pigs. Leonardo Murgiano*[1], Imke Tammen[2], Barbara Harlizius[3], and Cord Drögemüller[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]ReproGen, Faculty of Veterinary Science, Camden, Australia, [3]Institute for Pig Genetics, Beuningen, The Netherlands.*

The offspring of a clinically healthy Piétrain boar named ‘Campus’ showed a progressive postural tremor called Campus Syndrome (CPS). Backcross experiments (with a percentage of affected animals of 67%) suggested a dominant mode of inheritance, and the founder boar was believed to be a gonadal mosaic. A genome-scan mapped the disease-causing mutation to an 8 cM region of porcine chromosome 7 containing the MHY7 gene. Human distal myopathy type 1, a disease resembling partially CPS in pigs, has been associated with mutations in the MYH7 gene. The porcine MYH7 gene structure was predicted basing on the information obtained in comparison to the human ortholog. Mutation analysis of the genomic interval of more than 22 kb spanning the complete MYH7 gene revealed an in-frame insertion within exon 30 of MYH7 (c. 4320_4321insCCCGCC, predicted p.Ala1440_Ala1441insProAla) which was perfectly associated with the disease phenotype. Genotyping of the mutation confirmed the dominant inheritance. ‘Campus’ was shown to be a germline and somatic mosaic as assessed

by the presence of the mutant allele in 7 different tissues originating from the 3 germinal layers. We provide evidence that the spontaneous CPS mutation occurred during the early development of the boar 'Campus'. Therefore, this study provides an example of a germline mosaicism with an asymptomatic founder.

Key Words: germline mutation, pig, mosaicism

P5051 Investigation of DNA polymorphisms linked to cerebellar abiotrophy in Australian kelpie dogs. Annie Y. H. Pan*[1], Alan N. Wilton[1], Jeremy R. Shearman[1,2], Sven K. Delaney[1], and Barbara Zangerl[1], *[1]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia, [2]National Center for Genetic Engineering and Biotechnology, NSTDA, Pathumthani, Thailand.*

Inbreeding has been common within the Kelpie breed and persistent breeding of champion dogs (popular sire effect) has resulted in the spread of autosomal recessive conditions. One of these, Cerebellar Abiotrophy (CA) results in ataxia. Whole genome association and homozygosity analysis have mapped the CA locus to a 5 Mb region on chromosome 3 and identified a common SNP haplotype shared between all affecteds and some unaffected controls. Using 454 sequencing, 2019 differences were identified homozygous in the 2 affecteds compared with the control, 17 of which were synonymous substitutions in coding exons and substitutions in the untranslated regions of mRNA. On top of this, 22 differences in introns and intergenic regions with high sequence conservation between other mammals and dogs were identified and investigated as possible causative mutations for CA in Kelpies. PCR and Sanger sequencing were employed to fill in 454 sequencing gaps for 40 coding, non-coding exons and upstream regions of different genes within the candidate region. One intergenic deletion that is conserved between other mammals and dogs was found to be homozygous in the affecteds compared with controls and was predicted to disrupt a HSF2 transcription factor binding site for regulation of neighboring genes. These genes are currently under further investigation.

Key Words: cerebellar abiotrophy, Australian Kelpies

P5052 Omega-3 fatty acids inhibit proliferation in myoblasts and mouse muscle tissue via decreasing cyclin E expression through MEK-Erk pathway. Yunqian Peng,* Yu Zheng, Fei Chang, Xiaoxiang Hu, and Ning Li, *State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100193, P.R.China.*

Polyunsaturated fatty acids (PUFAs) are important molecules for human health. There is a lack of information on omega-3 PUFAs in the modulation of myoblasts and muscle. We indicate that different kinds of omega-3 PUFA differentially affected myoblast cell proliferation. DHA and EPA decreased myoblasts proliferation via decreasing cyclin E and CDK2 levels at both the protein and mRNA level. MAPK-ERK pathway is involved in this modulation. We also made a mouse model co-expressing fat-1 and fat-2 gene from C.elegans specific in muscle tissues, which can enable mice to synthesize PUFAs. Omega-3 fatty acids increased from 4.9% (wild type) to 14.2% (transgenic), especially DHA content increased from 4.5% to 14.1% in TG-mice. Then, we compared the cyclin E and ERK expression profile of skeletal muscles (quadriceps femoris and tibialis anterior) between TG-mice and normal mice. The cyclin E protein and phosphor-ERK decreased in TG-mice. This is consistent to our in vitro experiment. Our results indicate that very long chain omega-3 PUFA affected myoblast cell proliferation through a mechanism involving MAPK-ERK both in vitro and in vivo.

Key Words: omega-3 fatty acids, myoblast, MAPK

P5053 GWAS identifies FKBP6 as a susceptibility locus for impaired acrosome reaction in stallions. Terje Raudsepp*[1], Molly E. McCue[2], Pranab J. Das[1], Lauren Dobson[3], James N. Derr[3], Krista L. Fritz[4], Robert Schaefer[4], Aaron K. Rendahl[2,5], James R. Mickelson[4], Charles C. Love[6], Steve P. Brinsko[6], Bhanu. P. Chowdhary[1], and Dickson D. Varner[6], *[1]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX77843, USA, [2]Department of Veterinary and Biochemical Sciences, University of Minnesota, St. Paul, MN55108, USA, [3]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX77843, USA, [4]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN55108, USA, [5]School of Statistics, University of Minnesota, Minneapolis, MN55455, USA, [6]Department of Large Animal Clinical Sciences, Texas A&M University, College Station, TX77843, USA.*

Acrosome functions are under genetic control but the genomics of impaired acrosome reaction, a reproductive disorder causing male subfertility, is as yet poorly understood. Here we conducted Equine SNP50 genotyping and GWAS using 7 IAR-affected and 37 control TB stallions. Significant ($P < 6 \times 10^{-8}$) association was found in ECA13p in FKBP6. The gene belongs to the immunophilins FKBP family known to be involved in meiosis, calcium homeostasis, clathrin-coated vesicles, and membrane fusions. Direct sequencing and large cohort (n = 255) genotyping determined significant ($P < 7 \times 10^{-4}$) association of FKBP6 exon 4 SNPs g.11040315G>A and g.11040379C>A (p.166H>N) with the IAR phenotype, whereas all IAR stallions were homozygous for the A-alleles. The equine FKBP6 was exclusively and monoallelically expressed in testis

and sperm, with a tendency for upregulation in IAR stallions, and suppression of A-alleles in AC and AG heterozygotes. Two novel FKBP6 splice variants were found in testis and sperm. FKBP6 was considered as a susceptibility gene for IAR in stallions and a candidate gene for male subfertility in other mammals. A simple genotyping test is recommended for the detection of IAR susceptible individuals among potential breeding stallions. Successful use of sperm as a source of DNA and RNA propagates non-invasive sample procurement for fertility genomics in animals and humans.

Key Words: acrosome, stallion, FKBP6

P5054 DNA methylation analysis in sperm from infertile/subfertile boars. H. Acloque[1], A. Pinton[1], A. Congras[1], C. Delcros[1], F. Vignolles[1], S. Ferchaud[2], J. Riquet*[1], and M. Bouissou-Matet Yerle[1], *[1]INRA, Cell Genetics Laboratory, Castanet-Tolosan, France, [2]INRA, UEICP, Ruillé, France.*

Male infertility is an increasing health challenge for our societies, either for human or livestock's populations. As diffusion of the genetic progress goes through sires, male infertility consequently slows the improvement of animal selection schemas and farms' productivity. In the french pig sector, more than 35% of boars selected on agronomical criteria and to be diffused through Artificial Insemination Centers (AICs) are culled for bad sperm quality (including concentration, morphology and motility). We propose here to study the epigenetic marks in the sperm and somatic DNA from fertile and subfertile/infertile boars at two different levels. First, we started the production of genomewide methylome maps from fertile boars. Then, taking advantage of our collection of sperm and somatic cells from fertile and infertile boars, we compared DNA methylation levels at specific loci reported to be altered in infertile humans. Combining these studies, our aim is to define specific or common epigenetic signatures of farm animals' infertility. These epigenetic signatures will be an additional parameter to evaluate the sperm quality and will finally help to lower the number of potentially infertile males introduced in the selection schemas. Moreover acquisition of epigenetic information on germ cells may help to introduce epigenetic factors within genomic selection models.

Key Words: infertility, epigenetics, sperm DNA methylation

P5055 A fine genetic analysis of congenital diseases in pig. S. Rousseau[1], N. Iannuccelli[1], E. Pailhoux[2], B. Servin[1], and J. Riquet*[1], *[1]INRA, Cell Genetics Laboratory, 31326 Castanet-Tolosan, France, [2]INRA, Developmental Biology and Reproduction, 78352 Jouy-en-Josas, France.*

The most important congenital genetic defects that occur in piglets are hernias (umbilical hernia and inguinal or scrotal hernias), cryptorchidism and splay legs, and to a lesser extent intersexuality, hermaphrodism and anal atresia. They affect on average 3% of the commercial pig populations worldwide. It is important to stress out that, besides the direct economic loss, these defects have a serious impact on animal welfare and health. Some of the defects cause direct piglet mortality, while others lead to culling of piglets showing the disorder. For most of these defects there are strong indications for a genetic component. In SwAn project, funded by ANR (the French national agency of research) we propose to focus on the identification of genes underlying congenital / hereditary disorders in pigs. We propose (1) to construct a collection of affected samples obtained in collaboration with private breeding companies, (2) to use genome-wide association studies (GWA) with a SNP panel comprising 60 000 markers covering the entire genome at a density which is appropriate for population-based association studies, (3) to identify some of the causal mutations and genes of these congenital diseases and (4) to propose effective marker assisted selection (MAS) against genetic defects. First results obtained for intersexuality, scrotal hernia and cryptorchidism will be presented.

Key Words: pig, GWAS, congenital diseases

P5056 Genome-wide association study for cryptorchidism in dogs. X. Zhao, S. Onteru, D. Garrick, and M. Rothschild,* *Dept. Animal Science, Iowa State Univ., Ames, IA, USA.*

Cryptorchidism is a condition in which one or both testes fail to descend into the scrotum. The incidence in purebred dogs varies between 1 and 14%. The mode of inheritance of cryptorchidism is not known. To determine genes or genome regions responsible for cryptorchidism, DNA samples from 204 male Siberian Husky dogs (105 cases and 99 controls) collected from USA, Canada and UK were genotyped with the CanineHD BeadChip. The K-means clustering algorithm (K = 2) was applied to a difference matrix based on additive genetic correlations to separate dogs into 2 clusters to reduce population structure. Case-control analyses were performed separately for each cluster using PLINK and Bayes-B in Gensel. A 0.85Mb region on CFAX accounted for 1.06% of the genetic variance in cluster 1 and contained several genes involved in extracellular matrix (ECM) remodeling. It is known that ECM remodeling of the gubernaculum is involved in testicular descent. A genomic region covering 2Mb on CFA27 explained 3.21% of genetic variance in cluster 2. Fifty genes including 16 members of Keratin subfamily are located in that region. It appears that mutations on CFA27 and

CFAX might contribute to cryptorchidism separately or together in different dog populations.

Key Words: cryptorchidism, SNP, association

P5057 Diagnostic test for bovine brucellosis through molecular markers, integrated system of electronic identification and mapping of the origin of infected animals confirmed by DNA. K. Souza,* E. Silva, C. Trant, and C. Almeida, *Linhagen Biotecnologia, Belo Horizonte, MG, Brasil.*

Bovine brucellosis is a chronic disease, often asymptomatic; with difficult diagnosis and reduced mortality that threatens the Brazilian herds. Our goal was to develop a diagnostic kit gene that enables, by means of molecular markers, detection of infectious agent Brucella abortis, causing Brucellosis, and associated with an information system was confirmed by DNA, capable of performing the mapping of the identity and origin of the infected animals efficiently and auditable, from farm to supermarket. For the detection of virulent strain S2308 of B. abortus were designed two pair of primers, capable of differentiating between between the wild type strain and the vaccine strain S19. The target gene chosen for the detection of bacteria of the genus Brucella was the gene encoding the membrane protein immunogenic BCSP31 (Mayfield et al., 1988). This protein is specific for the genusBrucella and is present in all subtypes of bacteria this group. Another target was the gene encoding D-erythrulose-1-phosphate dehydrogenase (Ery C) vaccine strain S19 in a deletion of 702 bp (Sangari et al., 1994). A third pair of primers was also designed to differentiate another vaccine strain, strain RB51. This primer targets an insertion element IS711, which in this attenuated strain is interrupting the gene for glycosyltransferase (Vemulapalli et al., 1999). We used the DNAs of Brucella, strains S2308, S19 and RB51. We used specific primers for the genus,Brucella, and those showed no amplification of DNA fragments in the presence of other bacterial genera and were able to differentiate the vaccine strain S19, S2308 and RB51 strains.

Key Words: bovine disease, brucellosis, molecular detection

P5058 Promising loci for the susceptibility to both scrotal hernia and cryptorchidism in pigs. A. Stinckens*[1], S. Janssens[1], G. Spincemaille[2], and N. Buys[1], *[1]KU Leuven, Leuven, Belgium, [2]Rattlerow-Seghers Holding NV, Lokeren, Belgium.*

Congenital genetic defects are quite common in swine and cover a range of conditions. Two of the most important congenital defects that occur in piglets are scrotal hernia, a condition which is phenotypically characterized by an uncontrolled prolapse of the small intestine into the scrotum, and cryptorchidism, a sex-limited abnormality characterized by the fact that one or both testicles are not descended correctly into the scrotum. Scrotal hernia and cryptorchidism occur within the pig population at an average frequency from 1.7% to 6.7% and 1 to 1.4%, respectively, but in practice percentages of up to 10% are recorded in offspring of particular boars. For both disorders, in previous studies using microsatellites, several QTL were found. However, these QTL regions were still very wide (20–40 cM). To decrease the order of magnitude, in this study a whole genome scan was performed, using marker data obtained by the 60K porcine SNP beadchip. In total, 188 parent-offspring trios were genotyped for scrotal hernia and 300 parent-offspring triplets were genotyped for cryptorchidism. Based on the analysis with the free whole genome association toolset PLINK (http://pngu.mgh.harvard.edu/purcell/plink), significant loci for the susceptibility for both scrotal hernias and cryptorchidism in pigs could be detected.

Key Words: congenital genetic disorders, pigs, whole genome scan

P5059 Dogslife: A web-based study of canine health. K. M. Summers*[1,2], C. A. Pugh[1,2], I. G. Handel[1,2], B. M. Bronsvoort[1,2], and D. N. Clements[1,2], *[1]The Roslin Institute, Edinburgh, United Kingdom, [2]The Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom.*

Dogslife is a web-based, longitudinal study of the health of Labrador Retrievers in which participants complete an online questionnaire at defined intervals to capture phenotypic data and owner-reported information on the dog's environment, diet, exercise levels and health/illness. Since July 2010 over 2,300 dogs have been registered. This cohort was geographically representative of UK-based Labrador Retrievers. Rarer coat colors were slightly over-represented and the owner cohort comprised fewer smokers than the wider UK population. The recruitment rate was 2.2% of the (available) Kennel Club registered Labrador Retriever population. After 18 mo 2.6% of the registered dogs were permanently lost to follow-up either due to re-homing (1.5%) or death (1.1%). Illness or clinical signs were reported for 86% of dogs in the first year of life. To date, over 3,300 illnesses or clinical signs have been recorded, many of which did not require a veterinary visit and thus represent conditions perceived to be minor, but which may be associated with more severe presentation later in life. We are currently reviewing the data to identify groups of animals for genetic analysis. The breadth of illness data captured by Dogslife offers a unique opportunity to seek relationships between genetic factors,

the environment, early minor illness and subsequent ill health in a companion canine population..

Key Words: canine health, genetic disease, epidemiology

P5060 Genome-wide association mapping identifies several major susceptibility loci for Canine Lymphocytic Thyroiditis. K. Sundberg*[1], N. Kamgari[2], K. M. Ahlgren[3], A. Lobell[3], O. Kämpe[3], K. Truvé[1], E. Strandberg[1], G. Andersson[1], Å Hedhammar[4], G. Pielberg[2], and K. Lindblad-Toh[2,5], *[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Science for Life Laboratory, Dep of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [3]Dep of Medical Sciences, Autoimmunity, University Hospital, Uppsala, Sweden, [4]Dep of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, [5]Broad Institute of MIT and Harvard, Cambridge, MA, USA.*

Canine lymphocytic thyroiditis (CLT), an autoimmune disease resulting in hypothyroidism, is the most common immune-related disease in dogs and is believed to result from a complex interaction between genetic and environmental risk factors. To map susceptibility loci for CLT we performed a genome-wide association analysis in giant schnauzer dogs using the CanineHD Illumina SNP array in strictly classified cases (n = 74) and controls (n = 48). We identified several major susceptibility loci (praw = 10–6) located on chromosome 11 and X. The chromosome 11 region harbours 10 genes not previously associated with immunological processes, as well as the interferon α gene cluster. Type I interferons are key players in immune response activation, but are also known to be strong inducers of autoimmune thyroid disease in human. Next-generation sequencing revealed multiple mutations within and close to conserved elements. Currently, we are performing genetic and functional follow up of candidate mutations and genes.

Key Words: dog, thyroiditis, genome wide association analysis

P5061 Novel Th epitope of bovine leukemia virus detected in disease susceptibility cattle determined by BoLA-DRB3 allele. Shin-nosuke Takeshima*[1,2], Jiyun Kim[1], Kyoji Hagiwara[1], Yuki Matsumoto[1], Takashi Ohmori[3], Tetsuo Nunoya[3], Kazuhiro Matoba[4], and Yoko Aida[1,2], *[1]Viral Infectious Diseases Unit, RIKEN, Wako,Saitama,Japan, [2]Laboratory of Viral Infectious Diseases,The University of Tokyo, Wako,Saitama,Japan, [3]Nippon institute for biological science (NIBS), Ome, Tokyo, Japan, [4]National Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan.*

Bovine leukemia virus (BLV) induces Enzootic bovine leukemia (EBL) to infected cattle in low incidence rate. Our previously study determined that cattle which had BoLA-DRB3*1601/*1601 genotype tend to onset EBL and indicates high proviral load. Because of one of the major problem to develop the BLV vaccine is unstable effect of the vaccine because of the individual difference, we determined to develop the BLV vaccine for disease susceptibility cattle. We genotypes 90 heads of Japanese black cattle by using our developed BoLA-DRB3 PCR sequence based typing method and also detected BLV provirus by also our developed BLV-CoCoMo-qPCR method. One BLV infected susceptibility cattle, homozygote of BoLA-DRB3*1601 allele, was found and the cattle was used to immunological assay. To determine the Th epitope for BLV antigens from susceptibility cattle, we stimulated CD4 positive T cell obtained from susceptibility cattle with BLV antigen. This cell line was cocultured with irradiated, autologous antigen presenting cells and synthesis BLV overlattping peptides constructed from BLV env and gag sequences for 3 days for proliferation assay measured by [3H]-TdR incorporation. Four regions were detected as Th epitope including one novel region at the N-terminal of ENV Gp51 and GAG p12. Two region of three were positioned at near sequences but not itself. In this study, we identified Th epitope using BLV susceptibility cattle. Interestingly, the sequences were not corresponded with previously reported Th epitope. For design novel BLV vaccine for susceptibility cattle, candidate antigen will be designed based on the information of our determined Th epitope.

Key Words: Th epitope mapping, bovine major histocompatibility complex, bovine leukemia virus

P5062 Expression analyses of beta-carotene genes affecting beef fat colour. R. Tian*[1], N. Cullen[2], C. Morris[2], W. Pitchford[1], and C. Bottema[1], *[1]School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia, [2]AgResearch, Ruakura Research Centre, Hamilton, New Zealand.*

Beef with yellow fat is not acceptable in most European and Asian markets. Beta-carotene is the major carotenoid deposited in beef fat causing the yellowness. Beta-carotene 15,15'-monooxygenase 1 (BCMO1) and β-carotene oxygenase (BCO2) catalyze the symmetric and asymmetric oxidative cleavage of β-carotene in carotene metabolism, respectively. Epidermal retinal dehydrogenase 2 (RDHE2) belongs to a member of the short-chain alcohol dehydrogenase/reductase family and converts retinol to retinaldehyde as the first step in the retinoic acid synthetic pathway. An association study showed a significant effect of the BCMO1, BCO2 and RDHE2 genes on beef fat color. Given the genotypic effect of these 3 genes, the expression of the genes was measured in yellow and white fat samples

to investigate their role in fat color. The main findings from the real-time qPCR analyses were that 1) there was no difference in the expression of the BCO2 and BCMO1 genes, 2) the RDHE2 gene expression level was higher in white samples, 3) the expression of the BCO2 gene was associated with β-carotene concentration, and 4) the BCO2 variant W80X genotype was marginally associated with BCO2 gene expression levels. These observations indicate that the control of the retinol/retinoic acid pathway at the gene expression level is important in the accumulation of carotene in subcutaneous adipose tissue.

Key Words: cattle, carotenoid, yellow fat

P5063 Transcriptom variance of porcine renal epithelial cells induced by viral dsRNA stimulatory and DNA methyltransferases inhibitor. Xiaoshuo Wang, Linjing Bai, Liwei Zhai, Ying Yu, and Chuduan Wang,* *Department of Animal Breeding and Genetics, China Agricultural University, Beijing, 100193, P.R. China.*

Porcine viral-diseases cause great economic losses in the pig-raising industry. Since the low heritability of antiviral diseases, it is hard to improve the resistance of viral-diseases in swine population. DNA methylation builds up the crosstalk between virus and host, while DNA methyltransferases inhibitor 5-Aza-2′ deoxycytidine (5-Aza-dC, Aza) has demethylating effect. Here, we tested the hypothesis that genome-wide demethylation can influence the host gene expression induced by Aza and viral dsRNA stimulatory (Poly(I:C), Poly). We treated normal porcine renal epithelial cells with Poly and Aza agents to analyze genome-wide expression variation with Microarray techniques. A total of 860 (Poly), 5563(Aza) and 874 (Poly+Aza) porcine genes were showed differential expression compared with the controls (fold change ≥ 1.5, $P \leq 0.05$). Pathway analysis disclosed that the significant pathways were mainly concerned with antigen processing and presentation, long-term depression and T cell receptor signaling pathway via compared with 2 groups of Poly versus Control and Poly+Aza versus Poly. The results found that Aza has the negative effects on viral replication. Additionally, porcine CD4 gene expression was upregulated by DNA demethylation in the cell line, which is warranted to validate by bisulfite cloning sequencing and EMSA.

Key Words: methylation, CD4, porcine

P5064 Extended scrapie incubation time in goats singly heterozygous for PRNP S146 or K222. S. N. White*[1,2], J. O. Reynolds[1], D. F. Waldron[3], D. A. Schneider[1], and K. I. O'Rourke[1,2], *[1]USDA-ARS Animal Disease Research, Pullman, WA, USA, [2]Department of Veterinary Microbiology & Pathology, Washington State University, Pullman, WA, USA, [3]Texas Agrilife Research, San Angelo, TX, USA.*

Scrapie is the transmissible spongiform encephalopathy of sheep and goats. Goats may serve as a scrapie reservoir, and to date there has been no experimental inoculation confirming strong, lifelong genetic resistance in goats. Both S146 and K222 variants have been present in exposed flocks but significantly underrepresented in scrapie cases, and have low cell-free protein conversion efficiency to PrPSc. To test degree of resistance in live animals with consistent exposure, we performed the first oral scrapie challenge of goats singly heterozygous for either S146 or K222. All controls became clinically scrapie positive by an average of 24 mo, but all S146 and K222 heterozygotes remain scrapie negative by both rectal biopsy and clinical signs at longer incubation times ($P < 0.0001$). Recent reports indicate small numbers of scrapie positive S146 and K222 heterozygotes, suggesting heterozygotes will not have complete resistance but extended incubation. These oral challenge results confirm in a more natural exposure context the extended incubation observed in a recent intracerebral challenge of K222 heterozygotes, and to our knowledge provide the first demonstration of extended incubation in S146 heterozygotes. These results suggest longer relevant trace-back histories in scrapie-eradication programs for these animals and strengthen the case for additional experiments in both homozygotes.

Key Words: scrapie, goat, challenge

P5065 Estimates of SNP allele and genotype frequencies and whole-genome association analysis of scrapie incidence in sheep. Chunhua Wu*[1], Tracy Hadfield[1], James Kijas[2], and Noelle Cockett[1], *[1]Department of Animal, Dairy, and Veterinary Sciences, Logan, Utah, USA, [2]CSIRO Livestock Industries, Queensland Bioscience Precinct, Brisbane, Queensland, Australia.*

Scrapie is an infectious disease of sheep that affects the central nervous system and is classified as a transmissible spongiform encephalopathy (TSE). In this study, a genome-wide association analysis (GWAS) was performed using 37 sheep samples that were deliberately inoculated with scrapie-positive tissues under a case-control design. Of the 37 animals, 25 were classified as scrapie positive (i.e., case animals). These animals were genotyped with the Ovine SNP50 Beadchip. A comparison of allele frequencies between scrapie-positive animals and scrapie-negative animals identified 620 SNP loci which showed statistically significant frequency differences ($0.001 < P < 1.93E\text{-}05$). However, none of these SNPs were significant when genotype frequencies were compared between the 2 groups of animals. Chromosomal regions containing significant SNPs will be examined for positional candidate genes.

Key Words: sheep, 50K SNP chip, scrapie disease

P5066 Genotyping of genes responsible for 7 defective traits in Japanese Black by the DigiTag2 assay. H. Yasue*[1], T. Shimogiri[2], S. Niwata[3], M. Nishibori[4], and H. Mannen[5], *[1]Tsukuba GeneTechnology Laboratories, Tsukuba, Japan, [2]Kagoshima University, Kagoshima. Japan, [3]Kurabo Industries Ltd., Osaka, Japan, [4]Hiroshima University, Higashi-Hiroshima, Japan, [5]Kobe University, Kobe, Japan.*

In this study, we provide a genotyping system of genes responsible for 7 defective traits in Japanese Black cattle using the DigiTag2 assay, which had been developed as a 96-plex SNP typing system with a high conversion rate (>90%), high accuracy and low cost. Seven defective traits were Spherocytosis (Band3), Chediak-Higashi syndrome (CHD), renal tubular dysplasia (CL16–1 and CL16–2), Factor XIII deficiency (F13), Xanthinuria (MCSU), multiple ocular defects (MOD) and coat color variation (MC1R). Samples were 94 Japanese Black sire, one Japanese Black dam and one Holstein dam. Out of them, the numbers of carriers determined by the current genetic tests were 2 for Band3, 19 for CHS, 5 for CL16–1, one for CL16–2, one for F13, 2 for MCSU, one for MOD and one for MC1R. The DigiTag2 assay was performed according to the previous report. Genotyping by the DigiTag2 assay successfully discriminate between the carrier and non-carrier cattle in all traits. Genotypes from sequencing corresponded to those from the DigiTag2 assay. These results suggest that this system is efficient and reliable in the carrier identification of various hereditary diseases.

Key Words: DigiTag2, defective traits, Japanese Black

P5067 Genome-wide association study for blood MCV and MCH in an F2 intercross between Landrace and Korean Native pigs. Chae-Kyoung Yoo*[1], Jae-Bong Lee[1,2], Eun-Ji Jung[1], Beom-Mo Kim[1], Hye-In Kim[1], Hee-Bok Park[1,2], In-Cheol Cho[3], and Hyun-Tae Lim[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea, [3]National Institute of Animal Science, Rural Development Administration, Jeju 690-150, Korea.*

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are measured from the red blood cells and are used to reveal the causes of red blood cell problems and anemia. Our previous study reported quantitative trait loci (QTL) affecting blood-related traits in pigs. For these traits, genome-wide significant QTL were identified in SSC8 (SW444-S0069), SSC9 (SW2093-SW749), and SSC13 (SW38-S0215). To select single-nucleotide polymorphism (SNP) markers associated with the MCV and MCH, a genome-wide association study (GWAS) was conducted using an F2 intercross between Landrace and Korean native pigs (n = 1105). The porcine SNP 60K chip from Illumina (USA) and a mixed-effects model approach accounting for familial relationships between individuals were used to perform the GWAS. After implementation of quality control performance criteria, 42,773 SNP markers were left for GWAS. A genome-wide significant threshold was set using the Bonferroni method (P = 1.17 × 10–6). As a result, significant SNP markers were found in the QTL regions for SSC8 and SSC13, but nothing was found for SSC9.

Key Words: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), genome-wide association study (GWAS)

P5068 Study on experimental mastitis induced by *Staphylococcus aureus* in dairy cattle. Zhengrong Yuan*[1,2], Jiao Li[2], Junya Li[2], and Shangzhong Xu[2], *[1]Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of MedicalSciences & Peking Union Medical College, No. 151 Malianwa North Road, Haidian District,Beijing 100193, P. R. China, [2]Institute Animal Science, Chinese Academy of Agricultural Sciences, No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, P. R. China.*

Dairy mastitis is the most complex disease and major economic losses to dairy industry worldwide. *Staphylococcus aureus* is one of the most important pathogenic bacteria which causing dairy mastitis. The author has established the Chinese Holstein dairy clinical mastitis disease model which caused by infected with *S. aureus* and analyzed the genes expression profiles in the whole genome-wide by using cDNA microarray. Q-PCR, ELISA and Western Blot were used to verify the microarray results, then the differentially expressed genes were detected by using GO and Pathway analysis. Based on the differential proteomics method, we analyzed the protein expression and protein-related information by combining 2-D DIGE with MALDI-TOF-MS identification techniques. The pathological mechanism and screening of clinical mastitis disease molecular markers and key proteins have been observed through systematically analysis on the genome, transcriptome and proteome aspects. Gene regulation and protein interaction network has been investigated by bioinformatics and related experimental methods. We verified the function of differentially expressed genes and proteins from the molecular, cellular and overall level. This study has supported to further clarify the molecular mechanism of mastitis, explored the gene immunization approach to breeding for disease resistance to mastitis.

Key Words: mastitis, *Staphylococcus aureus*, dairy cattle

P5069 Principal component analysis of gene expression profiling to Campybacter infection in two distinctly genetic chicken lines. Huaijun Zhou* and Xianbo Jia, *Department of Animal Science, University of California, Davis, Davis, CA, USA.*

The generation of high throughput microarray data requires efficient exploratory tools to extract significantly important biological information and cope with the high dimensionality, high noise and a few samples. This is especially true in identifying a cluster of biological samples related to genetic resistance to bacterial infection from gene expression data of host response in different genetic lines. Principal component analysis (PCA) offers a great approach to reveal experimental characteristics by projecting the data into a new dimension that are uncorrelated and orthogonal. In this study, microarray data of splenic and cecal response to Campybacter infection in 2 distinctly genetic chicken lines (line A: resistant; line B: susceptible) were used. Birds from lines A and B were divergently clustered in both tissue data. However, within each line, infected birds were not separated from non-infected birds by including either all genes or only significantly expressed genes. For the infected birds within each line, there was considerable variation among them in terms of bacterial count in cecal content, and PCA analysis also could not differentiate them. The results suggest genetic difference rather than bacterial infection contributed to biological gene expression variation in this case. Further alternative approach using independent component analysis with better performance in separating different biological groups is underway to reevaluate this data set.

Key Words: principal component analysis, bacterial infection, chicken

P6000–P6015

Structural and comparative genomics

P6000 An American mink transcriptome. R. Anistoroaei,* L. Croft, and K. Christensen, *University of Copenhagen, Faculty of Health Sciences, Copenhagen, Denmark.*

In parallel with efforts of sequencing the American mink genome that are currently undertaken, a transcriptome was generated. Analysis of the long non-coding RNAs, microRNA, small peptides and elements unique to the mink are planned. A pool of RNAs originating from 4 different tissues from a wild type mink individual were deeply paired end sequenced on HiSeq 2000 Illumina. This yielded approximately 100 Gb of raw data. Ca. 260.000 unique contigs, covering 125 Mb, were assembled by means of Velvet and Phred Phrap respectively. Of these, 136.000 (52%) match the human genome and 55.000 contigs match the human transcriptome at 34.000 unique gene locations (~1.6 contig/gene). Several 222.000 contigs (85%) hit the dog genome but the annotation of genes in this species is still deficient. Subsequent searches against the mink genomic contigs developed and assembled so far (Anistoroaei et al. 2011; Mink Sequencing Project – draft in preparation) indicate that most of the transcripts have been recuperated and aligned against these contigs. Thus, the assembling and annotation of the transcriptome will be eased by the specific genomic sequence aiding in overcoming regions dismissed by the "de novo" assembling programs. In this context, our tests also indicate that ca. 20% of the contigs can be further assembled between them, hypothetically yielding one contig per annotated gene from the human reference transcriptome.

Key Words: American mink, transcriptome

P6001 Fine mapping of QTL for performance traits in the central part of SSC2 in Italian Large White. Stanislav Cepica*[1], Roberta Davoli[2], Filip Weisz[1,3], Ales Knoll[3], Paolo Zambonelli[2], Mila Bigi[2], Zuzana Vykoukalova[3], Martin Masopust[1], Maurizio Gallo[4], and Luca Buttazzoni[5], *[1]Institute of Animal Physiology and Genetics, AS CR, Libechov, Czech Republic, [2]DIPROVAL, Sezione di Allevamenti Zootecnici, University of Bologna, Reggio Emilia, Italy, [3]Mendel University in Brno, Brno, Czech Republic, [4]National Association of Italian Pig Breeders Association (ANAS), Rome, Italy, [5]Research Center for Meat Production and Genetic Improvement (CRA), Rome, Italy.*

Apart from QTL within the IGF2 gene located at the proximal tip of SSC2 other QTL affecting growth, carcass and meat quality traits have been documented between 55 and 77 cM, mostly in Meishan derived F2 populations. The SNPs were prepared either by comparative sequencing of 12 animals or were taken from literature. Genotyping was performed with 16 gene-tagged SNPs located between 43.99 and 77.49 Mb, with spacing 2.23 Mb, corresponding to 59.5 and 70.7 cM, with spacing 0.75 cM. In Italian Large White (ILW) pigs average daily gain, backfat thickness, lean cut, ham weight, feed conversion ratio values, pH1, pHu, CEI L*, a*, b* color measures and drip loss were determined. SNPs with MAF >0.05 and with significantly different allele frequencies in animals with the most divergent values for daily gain (50 plus and 50 minus variants) were used for association analyses. Preliminary association analyses in a random sample (n = 97 - 343) showed that UBL5 (68.93 Mb) was associated with ham weight, LDLR (70.18 Mb) with feed conversion rate, pHu, color L* and color b* and CNN1 (70.53 Mb) with feed conversion rate and drip loss. Results indicate that in ILW variation of studied performance traits was affected by genes located between positions 68.93 and 70.53 Mb on physical map of SSC2 based on Sscrofa10 assembly. GA CR (P502/10/1216)

Key Words: pig chromosome 2, fine mapping, carcass and meat quality

P6002 Predicting longevity and body temperature from the genetics of mammalian metabolic networks. M. Bekaert[1] and G. C. Conant*[2], *[1]Stirling University, Stirling, Scotland, UK, [2]University of Missouri, Columbia, MO USA.*

Metabolic networks attempt to describe the complete suite of biochemical reactions available to an organism. One notable feature of these networks in mammals is the large number of distinct proteins that catalyze the same reaction. While the existence of these isoenzymes has long been known, their evolutionary significance is still unclear. Using a phylogenetically-aware comparative genomics approach, we infer metabolic networks for 16 mammals as well as for their common ancestors. We find that the pattern of isoenzymes copy-number alterations (CNAs) in these networks is suggestive of natural selection acting on the retention of certain gene duplications. When further analyzing these data with a machine-learning approach, we found that that the pattern of CNAs is also predictive of several important phenotypic traits, including longevity, body temperature and milk composition. Integrating tools from network analyses, phylogenetics and comparative genomics both allows the prediction of phenotypes from genetic data and represents a means of unifying distinct biological disciplines.

Key Words: metabolic network, comparative genomics, longevity

P6003 Evolution, polymorphism and selection of TLR genes in the family Equidae. P. Frolkova[1], M. Bayerlova[2,3], N. Martinkova[2,3], B. Vokata[1], L. Vychodilova[1], and P. Horin*[1,4], *[1]Institute of Animal Genetics, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [2]Institute of*

Biostatistics and Analysis, Masayrk University, Brno, Czech Republic, [3]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic, [4]CEITEC-VFU, Brno, Czech Republic.

The toll-like receptors (TLRs) represent a multigene family of mammalian molecular pattern recognition receptors essential for recognition and defense against a variety of pathogen-related endogenous and exogenous ligands. In this study, a comparative genomic analysis of nucleotide sequences of genes encoding TLR molecules was performed in the members of the family Equidae. The protein coding sequence of the TLR4 gene showed high sequence similarity of the gene across the entire family. Phylogenetic trees constructed followed the general evolutionary pattern of the family. Similarly to other mammalian groups, striking conservation of the functionally important TIR domain was observed. Within-species polymorphisms were identified in 8 out of 10 taxonomic groups analyzed. Population distribution of selected SNP markers showed differences between various geographical populations of the same species. In contrast to TLR4 genes of other mammals, important effects of positive selection on this gene were not found in Equids. These data along with results of an ongoing analysis of the TLR 1, 6, 7 and 8 genes will be used for studying evolution of this important multigene family in Equids.

Key Words: toll-like receptor, Equidae, evolution and selection

P6004 Analysis of immunoglobulin transcripts in the ostrich *Struthio camelus*, a primitive avian species. Tian Huang*[1], Min Zhang[1], Zhiguo Wei[2], Ping Wang[1], Yi Sun[1], Xiaoxiang Hu[1], Liming Ren[1], Qingyong Meng[1], Ran Zhang[1], Ying Guo[1], Lennart Hammarstrom[3], Ning Li[1], and Yaofeng Zhao[1,4], *[1]China Agricultural University, Beijing, P. R. China, [2]Henan University of Science and Technology, Luoyang, Henan, P. R. China, [3]Karolinska University Hospital Huddinge, Stockholm, Sweden, [4]Qingdao Agricultural University, Qingdao, Shandong, P. R. China.*

Previous studies on the immunoglobulin (Ig) genes in avian species are limited but have revealed several interesting features, including the absence of the IgD and IgÎ° encoding genes, inversion of the IgA encoding gene and the use of gene conversion as the primary mechanism to generate an antibody repertoire. To better understand the Ig genes and their evolutionary development in birds, we analyzed the Ig genes in the ostrich (Struthio camelus)ã€,The ostrich expressed only 3 IgH chain isotypes (IgM, IgA and IgY) and Î» light chains. The IgM and IgY constant domains are similar to their counterparts described in other vertebrates. Although conventional IgM, IgA and IgY cDNAs were identified in the ostrich, we also detected a transcript encoding a short membrane-bound form of IgA (lacking the last 2 CH exons) that was undetectable at the protein level. No IgD or Î° encoding genes were identified. The presence of a single leader peptide in the expressed heavy chain and light chain V regions indicates that gene conversion also plays a major role in the generation of antibody diversity in the ostrich. Because the ostrich is one of the most primitive living aves, this study suggests that the distinct features of the bird Ig genes appeared very early during the divergence of the avian species and are thus shared by most, if not all, avian species.

Key Words: immunoglobulin, avian, evolution

P6005 Pig genome evolution: Chromosomal rearrangements and their impact on gene networks. J. Narayan, International Swine Genome Sequencing Consortium, and D. Larkin,* *Institute of Biological, Environmental and Rural Sciences, Aberystwyth, Ceredigion, UK.*

The genome sequencing and chromosomal assembling of various mammalian genomes opens a unique opportunity to study evolution in mammals and connect chromosomal rearrangements to adaptive changes gained by each species' genome during speciation. In the present study we performed classification of pig–specific evolutionary breakpoint regions (EBRs) using a newly developed algorithm. This led to detection of 192 pig EBRs. We later analyzed human gene content in and around the pig EBRs and found significant enrichment for the gene ontology categories related to sensory perception of taste, and keratinisation (FDR < 0.05; P < 0.05). This makes the pig genome different from the most other mammalian genomes where EBRs are commonly associated with the immune response genes duplicated by segmental duplications associated with genomic rearrangements. We also analyzed transposable elements distribution in the pig genome and found that LTR-ERV1 elements and satellite repeats contributed to formation of pig lineages-specific EBRs, while tRNA Glu-derived SINEs were uniquely involved formation of ancestral artiodactyl rearrangements (shared by pig and cattle). Extended to other genomes our approach will demonstrate how transposon activity associated with chromosomal rearrangements produces variations in gene networks used by the natural selection.

Key Words: pig, comparative genomics, evolutionary breakpoints

P6006 Identification of genome-wide CNV by resequencing Hanwoo and its comparison with Black Angus and Holstein. J. W. Choi[1], K. T. Lee*[2], X. Liao[1], P. Stothard[1], H. S. An[2], S. Ahn[3], S. Lee[1], S. Y. Lee[3], S. Moore[1], and T. H. Kim[2], *[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada, [2]Animal Genomics*

and Bioinformatics Division, National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Korea, [3]Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon 406-840, Korea.

Copy number variation (CNV) has received great attention as another important genetic variation which can be complementary to single nucleotide polymorphism (SNP) to account for economically important traits in mammals. Here we present genome-wide copy number variation regions (CNVRs) comparing the whole genome sequence of Hanwoo (HAN) with Black Angus (BA) and Holstein (HOL) sequence data. As a result, we identified in total 1,173 and 963 putative CNVRs across the whole genome representing ~16.7 and ~7.8 Mb from the comparison of BA versus HAN (BAvsHAN), and HOL versus HAN (HOLvsHAN) respectively. To analyze potential functional roles of those CNVRs, Gene Ontology enrichment analysis was performed and indicated that response to stimulus, immune system process, and cellular component organization were highly enriched among the genic CNVRs overlapped with annotated cattle genes. A total of 8 genic CNVRs were validated using RT-qPCR method in 7 breeds and all CNVRs polymorphic. The results in this study successfully presented genome-wide CNVs using massively parallel sequencing technology, and they can be valuable resources for the further study to correlate CNV and economically important traits in cattle.

Key Words: Hanwoo, resequencing, CNV

P6007 Mapping cattle copy number variation by population-scale genome sequencing. George Liu,* *USDA, ARS, ANRI, Bovine Functional Genomics Laboratory, Beltsville, MD, 20705, USA.*

Copy number variation (CNV) is abundant in livestock, differing from SNPs in extent, origin and functional impact. Despite progress in CNV discovery, the nucleotide resolution architecture of most CNVs remains elusive. As a pilot population study of cattle CNV, we started to sequence 100 representative cattle at 4 to 10-fold coverage. This panel comprises both dairy and beef breeds such as Holstein, Jersey, Angus, Brahman, Limosin, Nelore, Gir and others. We evaluated a dozen of screen methods developed by the 1000 Human Genomes Project. After customized and optimized for cattle, our best-performing pipelines (mrsFAST+WSSD and Genome STRiP) generate thousands of deletions and other CNVs, including insertions and tandem duplications. We further confirmed selected events with complementary CNV discovery approaches (high density CGH and SNP arrays) and experimental validations (PCR and FISH). With the price of next generation sequencing dropping, the genomes of influential livestock around the world will be generated. Our analytical framework and high resolution CNV map will serve as a resource for these sequencing-based comparative genomic studies. Combining with SNPs, CNVs in their haplotype contexts will support genome-assisted animal selection studies.

Key Words: copy number variation, comparative genomics, ruminant genomics

P6008 A genome-wide detection of copy number variations using SNP genotyping arrays in swine. J. Liu,* J. Wang, J. Jiang, L. Jiang, X. Ding, and Q. Zhang, *China Agricultural University, Beiing, China.*

Copy number variations (CNVs) have been shown important in both normal phenotypic variability and disease susceptibility, and are increasingly accepted as an important source of genetic variation complementary to SNP. Comprehensive identification and cataloging of pig CNVs would be of benefit to the functional analyses of genome variation. Herein we performed a genome-wide CNV detection based on the Porcine SNP60 genotyping data of 474 pigs from 3 pure breed populations and an Duroc × Erhualian F2 crossbred population. A total of 382 CNV regions (CNVRs) across genome were identified, which cover 95.76Mb of the pig genome and correspond to 4.23% of the autosomal genome sequence. These CNVRs contains 1468 annotated genes. To confirmation of these findings, 18 CNVRs representing different predicted status and frequencies were chosen for validation via qPCR. Accordingly, 12 of them was successfully confirmed. Our study first provides a comprehensive map of copy number variation in the pig genome, being of help for understanding the pig genome and provide preliminary foundation for investigating the association between various phenotypes and CNVs.

Key Words: copy number variation, SNP array, swine

P6009 Comparison of the skin transcriptome of white, brown and black alpacas by RNA-seq. K. A. Munyard* and R. Cransberg, *School of Biomedical Sciences, Curtin University, Perth, WA, Australia.*

Alpaca fiber is a luxury fiber that is strong, lightweight, has excellent thermal properties and comes in a wide range of natural colors. The value of alpaca fiber is determined by its fineness and by its color. Very little is known about color genetics in alpacas and even Mendelian inheritance patterns are largely unknown. However, alpaca breeders need this information to enable them to effectively and efficiently breed for desired colors. To identify genes that have a role in color determination in alpacas, the skin transcriptome of white, bay and black alpacas was investigated using RNA-seq. Over 5000 alpaca genes were expressed at levels greater than 10 copies in one or more of the 3 colors. Forty one

known color genes were expressed in at least one of the colors, with 25 of these being expressed equally in all colors. Eight genes; Rab38, Slc24a5, Tyrp1, Silv, Matp, Krt4, Oca2, and Tyr, were expressed in a common pattern: highest in black, moderate in bay and lowest in white samples. Mart-1 which controls trafficking of Oa1 and Silv to the melanosomes, was absent in white skin but expressed highly in bay and black skin. The promoter region of Mart-1 in alpacas was sequenced in representative animals of all 3 colors, however no variation was detected.

Key Words: alpaca, colour, RNA-seq

P6010 Identification of NF-κB transcription factor binding sites in promoters of miRNA genes in C2C12 myoblast. Lili Niu,* Yunxia Zhao, Xinyun Li, Jianhua Cao, and Shuhong Zhao, *Huazhong Agriculture University, Wuhan, China.*

Regulation of gene expression by sequence-specific transcription factors is central to developmental programs and depends on the binding of transcription factors with target sites in the genome. Nuclear factor κB (NF-κB) is a transcription factor that regulates various aspects. Recent data from knockout mice support that the classical NF-κB pathway functions as an inhibitor of skeletal muscle cell differentiation and muscle regeneration acting through multiple mechanisms. In this study genome-wide binding site of NF-κB(p65) transcription factor in C2C12 myoblast was analyzed by chromatin immuno-precipitation followed by sequencing. We identified 14,233 p65binding sites. Motif analysis revealed that an overwhelming majority of the identified binding sites contained the previously established consensus binding sequence. A panel of miRNA was inferred to be regulated through promoter binding of the NF-κB p65 subunit. Data analysis is performed using open source R Statistical software (version 2.6.1). The promoter sequence of miRNA gene between human and rodents was compared for the identification of many essential regulatory regions and binding sites for miRNA expression. Some binding sites were verified by EMSA. The results demonstrated a process that may be relevant to the regulation of skeletal muscle development through NF-κB(p65) regulated miRNA.

Key Words: NF-κB, miRNA, myoblast

P6011 Evolution and diversity of MHC class I and class II genes in suids and peccaries. A. Perdomo*[1], N. Mach[2], H-J. Megens[3], M. Moroldo[2], S. Marthey[2], J. Lecardonnel[2], P. Wahlberg[2], J. Estelle[2], M. Groenen[3], C. Rogel-Gaillard[2], and J. Gongora[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW 2006, Australia, [2]INRA, UMR GABI, CRB-GADIE, Domaine de Vilvert, 78350, Jouy-en-Josas, France, [3]Animal Breeding and Genomics Centre, Wageningen University, 6700 AH, Wageningen, The Netherlands.*

The major histocompatibility complex (MHC) is a gene-dense region in vertebrate genomes which plays an important role in the development and regulation of immune response to pathogens. This study aims to improve on our understanding of the diversity and evolution of class I and class II loci in 9 species of Suidae and 2 species of Tayassuidae. Initial MHC DNA data sets were sourced from a sequence capture project which used a 385K solid phase array based on the Sus scrofa MHC to capture this region across species. These data sets were aligned to perform phylogenetic analyses which showed that these loci overall cluster into 2 major clades representing the families Suidae and Tayassuidae. However, some loci (SLA5, SLA6, SLA7, SLA8, and DRB2, previously characterized in S. scrofa) appear to show highly conserved exon sequences across species. To validate these results, deep sequencing of amplicons from those loci for the same specimens is underway. In addition, MHC data sets from whole genome sequencing from 5 species of Suidae will be included. Further analyses will be implemented to assess the type of selection that has shaped the diversity of those loci.

Key Words: MHC, Suidae, Tayassuidae

P6012 Comparative proteomics of milk fat globule membrane proteins from transgenic cloned cattle. S. Sui,* J. Wang, C. Guo, T. Yu, and N. Li, *China Agricultural University, Beijing China.*

Use somatic cell cloning technology, we got 3 kinds of mammary gland bioreactor which specific expressed recombination human α-lactalbumin (LA), lactoferrin (LF) and lysozyme (LZ). Milk fat globule membrane (MFGM) is a bilayer structure which surrounding lipid in milk. It is said that the MFGM come from the apical plasma membrane of mammary epithelial cell. We use proteomics methods to investigate the protein expression of MFGM in transgenic cloned bovine milk include Two-dimensional gel electrophoresis (2-DE) Analysis and LC-MS/MS. In five groups (TC-LA, TC-LF, TC-LZ, Cloned and normal) of MFGM protein (include colostrum and ordinary milk), we have identified 1225 proteins, 939 proteins in colostrum and 910 proteins in ordinary milk respectively. Use Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) methods, we got the proteins relative quantitative information from five groups MFGM use iTRAQ method. There are 459 proteins which have relative quantitative in colostrum and 426 proteins in ordinary MFGM. We compare the proteins expression and relative quantitative of transgenic cloned bovine MFGM with cloned and normal MFGM. All data indicated that the expression of exogenous protein did not change the original

composition of MFGM and the status of mammary epithelial cell from transgenic cloned bovine is healthy.

Key Words: transgenic, MFGP, proteomics

P6013 Duplicated rainbow trout genes ISCU, NELL2, and MARCH5 show gene variant specific expression pattern. M. Verleih*[1], A. Rebl[1], B. Köllner[2], T. Korytár[2], C. Kühn[3], K. Wimmers[1], and T. Goldammer[1], *[1]Leibniz-Institut für Nutztierbiologie (FBN), Fachbereich Molekularbiologie, 18196 Dummerstorf, Germany, [2]Friedrich-Loeffler-Institut (FLI), Institut für Infektionsmedizin,, 17493 Greifswald, Insel Riems, Germany, 33 Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern (LFA-MV), Institut für Fischerei, Born, Germany.*

The holistic transcriptome comparison between a local rainbow trout selection strain, showing higher robustness to biotic and abiotic stressors and a commercial import strain, revealed remarkable differences in the expression profiles of several genes among them ISCU, NELL2 and MARCH5. We isolated and characterized the duplicated gene sequences and investigated their tissue specific gene expression. The encoded proteins play critical roles in various cell functions: ISCU is tasked with the building and mediation of iron-sulfur [Fe-S]-clusters for [Fe-S]-proteins; NELL2 encodes a glycoprotein, concerned in cell growth and differentiation; MARCH5 is a regulator of Toll-like receptor 7-mediated NFKB activation in mammals through protein ubiquitination. The coding sequences of ISCU, NELL2 and MARCH5 gene variants A and B are 91%, 83% and 65% identical, respectively. ISCUA and ISCUB showed remarkable differences in their transcript level with particular high cerebral mRNA-level of ISCUB. NELL2A gene is ubiquitous expressed, whilst NELL2B is abundantly expressed only in brain. Interestingly, in contrast to MARCH5B, MARCH5A gene is highly expressed in immunorelevant tissues and upregulated after viral infection. In addition, the investigated genes show tissue and strain-specific expression profiles indicating different functional roles of distinct rainbow trout gene variants.

P6014 Identification of novel transcripts and non-coding RNAs in bovine skin. R. Weikard,* F. Hadlich, R. Brunner, and R. Weikard, *Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

By generating a comprehensive transcriptomic landscape of cells and tissues, deep RNA sequencing has opened a new horizon for understanding global gene expression. Using this approach, we performed a whole transcriptome analysis on bovine skin to describe the complex transcript catalog of this tissue. Therefore, total RNA isolated from pigmented and non-pigmented skin areas from the same animals was subjected to deep paired-end mRNA sequencing analysis. Sequencing reads were mapped to the bovine reference genome assembly (UMD3.1, Ensembl release 64) (see poster Kühn et al.). A total of 49,524 different transcripts were assigned to the bovine reference genome. The majority of them could be mapped to reference transcripts (63%) with a high proportion displaying novel splice junctions (34%). In addition, a high number of novel transcripts (35%) were discovered that had not been annotated before. These novel transcripts (size range from 61 to 29,073 bp) were mapped predominantly in intergenic chromosome regions. The majority of them could be assigned to non-coding RNA types. Small RNAs were the most highly expressed transcripts in pigmented and non-pigmented skin: The results of our study demonstrated complex transcript patterns for bovine skin and indicated a functional relevance of novel transcripts in the modulation of pigmentation processes.

Key Words: RNAseq, annotation, cattle

P6015 Identification and characterization of copy number variations in a chicken F2-design resource population via SNP BeadChip. Rong Zhang, Liang Xie, Dexiang Zhang, Qinghua Nie, and Xiquan Zhang*, *South China Agricultural University, Guangzhou, Guangdong Province, China.*

A chicken F2-design resource population was used for SNP genotyping with a beadchip to investigate the distribution of copy number variations (CNV) in chicken genome with appropriate algorithms in this study. It will help to understand molecular mechanism of phenotypic differences. The F2-design resource population was made up of reciprocal cross between White Recessive Rock Broilers and Xinghua Chickens, and 554 birds totally, were genotyped by 60K Infinium II chicken SNP Beadchip of Illumina Inc. A total of 1875 CNVs were identified through PennCNV algorithm, and the average number of CNVs was 3.42. These CNVs encompass 16 megabases (1.3%) of the chicken genome, among which CNV regions overlap with function genes. Twenty-six CNVRs were overlapped with the CNVRs detected by chicken CGH study. After correction, 77 detected copy number duplications were found to be correlated with feather growing rates. Among these 77 detected copy number duplications, 42 are associated with early feathering, 16 are associated with equal feathering, and 3 are correlated with late feathering. An initial map of CNVs for the chicken has been first described via SNP chip. The mapping of CNVs in chicken could provide new opportunity for understanding genomic variation and related phenotypic differences.

Key Words: chicken genome, copy number variation, SNP BeadChip

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July 15–20, 2012, Cairns, Australia

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