

## Poster 4001

### **Title: Search for the imprinting mark required for parent-of-origin dependent *H19*ICR methylation using two different mouse models**

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#### **Abstract:**

A 2.4 kb DNA region, the *H19* imprinting control region (*H19*ICR), lies between the *Insulin-like growth factor 2 (Igf2)* and *H19* genes on mouse chromosome 7, and is important for normal imprinting of the two genes.

Interestingly, the *H19*ICR becomes only methylated in sperm. However, the mark required for paternal specific methylation is still not known. To learn more about the nature of this imprinting mark, we first wanted to know whether it is linked to the *H19*ICR, and whether this 2.4 kb region alone is sufficient for imprinting. We addressed these questions by generating two mouse models that carry the *H19*ICR upstream of the *Alpha fetoprotein (Afp)* on chromosome 5 and between the *CD3 gamma* and *delta* genes on chromosome 9, respectively. This approach allows us to study the unique function of the *H19*ICR outside an imprinting environment. Methylation analyses revealed that while the *H19*ICR becomes normally methylated on only the paternal allele at both exogenous loci in somatic tissues, it stays unmethylated in sperm. Thus, the imprinting mark is linked to the *H19*ICR, and the 2.4 kb region is sufficient to imprint somatic tissues. However, DNA methylation is not the imprinting mark that distinguishes the two parental alleles.

## Poster 4003

### **Title: Establishment of an in vivo model for canine prostate cancer and development of an AAV mediated gene therapeutic approach**

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#### **Abstract:**

The dog (*Canis lupus familiaris*) is the only mammalian species besides the man that spontaneously develops cancer of the prostate and thus is estimated as a unique model for the human neoplasia. The aim of this study was to develop an in vivo animal model that mimics the behaviour of an original tumour as basis for the development of therapeutic approaches. We used HMGA2 gene expression as marker due to the fact several human and canine tumours -including canine prostate carcinomas- were reported to show a correlation between their dignity and the expression of HMGA2 gene. We derived the cell line CT1258 from a highly malignant canine adenocarcinoma of the prostate showing high expression of HMGA2 and inoculated 1x10<sup>6</sup> cells in 9 NOD-SCID mice s.c. as well as 1x10<sup>5</sup> and 5x10<sup>5</sup> cells respectively i.p.. The induced tumours emulated the original tumour regarding biological behaviour, HMGA2 expression, and the presence of cytogenetic markers. Finally, we developed a gene therapeutic approach using recombinant HMGA antisense adeno-associated viruses (rAAV) resulting in significant inhibition of cell proliferation and apoptosis of CT1258 cell in vitro.

## Poster 4004

### **Title: Bovine *PRNP* polymorphisms in Asian native populations and Japanese breeds**

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We genotyped six polymorphic sites of a bovine prion gene (*PRNP*) and estimated haplotypes in Asian native populations and Japanese breeds. Six sites were 23-bp indel within promoter, 12-bp indel within intron1, nonsynonymous SNPs (K3T and S154N), octapeptide repeats within ORF and 14-bp indel within 3'UTR. At the 23-bp indel, the frequency of - (deletion) allele (0.55-1.00) was higher than that of + (insertion) allele in all groups except *Bos frontalis*. 12-bp indel frequency ranged from 0.00 to 0.93 for + allele and from 0.07 to 1.00 for - allele. + allele frequency was higher than - allele in all Asian populations except the Mongolian. In most Japanese breeds, - allele frequency was higher than + allele. For the 14-bp indel, + allele frequency (0.58-1.00) was higher than - allele. 6-repeats allele was common allele at the octapeptide repeats site. 5/5 genotype was detected in Japanese black and the Mongolian population. 4-repeats allele was detected only in *Bos frontalis*. K3T site was polymorphic in all Asian populations except the Mongolian. S154N was polymorphic in all Asian populations. We estimated 30 different haplotypes from these genotypic data. A '23-12-K6S14+' haplotype was found in all groups with frequencies from 0.05 to 1.00.

#### Poster 4005

##### **Title: *Bos indicus* mitochondrial DNA variation in East Asia.**

Presenting Author: Hideyuki MANNEN, Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Japan 657-8501.

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##### **Abstract:**

In order to elucidate the origin and genetic diversity of *Bos indicus* cattle, this study analyzed a large data set comprising mtDNA D-loop sequences of Asian *Bos indicus* derived from 10 countries. Phylogenetic analyses indicate the existence of two major haplogroups (I1 and I2) in *Bos indicus* mtDNA. The topology of I1 haplogroup illustrated a clear starburst phylogenetic pattern including one central haplotype

with numerical and topological predominance, while starburst pattern of I2 haplogroup was seemingly ambiguous and did not show numerous sequences at the center of this radiation. The plural mitochondrial haplogroups of *Bos indicus* and their topologies suggested that these are posited as the products of the domestication of two alternate types of *Bos indicus* mtDNA. The ancestry with I1 haplogroup would be a most contributed strain of cattle for the domestication of *Bos indicus*. The sequence diversity and the plural mitochondrial haplogroups suggested that major domestication center of *Bos indicus* is in Indian subcontinent and minor domestication or adoption from different strain of wild stock in Indian subcontinent.

#### Poster 4006

##### **Title: Development of DNA markers for discrimination between Japanese and Australian beef cattle**

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##### **Abstract:**

In the meat industry, correct breed information in food labelling is required to assure meat quality. As an element of traceability system, genetic markers are useful to identify breeds. The aim of this study was to develop the DNA markers discriminating between Japanese and Australian beef. SRY and ND5 were used as gene markers specific to *Bos indicus* and MC1R gene as to red coat color. In addition, amplified fragment length polymorphism (AFLP) method was employed to develop additional markers. A total of 1696 AFLP primer combinations provided four candidate polymorphisms that were converted into single nucleotide polymorphisms (SNPs) markers for high-throughput genotyping. The allele frequencies in cattle from both countries were investigated for estimating the discrimination ability. The allele frequencies in Australian beef were 0.194 (SRY), 0.471 (ND5) and 0.410 (MC1R). The allele frequencies of AFLP-SNPs ranged from 0.000 to 0.001 in Japanese cattle and 0.032 to 0.094 in Australian beef. Combining these seven markers, the probability of identifying Australian beef was 0.909 and probability of misjudgement was 0.008. The genetic system using these DNA markers could be a

powerful tool for breed identification and would contribute to prevent falsified display beef in Japan.

#### Poster 4007

##### **Title: Discovery of porcine microRNAs through comparative genomics approach and profiling from skeletal muscle tissues during development**

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##### **Abstract:**

MiRNA (microRNA) plays critical roles in many important biological processes such as growth & development in mammals. In this study, we used *in silico* prediction followed by microarray hybridization to identify porcine miRNAs. Alignment of porcine genomic sequence against human, mouse and rat miRNA stem-loop sequences identified 775 predicted porcine miRNAs. An initial screen by microarray hybridization with RNA samples prepared from the 33 days whole embryo and extra embryo membrane validated the expression of 296 of these candidates. To investigate the decisive miRNAs involved in muscle growth & development processes, we subsequently examined the miRNA expression profiles in longissimus muscle tissues collected from 33 days, 60 days of gestation fetuses and adult pigs. A total of 140 miRNAs were differentially expressed among the ages investigated. The differentially expressed miRNAs were classified into four categories of distinctive expression patterns, suggesting possible involvement of special biological processes respectively. Five of the differentially expressed miRNAs detected by the arrays were validated by real-time PCR method. Further bioinformatics analysis of the miRNA-mRNA interaction sites suggested that the potential mRNA targets of the differentially expressed miRNAs may play important roles in muscle growth & development. Our study have expanded the miRNA catalog of the pig, and for the first time, provided miRNA transcriptome landscape of the prenatal and adult porcine skeletal muscle. The novel data and the bioinformatics analysis presented in this study may facilitate the hypothesis-based functional studies of miRNAs in muscle.

#### Poster 4008

##### **Title: Muscle Fiber Typing of the Blind Subterranean Mole Rat Reveals Adaptive Features to Sustained Activity Under Hypoxia**

Presenting Author: Mark Band, University of Illinois, 1201 W. Gregory Dr., Urbana, IL, 61801

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##### **Abstract:**

Mammalian muscles are composed of three primary types of fibers, type I oxidative, type IIa oxidative and type IIb glycolytic. Most mammalian species present a distribution of the various fiber types allowing plasticity for adaptation to changing physiological conditions. Mole rats of the *Spalax ehrenbergi* superspecies are blind subterranean rodents that require intense activity under hypoxic conditions for survival. Fiber typing of neck (trapezius) and leg (gastrocnemius, quadriceps) muscles using standard histochemical techniques (succinic dehydrogenase, myosin ATPase) showed that the muscle fibers of mole rats in natural settings, as well as after extended captivity, were predominantly type IIa. The same muscles in laboratory rats showed the full range of fiber types. In contrast, the hearts of the mole rats and the laboratory rats were very similar. Previous investigations have shown a correlation of *VEGF* levels to a shift from type IIb to type IIa in mice under intense endurance activity. *VEGF* levels in mole rat skeletal muscle are naturally elevated as compared to other terrestrial mammals. Our results indicate that skeletal muscle in the mole rat appears to have evolved in response to specific environmental demands to permit intensive endurance burrowing activities under conditions of severe or chronic hypoxia.

#### Poster 4009

##### **Title: The association between Split Upper Eyelid and multiple horns observed in sheep is maintained in multihorned goats.**

Presenting Author: M.Teresa Tejedor Hernández, Department of Anatomy, Embriology and Genetics, Faculty of Veterinary Sciences of Zaragoza (Spain), C/Miguel Servet 177. 50013-ZARAGOZA (Spain)

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##### **Abstract:**

We have recently detected the existence of autochthonous populations of multihorned goats in the

region of Extremadura (Spain). The flocks contain around 7% multihorned females, since multihorned males are not selected for reproduction. Our first observations clearly indicate that this character is associated to Split Upper Eyelid (SUED). This was a well known association in several multihorned sheep breeds, such as Hebridean, Churro Navajo and Jacobs, but scientific reports on caprine populations carrying it were not published. As in sheep, caprine SUED shows a wide range of morphological alterations: Slight changes in the position of the eyelashes, small notches and finally, in a small proportion of individuals, completely split eyelids, exposing most of the eye. Multiple horns and SUED are completely associated, as the result of the observation of the modified eyelids at birth is completely concordant with the future eruption of multiple horns. At present, even if the exact inheritance model for this character remains undisclosed in both sheep and goat, its complete conservation in both species seems evident, suggesting that it appeared prior to their evolutionary segregation.

#### Poster 4010

##### **Title: Phylogeny relationship of GDF5 sequence between a number of mammalian species.**

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##### **Abstract:**

Growth differentiation factor 5 (GDF5) is a member of the bone morphogenetic protein (BMP) family and the TGF-beta super family. The members of this family are regulators of cell growth and differentiation in both embryonic and adult tissues. 441 base pairs exonic sequence of GDF5 gene, including GCA repeats was compared between 13 species belonged to Bovidae, Cervidae, Canidae, Suidae, and Equidae family and human as reference for primer design. Sixty nine single nucleotide differences were found in GDF5 sequence when compared between all species. Except to the region spanning tandem repeat of dog, (GCA)<sub>n</sub>GTA(GCA)<sub>n</sub> structure were common for GDF5 repeat region of all species, with the largest (GCA)<sub>7</sub> motive in domestic horse, Przewalski horse, and onager. Based on phylogeny distance data GDF5 sequence is the most variable inside Bovidae family in relation to other investigated taxa.

#### Poster 4011

##### **Title: Genomic organisation and mapping of the canine *High Mobilty Group A1 (HMGA1)* gene**

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##### **Abstract:**

The high mobility group A1 proteins (HMGA1a/HMGA1b) are highly conserved between mammalian species and widely described to participate in various cellular processes. In humans, chromosomal aberrations affecting the *HMGA1* gene locus on HSA 6p21 were described for various benign mesenchymal tumours, while high titres of HMGA1 proteins were shown to be associated with the neoplastic potential of various types of cancer. Due to the various similarities in biology and presentation of human and canine cancer, knowledge about the structure of cancer related genes and proteins is of significant value. Herein we report the characterisation of the genomic structure of the canine *HMGA1* gene consisting of 7 exons and 6 introns spanning in total 9524bp, the *in vivo* localisation of the HMGA1 protein to the nucleus, and chromosomal assignment of the gene by FISH to CFA12q11. Additionally, we evaluated a described canine *HMGA1* exon 6 SNP in 55 Dachshunds.

The performed characterisations will allow doing comparative analyses of aberrations affecting the human and canine gene and protein as basis for revealing mechanisms involved in HMGA1 related pathogenesis in both species.

#### Poster 4012

##### **Title: New loci and allele characterization of pig MHC (SLA) classical class I gene**

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**Abstract:**

Swine MHC (SLA) class I genes are classified into two groups, classical and non-classical ones. In pigs, the role for presentation of intracellular peptidic antigens to T cell receptors is mainly played by three types of class I antigens, SLA-1, SLA-2 and SLA-3. To date, 44 alleles have been reported for *SLA-1* locus, and the number of *SLA-1* alleles is considered to increase by further analysis. On the other hand, there are several observations for duplication of *SLA-1* locus in single haplotypes, and some haplotypes seem to lack expressed *SLA-1*. We classified the *SLA-1* alleles detected in newly sequenced SLA haplotypes in addition to the alleles known to date. One of the haplotypes we newly sequenced had two loci sharing characteristics with the known *SLA-1* alleles. Interestingly, we found loci that possessed some features distinguished from *SLA-1*, although they shared several characteristics with *SLA-1*, which have not been observed in *SLA-3* or other SLA loci. Sequences belonging to this type were also observed in dbEST for pigs. We have further to investigate locus organization of SLA classical class I genes on the genomic sequence in various haplotypes to build the appropriate classification of SLA loci and alleles.

**Poster 4013****Title: There are three TRBD-TRBJ-TRBC clusters in porcine TRB gene.**

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**Abstract:**

In the vertebrate immune system, T cells functions as a major factor for host defense against microbial or viral infection. Previous studies suggested that, at least, two sets of TRBD-TRBJ-TRBC clusters are harbored in the porcine genome. In this study, we isolated two BAC clones that were considered to cover the entire TRBC regions of a single chromosome, and we determined 212,193 bp of a continuous porcine genomic sequence. Nine TRBV, three TRBD and 21 TRBJ segments as well as three TRBC regions were identified in this sequence. EPHB6 and TRPV6 genes were also conserved in the vicinity of TRBD-TRBJ-TRBC clusters. However, we could find only one TRY gene between TRBV29-1 and TRBD segment, although five TRY genes had been identified in the corresponding region in human. Interestingly, three TRBD-TRBJ-TRBC clusters were observed in the single chromosome. Each

TRBD-TRBJ-TRBC cluster consisted of one TRBD and seven TRBJ segments, with one TRBC region composed of four exons. BLAST search with porcine TRB sequences available in the public databases showed that almost all of the TRBJ segments identified here had corresponding sequences in the databases. This suggests that all of the three TRBD-TRBJ-TRBC clusters are used in pigs.

**Poster 4014****Title: Evidence for Adaptive and Negatively Selected Chromosome Rearrangements in Amniote Evolution**

Presenting Author: Denis M. Larkin, Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

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**Abstract:**

The genome assemblies of 10 amniotes were compared at the level of chromosome organization; human, chimpanzee, macaque, cattle, pig, dog, rat, mouse, opossum, and chicken. Blocks of homologous synteny (HSBs) and evolutionary breakpoint regions (BR) were identified from pairwise comparisons of all genomes. The rates of rearrangements were compared. After the divergence from marsupials, an elevated rate of chromosomal rearrangements was observed in placental mammals (~1.3 breakpoint per My), followed by a decrease (~0.8 breakpoint per My) until the Cretaceous-Tertiary (K-T) boundary. After the K-T boundary the rate of chromosomal rearrangements in placental mammals increased to ~1.4 breakpoint per My. The rearrangement rate in marsupials is ~0.8 breakpoints per My, in birds it is ~0.7 breakpoints per My. We found that large HSBs conserved in all species contain significantly more genes controlling essential processes of organismal development than the genome average. One of these regions, a 23.6 Mbp HSB on HSA2, contains the *HOXD* cluster. In contrast, BRs contain a high frequency of genes associated with adaptive responses to the environment and sequence features that are characteristic of evolutionary hotspots. We propose that natural selection plays an important role in determining structural and functional features of amniote chromosomes.

## Poster 4015

### **Title: Three groups of Endogenous Retroviruses found in Crocodylians**

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#### **Abstract**

Two families, Crocodylidae, and Alligatoridae are unambiguously recognised, with gharials either lumped with Crocodylidae or assigned to a separate family Gavialidae. Endogenous retroviruses (ERVs) are copies or remnants of exogenous retroviruses that were integrated into the host genome at some stage in the past. ERVs are vertically transmitted from the host to its progeny. Although most ERVs are defective due to inactivating mutations, functional ERVs are potential agents of disease. Previous studies have identified the presence of a distinct clade of ERV from six species of crocodylians. Here we analyse the functionality, distribution and phylogenetic relationships of ERVs in thirteen species of Crocodylidae and seven species of Alligatoridae from across the world. The ERV reverse transcriptase (*pol*) gene fragment (0.8-1 kb) was amplified, cloned and sequenced. Preliminary analyses of sixty-six crocodylian ERV *pol* DNA sequences show that these retroelements possess stop codons and deleterious mutations. Thus, crocodylian ERVs are generally, if not universally, defective as has been observed in many other hosts. Phylogenetic analyses show that crocodylian ERVs cluster in three major clades, one Crocodylidae-specific, one Alligatoridae-specific and one found in all Crocodylians including gharials. Further analyses of the evolutionary relationships and evidence for ERV functionality within crocodylians are underway.

## Poster 4016

### **Title: A yeast-based assay to detect TP53 functional mutations: study on spontaneous feline tumors.**

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#### **Abstract:**

In feline neoplasms little is known about occurrence and nature of p53 mutations, although the few available data suggest a lower frequency compared to humans.

In order to understand the involvement of p53 mutations in feline carcinogenesis, we previously developed a yeast-based approach revealing that feline p53 (*fe\_p53*) can be studied in this model system and is equally susceptible to inactivating mutations as human p53.

In this report we examined 30 bioptic samples from spontaneous feline tumors using the yeast-based assay to score p53 mutations based on the frequency of phenotypically mutant *fe\_p53* cDNA alleles individually examined upon in-vivo cloning. In this assay non-transactivating p53 mutations result in the appearance of red colonies while white colonies are instead indicative of active p53.

Four samples resulted in a frequency of red colonies higher than 30% indicative of the presence of clonal p53 mutations in the biopsy. Sequencing of these cDNAs identified a splice donor site mutation (G>A) resulting in an 11 amino-acid insertion (exon 6) in an hepatocarcinoma and an in-frame deletion of 12 nucleotides in a schwannoma (exon 5 starting at codon 167). Direct DNA sequencing of genomic DNA is in progress and definitive results will be presented in poster.

## Poster 4017

### **Title: Comparative genomics of Toll-like receptor signalling reveals candidate genes for disease resistance traits**

Presenting Author: Oliver Jann, The Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Midlothian, EH25 9PS, Scotland

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**Abstract:**

Studies in human and mouse indicate that polymorphism in genes involved in Toll-like receptor (TLR) signalling can affect immune related traits and thus might explain part of the observed variation in disease resistance of livestock. Here we use an inter-species comparative approach to investigate the importance of 20 TLR related genes for resistance traits by associating their genomic localisation with previously published immune-related QTL regions and by analysing their transcript levels in breeds of divergent immune-related phenotypes. We report the localisation of these genes in cattle, sheep and pig and compare their positions to the corresponding genes in human and mouse. Several of the loci are located within regions affecting variation of related health traits in different species. Polymorphism in some of the genes have been directly associated with effects on relevant traits in humans and mice. Many of the genes display divergent transcript levels in animal breeds of different resistance status. Thus, this intergenomic approach has identified several strong candidate genes for a number of important immune traits like clinical mastitis, tolerance to *Trypanosoma* infection and general disease resistance in pig, sheep and cattle. Further studies are required to investigate the potential role of polymorphism within these genes and their direct effects on resistance traits.

**Poster 4018****Title: SPECIES IDENTIFICATION OF MEAT AND ESTIMATING THE PREVALENCE OF BUSH MEAT UTILIZATION IN RAW MEAT OUTLETS USING CYTOCHROME B SEQUENCES.**

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**Abstract:**

In Africa, identification of poached animal species relies greatly on morphological features, (Malisa *et al* 2005). In cases when the morphologically variable features are absent in confiscated carcasses, the accuracy in the identification reduces. DNA and protein based methods have been used in the identification of species with varying success rates. DNA techniques using either nuclear or mitochondrial DNA markers show greater potential for use in

conservation and wildlife management (Cronin *et al.*, 1991). Of the two, mitochondrial DNA analysis usually serves as a more powerful tool for detecting population genetic structure due primarily to its maternal mode of inheritance, lack of recombination, high mutation rate and the process of lineage extinction within populations (Brown *et al.*, 1982). In this study, the species of 565 randomly sourced samples of raw meat were determined by PCR amplification of genomic DNA using universal Cytochrome b primers, (Verma and Singh, 2003). The PCR products were sequenced and the nucleotide sequences were run on a BLAST search of sequence database in NCBI, (www.ncbi.nlm.nih.gov). The 550 samples analysed in this study were all identified as domestic animal species. Significant substitution of animal species was observed especially in the substitution of goat meat with sheep meat. The substitution between species is a clear indication that there is fraud with possible economic and health implications.

**Poster 4019****Title: Identification and characterization of a perfect mono-nucleotide microsatellite within 16S rRNA gene in chicken mtDNA sequence**

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**Abstract:** An alignment of 13 chicken and red junglefowl complete mtDNA sequences reveals a perfect mono-nucleotide microsatellite repeat unit consisted of between 8 and 13 Cs within the 16S rRNA gene in seven samples, however, the remaining six sequences have an interrupted non-polymorphic fragment ((C)<sub>4</sub>T(C)<sub>5</sub>). To further characterize this microsatellite, 350 samples of indigenous chickens, commercial breeds and wild red junglefowls were

genotyped. Thirty-five samples were also sequenced for the same region. Both genotyping and sequencing results confirmed the presence of these two types of structures in chicken mtDNA genome, with the wild red junglefowl samples showing the interrupted fragment. Among the domestic chickens analysed, the two structures show a clear geographic distribution pattern. The perfect repeats are detected in indigenous chickens from West Asia including Bangladesh, Myanmar and Pakistan and in the majority of commercial fowls. The interrupted fragment occurs among indigenous chickens from South and South-East Asia including China, Korea and Papua New Guinea. This perfect mono-nucleotide microsatellite, the first of its kind being validated in the avian mitochondrial genome, could be useful in providing insights into the evolutionary mechanisms of polymorphism and/or instability of mtDNA microsatellites and a better estimation of mutation rate of such microsatellites in birds.

#### Poster 4020

##### **Title: Sequences of MCW0371 reveal complex structures having alleles of either perfect mono-nucleotide repeats or compound microsatellite repeats**

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**Abstract:** A total of 378 samples from nine indigenous chicken populations and three commercial

breeds were genotyped using MCW0371 locus, a microsatellite mapped within MHC-B region on GGA16. Twelve alleles between 199 and 210 bp were detected. Some genotypes exhibited peaks that had a pattern similar to those of perfect mono-nucleotide repeats; however, irregular patterns predominated in the samples and it was impossible to determine their allele sizes accurately. To verify these results, PCR products from 24 samples with likely homozygous genotypes were selected for direct sequencing. Most of them turned out to be heterozygous. To further validate the sequence structure at this locus, 45 samples were chosen for sequencing after cloning. A total of 49 haplotypes were identified with two structures in their repeat units: the first being perfect mono-nucleotide repeats having 14 to 20 'As'; and the second, a compound microsatellite having 1 to 6 'AAC' repeats followed by 9 to 17 'As'. In addition, some SNPs and in/dels were present in flanking and repeat regions. These structures tend to complicate the accurate determination of allele sizes at this locus when using conventional capillary electrophoresis. This calls for thorough validation of such markers before wide application in genetic studies.

#### Poster 4021

##### **Title: Sequencing of full-length 5' end of canine *Ataxia Telangiectasia Mutated* cDNA and characterization of the promoter region**

Presenting Author: Maria Elena Turba; Genefast srl, Via della Pace 33/a 41050 Castelnuovo Rangone Modena, Italy

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##### **Abstract:**

The predicted canine *ATM* locus is annotated in a canine chromosome 5 region (Dog Genome assembly 2.0, Ensembl release 49) syntenic with human chromosome 11.

Using 5' RACE PCR, cloning and sequencing of mRNA purified from canine blood, the transcription start site of canine *ATM* was found on the reverse strand at CFA5: 27307661. To predict the putative promoter region, 700 bp genomic sequences upstream of the 5' cap site were analyzed using Proscan software v1.7. A putative TATA-less bi-directional promoter region was found in the 371bp region upstream of the cap site. The core promoter harbours different conserved regulatory motifs: CREB, CCAAT boxes, SP1, GCF, XRE, ETS, Cre and c-Myb. When compared to the human and pig *ATM/NPAT* promoter, the canine *cis*-regulatory elements are highly conserved.



Two exons, not annotated in the public database, were found in the 5'UTR. The cloned cDNA fragment extended from the transcription start site to exon 6. The putative translation start codon (XM\_845871.1) corresponding to the N-terminus of human isoform 1, was found in exon 3. Two additional in-frame Met codons were located downstream in exons 5 and 6. The cloning of 5' UTR could allow the investigation of the alternative splicing of canine *ATM* N-terminus.

#### Poster 4022

**Title:** Analysis of non-orthologous genes between cow and the genomes of other species.

Presenting Author: John L. Williams, IDRA, Parco Tecnologico Padano, Via Einstein, Lodi 26900, ITALY

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#### Abstract:

Automated annotation of the bovine genome sequence identified more than 25K genes. In the present study the Ensembl Biomart tool was used to identify genes that are orthologous between cattle and the human, mouse and dog genomes. Comparing the genes annotated in cattle with other species 865 were absent, whereas among the genes annotated in cattle 6,247 had no orthologs in the human, mouse and dog genome.

There are several reasons why genes annotated in cattle are missing in the other genomes; these include missing or incorrectly assembled bovine sequence and structural variations in the genes between species. However, there large number of genes in cattle for which orthologs are missing in the other species was not expected. An in silico pipeline was developed to make pair-wise alignments of the "missing" genes between genomes and the evidence from sequence alignments was used to cluster the genes into different classes. The possible systematic failure of the automated protocols to annotate particular classes of genes was investigated using annotation information available in Ensembl, ontology descriptions and manual curation for each subset of genes.

This work will help the annotation of the bovine genome and will assist in the development of

improved gene models for automated annotation protocols.

#### Poster 4023

**Title: High-resolution comparative mapping between X chromosomes in sheep, cattle, and human**

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#### Abstract:

X chromosome morphology and banding structure differ very strong between sheep and cattle, contrary to most other chromosomes in both species. We focused on the extensive analysis of the X chromosome in sheep (*Ovis aries*, OARX) to study the differences of chromosome organization in sheep and cattle and constructed a high-resolution physical map including 169 loci based on a comparative mapping approach. DNA sequences for mapping were selected by using the Virtual Sheep Genome Browser v1.1. Performed genotyping of DNA sequences in the USUoRH5000 ovine whole-genome radiation hybrid panel followed by RH map construction using the Carthagene1.1R software package revealed RH loci order on OARX. The RH map consists of four linkage groups with an average loci density of 1.3 megabases. Assignment of numerous microsatellite markers to the RH map of OARX anchors available linkage maps physically to the chromosome. The cytogenetic map based on FISH of BAC clones of the CHORI-243 library. FISH loci represent cytogenetic anchors and support loci order within the constructed RH map. Except for little rearrangements, the map is concordant with the virtual OARX map. A developed comparative map for sheep, cattle, and human X chromosomes identifies several new evolutionary break points and chromosome rearrangements.

#### Poster 4024

**Title: Mapping and sequencing of major histocompatibility complex class I loci in rabbit**

Order of the authors: Hassina OULD-HAMOUDA, Angélique TEILLAUD, Mark STAM, Claire ROGEL-GAILLARD

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#### **Abstract:**

The Major Histocompatibility Complex (MHC) is a conserved locus in jawed vertebrates and thus constitutes an interesting region for phylogenomic analyses and, more generally, for addressing the question of vertebrate evolution. Rabbit MHC referred to as RLA (Rabbit Leukocyte Antigen) complex, localized on OCU12q11 is still poorly characterized. Our aim was to produce data on the RLA complex in order to include rabbit in MHC comparative analyses among Mammals. We have built a physical map of the RLA complex and produced two contigs for class I and III adjacent regions. Contig1 spans 700 kilobases from MOG to VARSL and contig2 spans 1500 kilobases from CDSN to NOTCH4. We have identified two clusters of class I genes. A first cluster is intermingled between TRIM26 and TRIM39 in contig1 and a second cluster is situated between POU5F1 and BAT1 anchoring genes in contig2. Unlike the human MHC, no third class I gene cluster was detected close to ZNRD1. Six BACs covering the class I gene clusters were sequenced. Our results demonstrate the presence of six class I loci in each cluster and identification of putative functional loci is in progress. Phylogenetic studies of class I loci will be discussed.

#### **Poster 4025**

**Title: Goat mammary gland expressed genes representative of immunity function during pregnancy before the acquisition of its secretory phenotype**

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#### **Abstract:**

Mammary tissue differentiation into a secretory epithelium that synthesizes milk occurs during pregnancy. Impaired differentiation during pregnancy may provoke lactating default. Molecular mechanisms associated to mammary tissue terminal differentiation remained incompletely understood especially in ruminants. Morphological differences between mice and ruminant mammary tissues before pregnancy let's think that there are differences between these two mammals in the differentiation mechanisms. A transcriptional analysis on 5 physiological stages (4 during pregnancy and 1 during lactation) appeared as the best approach to get overview of the differentiation process. An appropriate experimental design was drawn to follow gene expression profiles during differentiation of mammary tissue. Using 3 nulliparous or primiparous goats per stage (a small ruminant model economically acceptable), the comparison was done on 9 dye-swaps, using 22k bovine oligoarrays. Statistical analysis revealed that 2 330 genes varied significantly at least once. These genes were divided into 19 clusters. Identification of biological functions revealed that mechanisms described for mice can be applied to goats. As for example the fall down in lactation of genes implicated in cell growth and proliferation and the increase in lactation of expression of genes implicated in lipid metabolism. A special observation, not described for mice, was the expression of immunity genes at mid-pregnancy.

#### **Poster 4026**

**Title :** In silico characterization of the genome of bovine endogenous retrovirus  $\gamma 4$  based on the bovine genome sequencing draft.

#### **Authors :**

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## Abstract

The genome of the replication-competent bovine endogenous retrovirus (BERV)  $\gamma 4$  provirus, which is the most abundant ERV family in the bovine genome, was characterized in detail. The BERV  $\gamma 4$  genome revealed that BERV  $\gamma 4$  harbors 8576 nucleotides and has a typical 5' long terminal repeat (LTR) gag pro pol env LTR-3' retroviral organization with a long leader region positioned before the gag open reading frame. Multiple sequence analysis showed that the nucleotide difference between 5' and 3' LTRs was 4.2% (mean value 0.042) on average, suggesting that the provirus formed at most 13.3 million years ago. Gag was separated by a stop codon from pro pol in the same reading frame, while env resided in another reading frame lacking a functional surface domain. According to the current bovine genome sequence assembly, the full-length BERV  $\gamma 4$  provirus sequences are only found in chromosomes 1, 2, 6, 10, 15, 26, 28, X, and unassigned, although the partial sequences are almost evenly distributed throughout the entire bovine genome. This is the first detailed study describing the genome structure of BERV  $\gamma 4$ . Combined with our recent reports on the characterization of bovine ERVs, this study will contribute to illuminating ERVs in cattle in which no information was previously available.

## Poster 4027

### Title: Polymorphism and evolution of the major histocompatibility complex DRA and DQA genes in Equidae

Presenting Author: Dr Petr Horin, Institute of Animal Genetics, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

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#### Abstract:

As a result of host and pathogen interactions, the major histocompatibility complex (MHC) genes coding for antigen binding and presenting molecules are the most polymorphic genes in the vertebrate genome. The diversity of MHC class II genes *DQA* and *DRA* was studied in the family Equidae. In addition to 11 previously reported *DRA* and 26 *DQA* alleles, 6 new *DRA* alleles and 11 new *DQA* alleles were identified in the genus *Equus*, species *Equus kiang*, *E. hemionus*, *E. zebra*, *E. grevyi* and *E. burchellii*. Phylogenetic analysis of both *DQA* and *DRA* sequences of the family Equidae provided further data supporting the trans-species hypothesis. The phylogeny trees constructed did not match the

standard *Equidae* taxonomy, expressing specific features of the MHC genes. Analysis of selection showed differences between the less variable *DRA* and more variable *DQA* genes. *DRA* alleles were more often shared by more species. The *DQA* sequences analyzed showed strong positive selection, the selected amino acid positions mostly corresponded to selected positions in rodent and human *DQA* genes.

## Poster 4028

### Title: Molecular characterization of the putative regulatory regions of the caprine prion protein gene (*PRNP*)

Presenting Author: Simone Peletto, National Reference Centre for Animal TSEs (CEA) – Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta

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#### Abstract:

Transmissible spongiform encephalopathies (TSEs) are a group of inevitably fatal neurodegenerative diseases of humans and animals. Several nonsynonymous single-nucleotide polymorphisms (SNPs) within the *PRNP* coding sequence have been associated with resistance/susceptibility to TSEs in humans, sheep, goats, deer and elk. Recent studies indicate that *PRNP* regulatory polymorphisms may also influence TSE occurrence. However, no basic information currently exists regarding the sequence composition and/or the frequency of genetic polymorphisms within the putative regulatory regions of the goat *PRNP* gene. Therefore, we generated sequences for the caprine *PRNP* gene regions that are homologous to known mammalian *PRNP* regulatory regions. Promoter prediction analysis using CpGPProD software yielded a single goat *PRNP* promoter that was homologous to regions of known promoter activity in sheep and cow. Sequence analysis and haplotype cloning revealed 25 nucleotide polymorphisms and 3 insertion/deletion (indel) polymorphisms in the putative promoter and intron I. No variation was detected within the predicted exon 1. The four conserved *PRNP* promoter motifs previously described for mammalian species were also conserved within goat sequence. The results of this study provide the initial genomic foundation for future investigations on the range of transcription factor binding motif profiles in the goat *PRNP* gene promoter.

## Poster 4029

### **Title: Polymorphism of PrP gene in Polish pig breeds.**

Presenting Author: Agata Piestrzyńska-Kajtoch, National Research Institute of Animal Production, Department of Immuno- and Cytogenetics, 32-083 Balice, Poland

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#### **Abstract:**

Host-encoded prion protein is believed to be a component of an infectious agent of transmissible spongiform encephalopathies (TSE). All analyzed mammalian genomes have a copy of *PrP* gene. Many polymorphisms within the coding region have an influence on the TSE susceptibility, pathogenesis and transmissibility. There are no reported TSE cases in pigs until today, but it was experimentally proved that pigs can become infected. The aim of our study was to investigate the polymorphism in the coding part of the *PrP* gene in Polish pig breeds.

DNA was isolated from different tissues of 152 pigs [*Sus scrofa domestica*] from 3 breeds (56 unrelated Polish Large White [WBP], 55 unrelated Polish Landrace [PBZ] and 41 Pulawska – some of which were half-siblings). The coding region of the gene was amplified, sequenced and analyzed in freeware genetic computer program.

Three silent polymorphisms were found (counted from the beginning of the coding region): T198C, C354T and C723G. 3,6% of WBP were homozygotes T198C, 30,3% of WBP and 9,1% of PBZ were heterozygotes T198C/T. 21,9% of Pulawska and 1 WBP pig were heterozygotes C354T/C. Only 1 WBP pig was heterozygote C723G/C.

These polymorphisms have not been published before.

## Poster 4030

### **Title: Comparative annotation of *Sus scrofa* chromosome 1**

Presenting Author: Zihua Jiang, Department of Animal Sciences, Washington State University, Pullman, WA 99164-6351 USA

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#### **Abstract:**

The pig whole genome is currently being sequenced by The Wellcome Trust Sanger Institute through funding provided by Cooperative State Research, Education and Extension Service at the United States

Department of Agriculture (CSREES-USDA). This project uses a clone-by-clone sequencing strategy, based on the MTP of BAC clones. To date, more than 1,200 clones have been selected and used to generate initial shotgun sequencing data for *Sus scrofa* chromosome 1 (SSC1). We have assigned these clones to their orthologous regions on human chromosomes 6, 9, 14, 15 and 18, which are consistent with the current comparative relationship between these two species. On the other hand, SSC1 might have some small regions orthologous to human chromosomes 1, 10, 11 and 20, respectively. Interestingly, the human orthologous regions harbor a total of 1,412 genes, including 917 known function genes, 170 genes similar to the other genes, 137 hypothetical genes, 114 ORF genes and 74 pseudogenes. However, one-to-one orthologous hits only confirmed 820 (89%) known function genes, 47 (28%) genes similar to other genes, 66 (48%) hypothetical genes, 82 (72%) ORF genes, and 27 (36%) pseudogenes in pigs. These results indicate that different categories of genes might have different evolutionary mechanisms.