Poster 1001

Title: Retention of Intron-1 in Cathelicidin-4 mRNA of Egyptian Native and Frisian Crossbred Cattle

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Abstract (maximum 200 words):
The antimicrobial peptide gene cathelicidin-4 (CATHL-4) was investigated in native and Frisian crossbred cattle reared in Egypt. The gene was tested for reaction with cDNA of various cattle tissues such as blood; lung; trachea; intestine; muscle; lymph and liver using PCR. A positive PCR product was seen in blood, lung, trachea, liver and lymph tissues, but not in intestine of both native and crossbred cattle. NCBI-Blast analysis of native and crossbred cattle CATHL-4 cDNA sequences (303 and 316bp, respectively) with *Bos taurus* CATHL-4 mRNA (gi: 31341226) and BTA22 (gi: 76649266) were almost the same, they both showed high sequence identity. Analysis of PCR products of CATHL-4 cDNA revealed the retention of intron-1 in both native and crossbred cattle, which is absent in *Bos taurus* CATHL-4 mRNA (gi: 31341226). This may have resulted from alternative splicing which increases the genetic diversity of the genome without increasing the overall number of genes. It may play a role in increasing their innate immunity by increasing gene expression, since intron-containing genes are expressed more efficiently than intronless ones. The results indicate that both native and crossbred cattle reared in Egypt may express cathelicidin gene more efficiently than *Bos taurus* or *Bubalus bubalis* reared abroad.

Poster 1002

Title: **Evaluation as therapeutics horse of KISO pony by behavioral related gene (DRD4 gene) polymorphism**

Presenting Author (name & address): HARUTAKA MUKOYAMA, Nippon Veterinary and Life Science University, Tokyo, Japan

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Abstract (maximum 200 words):
The ancestor of KISO pony which is a kind of Japanese native horses came from Mongolia grassland horse at the second century. KISO pony has been used as an employment animal for a long term. And this pony is utilized for animal therapy recently. The behavioral related gene polymorphism has been reported to be associated with the personality trait of novelty-seeking in humans.
We evaluated the temperament as therapeutics horse of KISO pony by the polymorphism of dopamine D4 receptor (DRD4) gene which is one of behavioral related gene.
The sequences of DRD4 exon III was determined 504bp in 21 KISO pony. All KISO pony samples had 9 repeats: no other repeat-type polymorphism of variable number of tandem repeats (VNTR) in DRD4 exon III was found. However, two SNPs was found in the amplified regions (147bp and 292bp).

Poster 1003

Title: **Epigenetic Case Studies in Agricultural Animals**
George Liu

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Abstract:
In many biological processes, the regulation of gene expression involves epigenetic mechanisms. An altered pattern of epigenetic modification is central to many animal diseases. Using animal disease models, we have studied one of the major epigenetic components: DNA methylation. We characterized the impacts of tissue types, ages and generations on the DNA methylation status of multiple genes in disease-resistant and susceptible animals. These results indicate that differential levels of DNA methylation of important candidate genes are related to the distinct traits of the animal models and could contribute to their resistance or susceptibility to diseases. We are also probing recent technological advances, which allow epigenetics to be studied at a genome-wide scale. Now it is time to apply epigenomics approaches to animal epigenetics research.

Poster 1004

Title: Genome wide SNP discovery in pig using 1G Genome Analyzer: How to play around with sequence length, quality level and mapping qualities

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Abstract (maximum 200 words):
Large scale sequencing of reduced representation libraries using the 1G Genome Analyzer has proven to be a reliable, fast method for the identification of large numbers of SNPs. Challenges arise, due to varying sequence quality, which is crucial during sequence selection. For instance, quality scores decrease with increasing sequence length, thereby increasing the number of unique sequences due to sequencing errors and leading to the identification of false SNPs in the 3'end of the sequence. On the other hand, shorter sequences lead to a higher proportion alignment of paralogous sequences.
This study shows the impact of varying sequence length and quality level on mapping quality and identification of true SNPs. A DNA pool of 5 animals from a boar line was digested with *DraI* and fragments of around 200bp were end-sequenced using 1G Genome Analyzer, yielding 70.348.064 sequences of 36bp. Output from BUSTARD, was used to select sequences which were uniquely mapped to a reference genome. Sequences with unknown bases and polymers were removed and sequence uniqueness ranged from 35% at 29bp to 55% at 35bp indicating a high error rate. Results, show the reliable identification of large number SNPs in spite of a relatively high error rate.

Poster 1005

Title: **CRB GADIE, a Biological Resources Centre dedicated to livestock genomics**

Order of the authors: ESQUERRE Diane, MARTHEY Sylvain, NEAU André, GAO Yu, ROGEL-GAILLARD Claire, HUGOT Karine

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Abstract (maximum 200 words):
CRB GADIE has a double-objective related to livestock genomics: (1) to maintain and valorize DNA collections and (2) to prospect, develop and distribute high throughput genomics tools. CRB GADIE conserves 2.5 millions BAC and cDNA clones for eight species including pig, chicken, cattle, trout, horse, rabbit, goat and sheep. Until 2001, our team has set up protocols, automates and LIMS to manage and screen libraries in optimal and secure conditions. More than 150 worldwide laboratories have been using INRA libraries thanks to CRB GADIE activities. Until 2004, CRB GADIE produced and distributed generic cDNA and oligonucleotide microarrays dedicated to pig, cattle, chicken and trout species. The CRB GADIE has been identified by REX EADGENE as a reference platform to produce chicken and pig microarrays and is currently working with two other European platforms (ARK-Genomics/Roslin, UK and University of Aahrus, DK) to develop common quality controls. In addition to a generic activity on clone management and chip production, CRB GADIE is involved in the development of promising new tools and methods such as genomic tiling arrays and sequence enrichment of targeted genomic regions by capture on oligonucleotide microarrays. CRB GADIE activities are presented at http://www-crb.jouy.inra.fr/BRC/index.html.

Poster 1006

Title: Automated SNP calling overestimates the number of SNP in the bovine genome

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Single nucleotide polymorphisms (SNP) have been identified from the Bovine Genome Sequencing Project and over 2 million have been identified at the Baylor College of Medicine (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/). The SNP were identified using text based methods that included a series of filters as described in the data set. We generated assays for 42 SNP from the genome sequence SNP set with the ABI SNPlex™ technology. We sampled genotypes from 804 animals of taurine dairy and 379 animals of taurine, zebu and composite beef breeds. Two of the SNP assays did not work and 22 of the SNP were monomorphic in all breeds, both taurus and zebu. That gives an estimate of the proportion of 0.45 ± 0.08 (SE) of the genome sequence SNP being valid. There were significant differences in the proportion of successful SNP assays from the three genetic regions that were tested. The sequence traces for the successful SNP, when compared to the traces of the unsuccessful SNP, showed that automated text based SNP calling had overestimated the number of SNP in the genome sequence database. Using a simple algorithm based on the sequence traces we were able to reduce the false calls to zero in our data set.
Financial support: L.R.P.N. is a Ph.D. student at the University of Queensland and receives an Endeavour International Postgraduate Research Scholarship, a UQ International Student Living Allowances and a scholarship from the Beef CRC. W.B. receives financial support from CSIRO, the Beef CRC, and Meat and Livestock Australia.

Poster 1007

Title: **From public sequences to custom microarrays: an easy and affordable tool.**

Presenting Author (name & address): Lorraine Pariset, Department of Animal Production, Università della Tuscia, Viterbo, Italy

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Abstract (maximum 200 words):
Expression data of more than 100 species are present in Gene Expression Omnibus at NCBI, with most platforms represented by spotted DNA/cDNA or oligonucleotides. This evidences a weak point in microarray generation, since starting from cDNA libraries or oligonucleotides synthesis implies a considerable lag between the availability of sequences and microarray preparation. In situ generated oligonucleotides are quicker to develop, but being mostly produced by big companies, only species for which there is high interest worldwide are considered.
We developed a pipeline of software instruments that allow to start from unannotated, redundant sequences, as those found in public databases or generated by parallel sequencing, to yield oligonucleotides suitable for in situ generation on chip. As an example, we generated a chip from sheep (*Ovis aries*) ESTs deposited at NCBI. The chip carries about 23,000 non-redundant features in quadruplicate and represents the first fully annotated sheep microarray with a large genome covering. In slide replicates show a coefficient of variation<0.25 for genes that are differentially expressed with P<0.01 of fold change.
The procedures are being installed in a portal with public access and will allow researchers to develop their own microarray from any species provided there are available sequences.

Poster 1008

Title: The validity of SNP markers for Bovine Parentage Test in Japan

Presenting Author (name & address): YOSHIYUKI MIYAZAKI, Maebashi Institute of Animal Science, Livestock Improvement Association of Japan, Inc., Maebashi, Japan.

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Abstract (maximum 200 words):
We have been conducting parentage testing using 17 microsatellite (MS) markers that include ISAG preferred 9 markers, to carry out tests with PE1* scores at > 0.99998. Recently, we have been able to confirm progress in the technology for the SNP typing that has also resulted in lower costs than before. In this study, we selected 91 SNP markers for parentage test in order to calculate excluding probability.
The SNP were selected based on the date of 87 Japanese Black and 84 Holsteins genotyped using Bovine Mapping 10K SNP Kit (Affymetrix GeneChip®) under the following conditions, (1) heterozygosity at ≧0.4, (2) located in a unique position on the genome, (3) 2~4 SNPs that were more than 5.7Mb apart on each chromosomes. Samples were 175 major sires (104 Japanese Black and 71 Holsteins) used in Japan. SNP was genotyped using the Illumina GoldenGateAssay technique, and analyzed by the Illumina BeadStadio3 software. PE1* score using the 91 SNP markers was >0.99999997. Equivalent scores for the 17 MS markers were achieved using 60~65 SNP markers. Finally, we were able to achieve higher degrees or precision on parentage test in Japan using the SNP markers.

PE1*:Given two parents and one offspring, excluding a parent

Poster 1009

Title: Comparative analysis of a BAC-based physical map of the horse genome

Authors: A. Wöhlke[1], G. Nordsiek[2], M. Scharfe[2], M. Jarek[2], F. Schrader[2], J. Wrede[1], J.P. Pook[1], B. Zhu[3], P. J. De Jong[3], B. P. Chowdhary[4], T. Leeb[1,5], H. Blöcker[2], O. Distl[1]
Presenting Author (name & address): Ottmar Distl, [1]Institute of Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Bünteweg 17p, 30559 Hannover, Germany

Abstract (maximum 200 words):
The second horse genome assembly is based on whole genome shotgun sequences (Broad Institute) and BAC end sequences (Hannover). We developed a high-resolution BAC-based physical map to further improve the horse genome assembly and to provide further SNPs. This physical map of the horse genome has been created using a combination of fluorescent fingerprinting and end sequencing of 150,000 BAC clones from the CHORI-241 equine BAC library (10X genome coverage). The results of 314,972 BAC end sequences are accessible at http://www.tiho-hannover.de/einricht/zucht/hgp/index.htm and public databases (NCBI, Ensembl). Fluorescent fingerprints were obtained by using the 4-restriction enzyme 4-color technique and separating the resulting fragments on capillary sequencers. Simultaneously with the collection of raw data we optimized the parameters for the assembly of the fingerprint contigs using the software FPMiner 2.0 and FPC V8.5.3. In addition, we used BAC end sequences and horse genome draft sequences for anchoring the fingerprint contigs, for closing gaps and for selecting the minimal tiling path of the physical horse map. So we can compare an assembly solely based on fingerprints with an assembly using fingerprints and equine sequences. We will present an updated status of this fingerprint map and comparative analyses with other genomes.

Poster 1010

Title: The SLA-immune long oligonucleotide set: a new tool for functional studies of immunity and disease resistance in pig

Presenting Author (name & address): Yu GAO [1, 2]
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Abstract (maximum 200 words):
Several genome wide generic arrays have been developed in pig but they only partially cover the genome and lack many immune response genes. Our aim was to design and validate a long oligonucleotide set dedicated to the immune response. The set comprises 3773 unique probes and includes all genes and putative transcripts localized in the swine leukocyte antigen (SLA) complex region (826 probes) as well as immune response genes outside SLA (2947 probes). The SLA subset contains targeted sense and anti-sense sequences of 407 transcripts and 6 non-coding RNA genes. The SLA-immune set represents 3104 porcine genes or transcripts among which 95% have one assigned GO term at least. The immune response pathways referred in KEGG are all covered and 38 other pathways are represented. The SLA-immune set was spotted onto glass slides together with the Qiagen-NRSP8 set and a series of control elements. We have analyzed transcriptome variations between unstimulated PBMCs and PBMCs stimulated by either LPS or PMA/ionomycin. We will present our results using this generic chip enriched with probes targeting immune response and will discuss the porcine SLA-immune oligonucleotide set as a promising tool for studying immunity and disease resistance in pig.

Poster 1011

Title: **Development of a 25-plex SNP assay for traceability in cattle**

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Abstract (maximum 200 words):
Single Nucleotide Polymorphisms (SNPs) are amenable to automation and therefore become the marker of choice for DNA profiling. *SNaPshot*, a primer extension-based method was used to multiplex 25 SNPs that have been previously validated as useful for identity control. Detection of extended products was based on four different fluorochromes and extension primers with oligonucleotide-tails of differing lengths, thus controlling the concise length of the entire chromatogram to 81 bases. Allele frequencies for Holstein, Simmental, Limousine, Angus, Charolais and Tux Cattle were estimated and significant positive Pearson-correlation coefficients were obtained among the analyzed breeds. The probability that two randomly unrelated individuals would share identical genotypes for all 25 loci varied from 10^{-8} to 10^{-10} for these breeds. For parentage control, the exclusion power was found to be 99.9% when the genotypes of both putative parents are known. A traceability test of duplicated samples indicated a high genotyping precision of greater than 0.998. This was further corroborated by analysis of 60 cases of parent-sib pairs and trio families. The 25-plex *SNaPshot* assay is adapted for low and high-throughput capacity and thus presents an alternative for DNA-based traceability in the major commercial cattle breeds.

Poster 1012