Proceedings of the 30th International Conference on Animal Genetics ISAG 2006

■ Biodiversity, the future pass through preservation ■

Porto Seguro, BA, Brazil, August 20-25, 2006

International Society for Animal Genetics

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18. Genetics of Immune Response Committee (*Herman Raadsma*)
19. Swine Lymphocyte Antigen Committee (*Douglas Smith*)
20. Comparative MHC Steering Committee (*Shirley Ellis*)
21. Animal Forensic Genetics Committee (*Sree Kanthaswamy*)

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Acknowledgment

Dear friends and colleagues,

We are very proud to host, for the first time in South America, the ISAG Conference. Specially here in Porto Seguro, one of the most hospitable Brazilian cities. We believe that you will have an unique opportunity, to know our culture and to exchange knowledge.

The Organizing Committee wishes to thanks to all the individuals who helped us to achieve our objectives. Our sincere gratitude to the members of the scientific committee; to the plenary sessions, workshops and poster oral presentation chairpersons; the invited speakers and volunteer students.

It is also the proper time for our gratefulness to the ISAG Executive Committee and all the ISAG members, who gave us, six years ago, during the conference in Minneapolis, U.S.A., a vote of confidence to organize this conference in Brazil.

We also address our thanks to all the sponsors and exhibitors, whose support was essential for the realization of this event.

Finally, our gratitude to the Brazilian College of Animal Reproduction (CBRA) staff, especially to Rômulo Cerqueira Leite, Maria Helena Chaves Silva, Simone Martins Monteiro, Marta Lúcia Oliveira Paiva, Eunice Faria Lopes, Marc Roger Jean Marie Henry e Antônio de Pinho Marques Júnior.

On behalf of the Local Organizing Committee,

Denise A. A. de Oliveira
Chair of ISAG 2006
Veterinary College, Universidade Federal de Minas Gerais

Marcelo Y. Kuabara
General Secretary of ISAG 2006
Parentage LTDA.

Timetable of the Conference

TIME	SUNDAY			MON			TUE				WED				THU		FRI		
	20-Aug-06			21-Aug-06			22-Aug-06				23-Aug-06				24-Aug-06		25-Aug-06		
09:00 - 10:00	registration			registration			registration				conf.tour				registration		registration		
10:00 - 11:00	plenary session 1		poster mounting (all sessions)	animal forensic		poster exhibition	plenary session 2			poster exhibition				poster exhibition	plenary session 3		cattle,sheep, goat gene map	domest anim gen seq	immune response
11:00 - 12:00																			
12:00 - 13:30	lunch			lunch			lunch				lunch				lunch		lunch		
13:30 - 14:30	pig gene map	appl gen companion animals		poster viewing			horse gen map	poultry gen map	appl gen sheep goat		poster viewing				ISAG gen business meeting		cattle,sheep, goat gene map	dog gen map	
14:30 - 15:30																			
15:30 - 16:15	coffee break		poster exhibition	coffee break			coffee break				coffee break			poster removing	coffee break				
16:15 - 17:15	pig gene map	equine genetics parentage		cattle molecular parentage	ISAG-FAO		horse gen map	poultry gen map			comp genomics	comparative MHC Cancelled by the Chair	poster oral presentation		animal forensic	poster oral presentation			
17:15 - 18:15																			
18:15 - 19:15	welcome reception																		
19:15 - 19:45																			
20:00 - 24:00															banquet				

Plenary Sessions

Plenary Session 1: **Biodiversity research: understanding the past, designing the future.**
August 20 (Sunday) 10:00-12:00 at Room 1
***Chair**: Henner Simianer, Inst.Animal Breeding & Genetics, Germany.*

- **The value of diversity rational concepts on how to asses diversity and develop global strategies to maintain it**
  Martin Weitzman, Department of Economics, Harvard University, USA
- **The history of the molecular diversity of livestock genetic resources: overview of our current knowledge**
  Olivier Hanotte, International Livestock Research Institute, Kenya.
- **Conservation genetics in a megadiversity country**
  Fabrício R. Santos, Universidade Federal de Minas Gerais, Brazil.

Plenary Session 2: **Genomics and biodiversity.**
August 22 (*Tuesday*) 10:00-12:00 at Room 1
***Chair:** Harris A. Lewin, Department of Animal Sciences, University of Illinois, USA.*

- **Evolution Highway: large-scale analysis of vertebrate chromosomal evolution**
  Denis Larkin, University of Illinois, USA.
- **Combined technologies of assisted reproduction, cloning and the state-of-the-art of microarray analysis**
  Jerry Yang, University of Connecticut, USA.
- **Gene Identification Signature (GIS) analysis: a novel cloning technology using paired-end 5' and 3' tags to characterize mammalian transcriptome and genome**
  Ruan Yijun, Genome Institute of Singapore, Singapore.

Plenary Session 3: The functional genome.
August 24 (Thursday) 10:00-12:00 at Room 1
***Chair**: Luiz Lehmann Coutinho, Department of Animal Science, Universidade de São Paulo, Brazil.*

- **Functions of microRNAs in C. elegans development and human cancer**
  Eric Miska, Dept. Biochemistry, University of Cambridge, United Kingdom.
- **Genome adaptations of TRIM5 for retroviral defense in ruminants** – *Timothy Smith, USDA, USA.*
- **Physiological genomics Identifying genes for endoparasite resistance**
  Allan Crawford, AgResearch, New Zealand.

Workshops

Workshop 1: **Pig Gene Mapping and Applied Genetics**.
August 20 (Sunday) 13:30-15:30/Coffee break/16:15-18:15 at Room 1
***Chair**: Gary Rohrer, USDA-MARC, Clay Center, NE, USA*

- **Session 1: Status of Pig Genomic Research**
  - **Welcome** [5']
  - **Overview of Pig QTL and Identification of QTN** [25']
  - **An integrated RH map of the porcine genome with more than 5000 anchoring points on the human genome provides a framework for the sequencing of the pig** [B520]
    Denis Milan, INRA/Laboratoire de Génétique Cellulaire, France
  - **Construction of pig gene catalog: determination and application of porcine full-length cDNA sequences** [B283]
    Hirohide Uenishi, National Institute of Agrobiological Sciences, Japan
  - **Genetics of porcine Geneexpression**
    Christian Bendixen, Danish Institute of Agricultural Sciences, Denmark
  - **Sequencing the Pig Genome using a BAC by BAC approach** [B404]
    Sean Humphray, The Wellcome Trust Sanger Institute, UK

- **Session 2: Highlights of Posters**
  - **Improved annotation of the porcine Affymetrix GeneChip(R) and functional comparison to QIAGEN-NRSP8 oligonucleotide array data** [E246]
    Oliver Couture, Iowa State University, Ames, USA
  - **Microsatellite and chromosome Y sequence analysis of wild boar and autochthonous pig breeds from Asia, Europe, South America and Africa** [A320]
    Oscar Ramirez, Universitat Autònoma de Barcelona, Spain
  - **QTL scan for physiological variables related to vitality in newborn piglets** [D347]
    Anna Tomas, UAB, Spain
  - **Characterization of the aldo-keto reductase 1C gene cluster on pig chromosome 10 and association with age of puberty and ovulation rate** [D277]
    Dan Nonneman, USDA/ARS/US Meat Animal Research Center, Clay Center, USA
  - **Confirmation of QTL for the inverted teat defect in porcine dam lines** [D299]
    Klaus Wimmers, Research Institute for the Biology of Farm Animals (FBN Germany
  - **Refined linkage mapping of the Escherichia coli F4ac receptor gene on pig chromosome** 13 [B512]
    David Joller, Institute of Animal Sciences, Switzerland
  - **Business meeting and election of new committee**

Workshop 2: **Applied Genetics Committee of Companion Animals**

August 20 (Sunday) 13:30-15:30 at Room 2

***Chair**: Hein van Haeringen, Dr.Van Haeringen Laboratorium B.V, Wageningen, The Netherlands*

- **Welcome**
- **Comparison test: Comments from Duty Laboratory South Africa; Comments from Computing Laboratory The Netherlands; Discussion**
- **ISAG panels: Do they work well? How many laboratories are using these panels in the routine? Do we need changes? Standard / Reference samples?**
  - **Nomenclature: Is there a need to change or do we continue?**
- **Next Comparison test: Do we need another one? If so, when? Duty laboratory; Computing laboratory**
- **Cat Phenotypic and Health Information Registry (PHIR): Presentation by Leslie Lyons**
- **Elections: Hein van Haeringen will step down; Matthew Binns and Andrea Rosati have changed their position**
- **Any other business**
- **Closing remarks**

Workshop 3: **Equine Genetics and Parentage Testing Standardization**

August 20 (Sunday) 16:15-18:15 at Room 2

***Chair**: E. Gus Cothran, Texas A & M University, College Station, TX, USA*

- **Introduction.**
- **Horse Comparison Discussion**
- **Duty Lab Report**
- **Analysis Lab Report**
- **Discussion of Results**
- **Short Presentations**
  - **Diversity and performance of a standard set of 17 microsatellites for paternity testing in Brazilian Crioulo, Campolina and Thoroughbred horses** [A352]
    Dario Grattapaglia, Genomax/ Heréditas Tecnologia em Análise de DNA, Brasília, DF, Brazil
  - **Equine Genotyping: Minimizing the risk of parentage errors** [A375]
    Wim A van Haeringen, Dr. Van Haeringen Laboratorium BV, Wageningen, The Netherlands
  - **Development of mutagenically-separated PCR assays for equine genetic screening** [D377]
    Ann E.O. Trezise, Australian Equine Genetics Research Centre & School of Biomedical Sciences, University of Queensland, Australia
  - **Molecular and evolutionary study of genetic variability in Equids DRA gene** [A536]
    Silvina Díaz CIGEBA, Facultad Ciencias Veterinarias. Universidad Nacional de La Plata. Argentina
- **Election of Committee**
- **Other Business**
- **Close**

Workshop 4: **Animal Forensic Genetics – Part 1**

August 21 (Monday) 10:00-12:00 at Room 1

***Chairs**: Wim van Haeringen, Dr.Van Haeringen Laboratorium B.V, Wageningen, The Netherlands and Sree Kanthaswamy, University of California-Davis, USA.*

- **Resequencing microarray technology for nucleotide sequence analysis of the entire mitochondrial genome** .[W581]
  Matthew C Lorence, Affymetrix, USA
- **Wildlife DNA forensics in the UK: a partnership approach** [W584]
  Ross McEwing, Wildlife DNA Services, UK.
- **Improved genomic typing for animal identification and breed estimation** [W583] *Peter Kesners, CY O'Connor, Village Foundation, Australia*

Workshop 4: **Animal Forensic Genetics – Part 2**

August 24 (Thursday) 16:15-18:15 at Room 1

***Chairs**: Wim van Haeringen, Dr. Van Haeringen Laboratorium B.V, The Netherlands and Sree Kanthaswamy, University of California-Davis, USA.*

- **An STR Forensic Typing System in the Domestic Cat and Population Genetic Database in 38 Cat Breeds** [W580]
  Marilyn Menotti-Raymond, National Cancer Institute, USA
- **Development of STR assays for identification and forensic testing** [W582]
  Mikko T. Koskinen, Finnzymes Diagnostics, Finland
- **Round Table Discussion - Recommendations for Animal DNA Forensic and Identity Testing**

Workshop 5: **Cattle Molecular Markers and Parentage Testing**

August 21 (Monday) 16:15-18:15 at Room 2

***Chair**: Marie-Yvonne Boscher, Labogena, France*

- **Welcome Introduction.**
- **Comparison test: Comments from Duty Laboratory; Comments from Computing Laboratory**
- **Discussion of Results : STR and genes,**
- **Next comparison test**
- **Other Business: Advance in the use of SNPs for cattle identification, need for accreditation**
- **Election of Committee**
- **Close**

Workshop 6: **ISAG-FAO Advisory Group on Animal Genetic Diversity**

August 21 (Monday) 16:15-19:15 at Room 1

***Chair**: Henner Simianer, Institute of Animal Breeding & Genetics, Germany*

- **Report on activities of FAO and the working group on animal genetic diversity**
  I. Hoffmann and H. Simianer
- **A mitochondrial survey of South American goat breeds reveals the existence of an ancient Canarian genetic signature** [A240]
  Marcel Amills, Universitat Autònoma de Barcelona, Spain
- **Genetic variation at 23 STRs loci in five Brazilian populations of Santa Inês hair sheep breed** [A467]
  Carla A. Souza, Embrapa Genetic Resources and Biotechnology, Brazil
- **Mitochondrial DNA sequences reveal a putative East Asian ancestry for old Chilean chickens** [A443]
  Jose Alcalde, Pontificia Universidad Catolica de Chile, Chile.
  Phylogeography of three stocks of the Amazonian fish Peacock bass (Cichla - Pisces) introduced in Minas Gerais State, southeast Brazil [A276]
  Daniel Cardoso de Carvalho, Universidade Federal de Minas Gerais, Brasil

Break

- **Microsatellites and Y-chromosomal haplotypes of European and Middle-Eastern cattle, sheep and goats: geographic clines, clusters, male introgression and conflicting views on diversity** [A380]
  J.A. Lenstra, Utrecht University, The Netherlands

- **Genome-wide SNP analyses of Holstein Friesian cattle reveal new insights into Australian and global population variability** [A481]
  Kyall R. Zenger, The University of Sydney, Australia
- **Estimation of genetic distances from two partly overlapping microsatellite marker data sets** [A238]
  Helge Taeubert, Trinity College Dublin, Ireland
- **Weitzman´s approach and the diversity within and between chicken populations** [A574]
  Tamina Pinent, Georg-August-University, Germany
- **Accounting for concurrence between breeds in the derivation of conservation priorities based on Weitzman's diversity concept** [A187]
  Henner Simianer, Georg-August-University, Germany

Workshop 7: **Horse Genome Mapping**

August 22 (Tuesday) 13:30-15:30/Coffee break/16:15-18:15 at Room 1

***Chair**: Telhisa Hasegawa, Laboratory of Molecular and Cellular Biology, Equine Research Institute, Japan*

Session 1: Whole Genome Mapping and Sequencing

- **Whole genome linkage maps**
  Domenico Bernoco, Professor Emeritus, Dept of Population Health and Reproduction, University of California, USA
  - **Integrated map** [B553]
    Kao Castle, University of Sydney, Australia
  - **Y chromosome BAC contig** [B410]
    Terje Raudsepp, Texas A & M University, USA
  - **BAC end sequencing** [B233]
    Tosso Leeb, University of Berne, Switzerland & University of Veterinary Medicine Hannover, Germany
  - **Whole Genome Sequencing** [B551]
    Ernest Bailey, University of Kentucky, USA
  - **Discussion**

Session 2: Mapping traits

- **QTL mapping for OC**
  Catherine Wittwer
  - **Whole genome LD analysis** [D037]
    Teruaki Tozaki, Laboratory of Racing Chemistry, Utsunomiya, Japan
  - **Mapping Grey coat colour** [B371]
    Gerli Pielberg, Uppsala University, Sweden
  - **Mapping silver coat color** [C357]
    Sofia Mikko, Swedish University of Agricultural Sciences, Sweden
  - **Brindle color and chimerism** [B521]
    M. Cecilia T. Penedo, University of California, USA
  - **Discussion**

Workshop 8: **Poultry Gene Mapping**

August 22 (Tuesday) 13:30-15:30/Coffee break/16:15-19:15at Room 2

***Chair**: Richard Crooijmans, Wageningen Univ, Anim. Breeding & Genetics Group, The Netherlands*

Chicken *genome* (Topics)

- **Genome Sequence update**
  Richard Crooijmans
- **Use of large scale SNPs (Genetic maps, LD)** [B389]
  Richard P.M.A. Crooijmans, Wageningen University, The Netherlands
- **RH-maps** [B466]
  A. Vignal, INRA Laboratoire de Génétique Cellulaire, France
- **(Fine) mapping QTLs**
  - **QTL mapping for resistance** [D465]
    Marie-Helene Pinard-Van Der Laan, UMR INRA/INA-PG Génétique et Diversité Animales, France
  - **SNP allele frequencies between lines** [D197]
    Chris M. Ashwell, North Carolina State University, USA

- **Candidate genes of myogenic factors** [C518]
  Helena J. Alves, Animal Biotechnology Laboratory USP/ESALQ
- **Differential expression** [C168]
  Anna K. Bennett, Iowa State University, Ames, IA USA

▪ **Regulation**
- **MircoRNA prediction** [E434]
  Shu-Hong Zhao, Huazhong Agricultural University, China

***MHC* (C*hair Janet Fulton*)**

▪ **MHC haplotypes** [A543]
Janet E. Fulton, Hy-Line International, USA

▪ **Genotyping MHC class-I locus BF1**[C456]
Bertrand Bed'Hom, UMR INRA / INA-PG Génétique et Diversité Animale, France

▪ **Genomic analysis of the MHC in turkey** [B333]
Lee D. Chaves, University of Minnesota, USA

Quail genome

▪ **Update genetic resources**
Alain Vignal

Duck genome

▪ **Update genetic resources**
Ning Li

▪ **Genetic relationships among Chinese ducks** [A211]
Ning Yang, China Agricultural University, China.

Turkey genome

▪ **Update genetic resources**
Kent Reed, University of Minnesota, USA

Workshop 9: Applied Genetics in Sheep and Goats

August 22 (Tuesday) 13:30-15:30 at Room 3
***Chair**: Liliana Di Stasio, Dipartmento di Scienze Zootecniche, Italy*

▪ **Comparison test: report from the Duty Laboratory (LABOGENA, France)**
▪ **Discussion of results**
▪ **Poster presentation**
  ▪ **Novel Y Chromosomal Haplotypes Reveal Wild and Domestic Sheep Diversity** [A070]
  Jennifer R. S. Meadows, CSIRO Livestock Industries, Australia
  ▪ **Enhanced mapping tools for the sheep genome – BACs, SNPs and the virtual map** [E507]
  Jillian F. Maddox, University of Melbourne, Australia
▪ **Election of Committee**
▪ **Any other business**
▪ **Close**

Workshop 10: Comparative Genomics

August 23 (Wednesday) 16:15-19:15 at Room 3
***Chair**: Christopher Tuggle, Department of Animal Science, Iowa State University, USA*

▪ **Combining mouse mammary gene expression and comparative mapping for the identification of candidate genes for QTL of milk production traits in dairy cattle** [C317]
Ron Micha, Agricultural Research Organization, Israel

▪ **A procedure to finemap mouse QTLs using dense SNP data** [abstr B402]
Gudrun A Brockmann, Humboldt-Universität zu Berlin, Germany

▪ **Comparative Functional Genomics: Endometrial gene expression profiling during the estrous cycle and early pregnancy of Yorkshire gilts using the Affymetrix porcine GeneChip** [C263]
Shu-Hong Zhao, Huazhong Agricultural University, China & Department of Animal Science, Iowa State University, USA

▪ **Comparative profiles of gene expression of Bubalus bubalis and Bos taurus** [C548]
Maria Paula Schneider, Universidade Federal do Pará, Brazil

Break

- **Systematic identification of regulatory elements in cattle** [C448]
  George E. Liu, USDA, ARS, ANRI, Bovine Functional Genomics Laboratory, BARC-East, USA
- **Phylogenetic relationships among wild boar (Sus scrofa) populations in Italy** [A308]
  Longeri et al. Maria Longeri, University of Milan, Italy
- **FUGATO - Functional Genome Analysis in Animal Organisms** [D328]
  Kirsten Sanders, Managing office FUGATO, Germany,
- **Open discussion regarding possible collaborative projects, 2008 Workshop format, new election of Committee members**

Workshop 11: **Comparative MHC:** levels of diversity and mechanisms involved in its generation
August 23 (Wednesday) 16:15-18:15 at Room 2
***Chair**: Shirley Ellis, Institute for Animal Health, UK*

Cancelled by the Chair. Selected posters moved to Session **"Oral Presentations of Selected Papers one"** - August 23 (Wednesday) 16:15-18:15 at Room 1

Workshop 12: **Cattle, Sheep and Goat Gene Mapping**
August 25 (Friday) 10:00-12:00/Lunch time/13:30-15:30 at Room 1
***Chair**: Eduardo Casas, Molecular Genetics Research Unit, US Meat Animal Research Center, ARS-USDA, USA*

- **Welcome and introduction**
  Eduardo Casas
- **Development of an ovine 20K SNP chip and its use to construct an ovine HapMap**
  James Kijas, CSIRO, Australia
- **Differential gene expression in the muscle of callipyge sheep**
  Chris Bidwell, Purdue University, USA
- **A bovine whole genome long *oligonucleotide* expression array**
  Chris Elsik, Texas A& M University , USA
- **Linkage disequilibrium: Why do we care and now what?**
  Stephanie McKay, University of Alberta, Canada
- **Discovery and profiling of bovine microRNAs from immune-related and embryonic tissues**
  Luiz Coutinho, University of Sao Paulo, Brazil
- **Characterization and validation of in silico developed SNP mapping to three different bovine genomic regions on 15 different cattle breeds**
  Mathieu Gautier, INRA, France
- **Validation of molecular markers: the industry's perspective**
  Brent Woodward, Merial Limited
- **Business meeting**
- **An overview of the bovine HapMap project**
  Curt Van Tassell, ARS-USDA, USA
- **The bovine gene atlas**
  Tad Sonstegard, ARS-USDA, USA
- **Genomics approches to genetic disorders in France: Identification of a doublet mutation responsible for Syndactyly in Holstein cattle and primary localisation of an Hypoplasia syndrome in Montbeliard cattle**
  André Eggen, INRA, France
- **Allelic variation in gene expression is correlated with phenotypic variation**
  Hasan Khatib, University of Wisconsin, USA
- **Prediction of putative imprinted genes influencing birth weight in the bovine genome**
  Ikhide Imumorin, Spelman College

Workshop 13: **Domestic Animal Genome Sequencing**
August 25 (Friday) 10:00-12:00 at Room 2
***Chair**: Lawrence Schook*

- **Introduction and Call to Order**
  Lawrence B. Schook, University of Illinois, USA

- **Chicken Genome Project**
  Richard Crooijmans, Wageningen University, The Netherlands
- **Bovine Genome Project**
  Curt Van Tassell, ARS-USDA, USA
- **Porcine Genome Project**
  Jane Rogers, The Wellcome Trust Sanger Institute
- **Ovine Genome Project**
  Noelle Cockett, Utah State University, USA
- **Canine and Equine Genome Projects**
  Matthew Binns, Royal Veterinary College, University of London, UK
- **Closing Remarks**

Workshop 14: **Dog Genome Mapping**

August 25 (Friday) 13:30-15:30 at Room 2

***Chair**: Kathryn Graves, Equine Parentage Testing and Genetic Research Lab, University of Kentucky, USA*

- **Population structure and complex traits: methods for mapping in the dog genome**
  Heidi G. Parker.
- **Molecular characterization of the canine RAGE gene** [B415]
  Hugo Murua Escobar, University of Veterinary Medicine Hanover, Germany
- **Two novel mutations in the canine bestrophin gene are associated with autosomal recessive multifocal retinopathy** [B316]
  Karina E. Guziewicz, University of Pennsylvania, USA.
- **Use of autozygozity mapping in dogs:Mapping the gene for Trapped Neutrophil Syndrome, an immune dysfunction in Border Collies** [B318]
  Alan N. Wilton, University of New South Wales, Australia

Workshop 15: Immune Response and Disease Resistance

August 25 (Friday) 10:00-12:00 at Room 3

***Chair**: Herman Raadsma, ReproGen, University of Sydney, Australia*

- **Microarray analysis of host gene expression in response to bovine Leukemia Virus Infection** [C554]
  Rosane Oliveira, University of Illinois at Urbana-Champaign, USA
- **Quantitative trait loci associated with parasitic infection in a bovine F2 population** [D348]
  Marcos V. G. B. Da Silva, Embrapa Dairy Cattle Research Center, Brazil
- **Putative QTL for Parasite Resistance in Sheep** [D407]
  Tracy S. Hadfield, Utah State University, USA
- **Quantitative trait locus affecting susceptibility to mycobacterium paratuberculosis infection identified on BTA 20 in US Holsteins** [D472]
  Michael G. Gonda, University of Wisconsin, USA
- **Genome scan for QTLs related to tick resistance in bovine** [D267]
  Marco A. Machado, Embrapa Dairy Cattle Research Center, Brazil
- **Fine mapping of QTL affecting mastitis resistance in Nordic dairy cattle** [D454]
  Mogens S. Lund Danish Institute of Agricultural Sciences & MTT Agrifood Research Finland
- **Wide-response variation to *Babesia bovis* infection can be used to select babesiosis-resistant cattle** [A458]
  Magda V. Benavides, Embrapa Sheep and Cattle Research Centre, Brazil
- **Comparative transcriptomic analysis of the immune response in Large White pigs differing by 20 years of selection** [C345]
  Valentina Mariani, INRA CEA, Laboratoire de Radiobiologie et Etude du Génome, France
- **Gene *expression* analysis in cattle resistant and susceptible to gastrointestinal nematode infections by real-time RT-PCR** [C066]
  Lilian G. Zaros, University of São Paulo/ESALQ, Brazil
- **Lack of a strong NFkB-dependent transcriptional response during infection with Salmonella enterica serovar Typhimurium as compared to that observed in S. Choleraesuis infection** [C264]
  Chris Tuggle, Iowa State University, USA

- **Fine mapping of *trypanosomosis* resistance loci, Tir2 and 3, sequence and expression variation of TLR as candidate genes underlying the QTL**
  Joseph Nganga, International Livestock Research Institute & Jomo Kenyatta University of Agriculture and Technology, Kenya

Oral Presentation of the Selected Posters

Oral Presentations of Selected Papers – Part 1

August 23 (Wednesday) 16:15-18:15 at Room 1
***Chair**: Ernest Bailey,University of Kentucky, USA*

- **Alleles of a bovine *DGAT1*-promoter variable number of tandem repeat associated with a milk fat QTL at chromosome 14 can stimulate gene expression** [abstr. C186]
  Rainer Fürbass, Research Institute for the Biology of Farm Animals, Germany
- **Preliminary results on the genetic background of meat quality differences between two muscles in Avileña Negra-Ibérica calves, using cDNA microarrays** [abstr. C311]
  Natalia Moreno-Sánchez, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain
- **Fine-mapping of a bovine QTL for marbling on BTA 4 using association study** [abstr. D557]
  Kou Yokouchi, Shirakawa Institute of Animal Genetics, Japan
- **Myosin heavy chain isoform transcript abundance and muscle fiber type distribution of *Longissimus Dorsi* in high and low performing pigs and in different pig breeds** [abstr. C272]
  Nguyen T. Ngu, University of Bonn, Germany
- **Transcript profiling of chondrocytes in a porcine impact injury model of osteoarthritis** [abstr. C198]
  Melissa S Ashwell, North Carolina State University, USA

Oral Presentations of Selected Papers – Part 2

August 24 (Thursday) 16:15-18:15 at Room 2
Chair: Ernest Bailey,University of Kentucky, USA

- **Genomic characterization of a defensin gene cluster on ECA 27q17** [abstr. B242]
  Christian Looft, The Royal Veterinary and Agricultural University, Denmark
- **Analysis of novel equine defensins and psoriasin as another antimicrobial peptide** [abstr.C174]
  Oliver Bruhn, University of Kiel, Germany
- **mtDNA sequences shows Japanese native chickens have multiple origins** [abstr.A209]
  Takashi Amano, Tokyo University of Agriculture, Japan
- **Evidence for a separate lineage of the insulin gene in tetrapods and fish** [abstr. B441]
  Geoffrey C Waldbieser, USDA/ARS, Catfish Genetics Research Unit, Stoneville, USA
- **An intronic insertion in *KPL2* results in abnormal gene expression and causes the immotile short tail sperm defect in Finnish Large White** [abstr. C289]
  Anu Sironen, MTT/ Biotechnology and Food Research, Finland
- **Microsatellite diversity within the SLA region of homozygous and heterozygous samples** [abstr. A229]
  Asako Ando, Tokai University School of Medicine, Isehara, Japan
- **Polymorphism and gene organization of Bubalus bubalis MHC-DQB show homology to BoLA DQB region** [abstr A313]
  Leonardo Sena, Instituto de Estudos Superiores da Amazônia, Brazil

Best Posters

- **Comparative transcriptomic analysis of the immune response in Large White pigs differing by 20 years of selection** - Mariani V, Lefèvre F, Bidanel J-P, Flori L, Chardon P, Rogel-Gaillard C. [C345]
- **Fine mapping of the porcine Arthrogryposis Multiplex Congenita (AMC) region on SSC5 and development of a genetic marker test** - Genini S, Haubitz M, Nguyen TT, Joller D, Malek M, Anderson S, Archibald A, Stranzinger G,Voegeli P. [D419]
- **Two novel mutations in the canine bestrophin gene are associated with autosomal recessive multifocal retinopathy** - Guziewicz KE, Zangerl B, Grahn BH, Lindauer SJ, Acland GM, Aguirre GD. [B316]
- **Genome-wide SNP analyses of Holstein Friesian cattle reveal new insights into Australian and global population variability** - Zenger KR, Khatkar MS, Cavanagh JAL, Hawken R, Raadsma HW. [A481]
- **Differential expression of genes in the bone marrow of layer and broiler chickens** - Bennett AK, Hester PY, Spurlock DM. [C168]
- **A porcine model of T-cell lymphoma** - Kuzmuk KN, Rund LA, Adam SJ, Zachary JF, Counter CM, Schook LB. [E266]
- **What sort of genes affect milk production?** - ChamberlainAJ, Goddard ME. [D363]
- **Use of SNP markers for individual identification and genetic traceability in the pig based on the SNPlexTM Genotyping System** - Ballester M, Pérez-Enciso Ml, Van Haandel B, Santamartina J, Sánchez A. [A497]
- **Investigation on the genetic background of coat colour inheritance in a Charolais x German Holstein F2 resource population** - Kühn C, Weikard R. [D239]
- **Gene expression profiling of two hypertrophied muscles in callipyge lambs** - Fleming-Waddell JN, Wilson LM, Olbricht GR, Vuocolo T, Byrne K, Craig BA, Tellam RL, Cockett NE, Bidwell CA. [C514]
- **Haplotypes in the bovine leptin gene associated with serum leptin, feed efficiency, growth and carcass merit** - Mujibi FN, Nkrumah DJ, Murdoch B, Li C, Moore SS. [D516]
- **Polymorphism and gene organization of Bubalus bubalis MHC-DQB show homology to BoLA DQB region** - Sena L, Schneider MPC, Honeycutt RL, Womack JE, Honeycutt D, Skow LC. [A313]

Guest Chairs and Speakers

Allan Crawford
(AgResearch, New Zealand)
Speaker: Plenary Session 3

Allan is a senior scientist in the Animal Genomics section of AgResearch. He completed his PhD in microbiology at the University of Otago, and then spent the next 12 years in labs in Canada, New Zealand and the USA, working on projects involving bio-control of insects using natural pathogens. Sixteen years ago he joined a new group set up by AgResearch and the University of Otago to study the genes of livestock. His major contribution has been the construction of the first genetic linkage map of sheep and its use in the discovery of regions of the genome that make sheep resistant to diseases.

Denis M Larkin
University of Illinois at Urbana-Champaign
Speaker: Plenary Session 2

Education: Institute of Cytology and Genetics (Novosibirsk, Russia): Ph.D. – 2000 – Genetics Ph.D. thesis is titled: *Comparative mapping of human chromosome 17 genes in pigs and common shrews*.

Novosibirsk State University (Novosibirsk, Russia): M.S.: – 1997 – Biology Major subject: Genetics / Animal Science Minor subjects: Microbiology, Philosophy Diploma project was titled: *Chromosomal localization and synteny analysis of several genes in pigs, cattle, and sheep.*

Research experience: April 2004 – present: Visiting Assistant Professor in Genetics and Bioinformatics, in the Laboratory of Immunogenetics of Dr. Harris A. Lewin, Department of Animal Sciences, University of Illinois at Urbana-Champaign.
The project is titled: *Construction of 1Mbp resolution RH comparative map of the cattle, human, and mouse genomes*. The goal is to build 1Mbp resolution RH comparative map of the cattle genome linked to the human and the mouse genome sequences. End sequencing of 20,000 clones from bovine RPCI-42 library for SNP search and analysis. May 2001 – April 2004: Postdoctoral Research Associate in Genetics and Bioinformatics, in the Laboratory of Immunogenetics of Dr. Harris A. Lewin, Department of Animal Sciences, University of Illinois at Urbana-Champaign. January 2001 – April 2001: Postdoctoral Research Associate in Genetics, in the Laboratory of Developmental Genetics of Dr. Oleg Serov, Institute of Cytology and Genetics, Novosibirsk, Russia.

Eric Miska
(Dept. Biochemistry, University of Cambridge, United Kingdom)
Speaker: Plenary Session 3

Was born in 1971 in Bitburg, Germany. He studied physics and mathematics at Heidelberg University, Germany, and received a BA in Biochemistry from Trinity College, Dublin, Ireland in 1996. He received his PhD in Pathology from the University of Cambridge, Cambridge, UK in 2000. He was a postdoctoral fellow in the laboratory of Bob Horvitz at the Massachusetts Institute of Technology, Cambridge, MA, USA from 2000 to 2004. He started his own laboratory at the Wellcome Trust/Cancer Research UK Gurdon Institute at the University of Cambridge, Cambridge, UK in 2005. His laboratory is studying the role of non-coding RNA genes in animal development and human disease.

Ernie Bailey

Chair: Oral presentations of selected papers sessions

PhD, is a professor in the Veterinary Science Department at the University of Kentucky. He completed his doctorate at the University of California, Davis under the direction of Professors Clyde Stormont and Domenico Bernoco in 1980. He took a faculty position at the University of Kentucky and conducted research on the major histocompatibility complex of horses. In the 1990s he began genomics research in horses and served as coordinator for the International Horse Genome Project. He serves on the editorial board of Animal Genetics as well as the editorial boards of the Journal of Heredity, Animal Biotechnology and BMC-Genetics. Dr. Bailey, his coworkers and his students conduct research on gene mapping, cytogenetics, coat color genetics, and immunogenetics of horses.

Fabrício R. Santos
Universidade Federal de Minas Gerais, Brazil.
Speaker: Plenary Session 1

Fabrício R. Santos - Biologist (B.Sc. in Genetics, 1990), Ph.D. in Molecular Biology and Biochemistry in 1995 (Universidade Federal de Minas Gerais - UFMG), Research Associate in Oxford University, UK, from 1995-1997, Adjunct Professor of Genetics and Evolution in UFMG, Belo Horizonte, Brazil, since 1997. He is author of more than 50 original articles in scientific journals, author of 4 books and more than 10 book chapters. He is Researcher of CNPq (Brazil) and advisor in the Graduate courses of Genetics, Bioinformatics and Ecology of UFMG. His main research interest is evolutionary genetics of humans and other animals.

Gordon Luikart (University Joseph Fourier, Grenoble, France)
Speaker: Plenary Session 3

Current appointments: Visiting Professor, Center for Investigation of Biodiversity and Genetic Resources (CIBIO) University of Porto, 4485-661 Vairão, Portugal; Research Associate Professor University of Montana, Division of Biological Sciences - Missoula, MT, 59812, USA: gordon.luikart@mso.umt.edu; **Professional Preparation and employment:** Research Scientist (CR1), CNRS (Centre National Recherche Scientifique), France 2001-2005; Research Scientist, Montana Conservation Science Institute (MOCSI), USA, 2004-2005; NSF-NATO Post Doctoral Fellowship, Evolutionary and Conservation Genetics, France, 1998-1999; Université Joseph Fourier, European Union Postdoctoral Fellow, Evolutionary Genetics, France, 1997-1998; University of Montana, Ph.D., February 1997, Organismal Biology and Ecology. University of Montana, M.S., 1992, Zoology Iowa State University, B.S., 1988, Biology.

Harris Lewin
(Department of Animal Sciences University of Illinois, USA)
Chair: Plenary Session 2

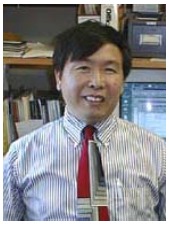
Harris Lewin is Professor of Immunogenetics, with a primary appointment in the Department of Animal Sciences, and holds the Gutgsell Endowed Chair. Lewin was the founding Director of the W. M. Keck Center for Comparative and Functional Genomics, and is currently Director of the Institute for Genomic Biology at the University of Illinois. Professor Lewin's current research interest is mammalian comparative and functional genomics. His research has resulted in the identification of the gene responsible for resistance and susceptibility to bovine leukemia virus infection, the development of high-density comparative maps for mammalian genomes, and co-discovery of a gene affecting milk fat composition in dairy cattle. In addition, his group produced the first large-scale cattle cDNA and oligo microarrays, and applied the technology for understanding the genomic effects of nuclear transfer cloning and the elucidation of the dietary effects on gene expression in the periparturient period. Lewin is Associate Editor of the journal *Animal Biotechnology* and serves on the Editorial Board of *Physiological Genomics*. In 2004, he was elected as a Fellow of the American Association for the Advancement of Science.

Henner Simianer

(Inst. Animal Breeding & Genetics, Germany)
Chair: Plenary Session 1

Was born in 1957 in Karlsruhe, Germany. He studied agricultural sciences and obtained his doctor degree at the University of Giessen and later worked at the Universities of Stuttgart-Hohenheim, Guelph (Canada) and ETH Zurich (Switzerland). After four years work as consultant to the industry, he was appointed as full professor for animal breeding at the University of Goettingen, Germany, in 2001, where he is Director of the Institute of Animal Breeding and Genetics. He is chairman of the German society of animal breeding scientists and of the ISAG/FAO advisory group on farm animal genetic diversity. Prof. Simianer's scientific background is in quantitative genetics and selection theory, and he is also active in research on farm animal biodiversity and conservation genetics. He was one of the first to adopt Weitzman's diversity concept to design cost-effective conservation programs in farm animals.

Jerry Yang



(Dept. of Animal Science, Center for Regenerative Biology University of Connecticut, USA)
Speaker: Plenary Session 2

Xiangzhong Yang, known as Jerry to his friends, was born and raised in a rural Chinese village. Upon his graduation from high school, he joined the labor force in his poor village initially as a herdsman, a "bear-foot" veterinarian and later as the deputy mayor. Upon the re-instatement of the national entrance examination for college education in 1977, he was admitted to the Department of Animal Science, Beijing Agricultural University in 1978. With a prestigious national fellowship, he came to the United States in 1983 and received his MS and Ph.D. degrees at Cornell University in 1986 and 1990 respectively. Following a short postdoctoral training at Cornell University in the areas of animal biotechnology in 1991, Jerry was offered a research scientist position as a principal investigator and program director in the Cornell's Department of Animal Science. In June 1996, Jerry joined the faculty of the University of Connecticut as Associate Professor of Animal Science and Head of the Biotechnology Center's Transgenic Animal Facility. In 2000, he was promoted to the rank of full professor. In 2001, at the University of Connecticut, was appointed as the founding director of the Center for Regenerative Biology.

Luiz Lehmann Coutinho

(Dept. of Animal Science, Universidade de São Paulo, Brazil)
Speaker: Plenary Session 3

Dr. Luiz Lehmann Coutinho received a B.S. in agricultural science from the Luiz de Queiroz Superior School of Agriculture of the University of São Paulo, Brazil (ESALQ-USP) (1983), a MS (1987) and PhD (1990) at Michigan State University and post doctoral training at the University of Georgia (1991-1992) and the United States Department of Agriculture (2005-2006). He joined the staff of ESALQ-USP in 1990, where as an associate professor teaches animal nutrition, growth biology, gene expression and genomics at both undergraduate and graduate levels. As the coordinator of the Animal Biotechnology Laboratory at ESALQ-USP, has participated in several Brazilian bacterial, plant and animal genome projects, including Xylella fastidiosa, Sugarcane, Eucalyptus, coffee, chicken as well as coordinated the Bos indicus est project. The Animal Biotechnology Laboratory also has projects in collaboration with businesses such as Agroceres-PIC and Agroceres-Aviagen. Dr. Coutinho is currently coordinator of the Nucleus of Research in Cellular and Molecular Biology in Agriculture at the University of Sao Paulo, and the Collaborating Centre of Animal Genomics and Bioinformatics of the International Agency of Atomic Energy.

Martin L. Weitzman
(Department of Economics, Harvard University, USA)
Speaker: Plenary Session 1

Professor of Economics at Ernest E. Monrad - Harvard University - Cambridge, MA, USA. Received a BA in Mathematics and Physics from Swarthmore College, Stanford University, in 1963. M.S. in Statistics and Operations Research in 1964. He received his PhD in Economics from the Massachusetts Institute of Technology, in 1967. His areas of interest are Environmental and Natural Resource Economics, Green Accounting, Economics of Biodiversity, Economics of Environmental Regulation, Discounting the Distant Future, Comparative Economic Systems, Economics of Profit Sharing, Economic Planning, Micro foundations of Macro Theory, Economic Development. Among his professional activities are: Consultant to The World Bank, Stanford Research Institute, International Monetary Fund, Agency for International Development, Arthur D. Little Co., Canadian Parliamentary Committee on Employment, Icelandic Committee on Natural Resources, National Academy Panel on Integrated Environmental and Economic Accounting. Is also the Associate Editor of the Journal of Comparative Economics, Economic Letters, Journal of Japanese and International Economics, and member of the Executive Board of the Association for Comparative Economic Systems.

Olivier Hanotte

(International Livestock Research Institute, Kenya)
Speaker: Plenary Session 1

Studied zoology at the Free University of Brussels (Belgium) from 1979-1983. He obtained a PhD in 1991 from the University of Mons-Hainaut (Belgium), through a collaborative research work with the departments of zoology and genetics of the University of Leicester (UK) in 1991. He joined the International Livestock Research Institute (ILRI, Kenya), a member of the Consultative Group on International Agricultural Research (CGIAR), in 1995, where he is currently holding the position of senior scientist – project leader ''Improving Animal Genetic Resource Characterization''. For the past 10 years, his research team has been studied, using molecular markers, the origin and distribution of livestock genetic diversity from the African and the Asian continent. His research interests also includes the understanding of mechanism of adaptation in livestock, disease tolerance-resistance, with a particular on trypanosomosis in cattle, gastro-intestinal nematode parasites in small ruminants and more recently avian influenza viral infection. He is currently member of the editorial board of Animal Genetics and the Journal of Animal Breeding and Genetics.

Ruan, Yijun (Genome Institute of Singapore)
Speaker: Plenary Session 2

Associate Director
Genome Institute of Singapore
ruanyj@gis.a-star.edu.sg
Research Focus: My primary interest is to elucidate the structures and dynamics of all functional DNA elements in complex genomes through transcriptome characterizations. To facilitate such understanding we have been developing high throughput and high precision DNA sequencing and mapping methodologies in both "wet lab" and "dry lab" to characterize transcriptomes and annotate genomes. We also push to apply these sequencing-based technologies for addressing complex biological questions such as how cancer cells progress and how stem cells maintain their unique properties. Another major interest of mine is to discover previously uncharacterized viruses that are relevant to human health. To this end, we have developed a metagenome analysis capability that use shotgun DNA sequencing and genome sequence assembling techniques to uncover viral genome sequences from uncultured viral particles. We are currently trying to characterize the viral flora in human GI system. Education: 1995-1996 - Post-doctoral Fellow, Monsanto Company: 990-1994 - Doctor of philosophy in Plant Molecular Biology University of Maryland at College Park College Park, Maryland, USA: Professional Appointments: 2002 - Senior Group Leader, Genome Institute of Singapore; 2003 - Associate Director, Genome Technology; 1999-2002 - Director, Large Scale Biology Corporation, Vacaville, CA, USA; 1996-1999 - Senior Scientist, Monsanto Company, St. Louis, Missouri, USA.

Plenary Sessions

P578

The history of the molecular diversity of livestock genetic resources: overview of our current knowledge

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The last two decades have seen an explosion in our knowledge on the origin and distribution of diversity of indigenous livestock genetic resources, through the intensive use of molecular tools (mitochondrial, *Y*-specific and autosomal DNA markers), in an increasing number of livestock populations. These studies are revealing a remarkably complex picture of the origin of our domesticated species and subsequently of their colonization of the world following the expansion of farmers' societies, human migrations and trading. They are continuously challenging our assumptions on the origin, history and distribution of the today molecular diversity of farm animal genetic resources.

Mitochondrial studies have not only revealed the ancestral wild species of most of our livestock species but also that multiple domestication and/or maternal introgression is the rule not the exception. The large number of mitochondrial control region sequences now available is providing new and unique opportunities to untangle the distribution of maternal diversity and to compare the pattern of domestication and migration among our major livestock domesticates. *Y*-specific markers similarly reveal multiple male lineages and caution the extrapolation of history of livestock from single gene. Autosomal microsatellite markers applied at large geographical scale and on geo-referenced indigenous livestock populations allow the mapping of the continental distribution of livestock diversity. Ancient DNA studies unravel the local contribution of now extinct populations of the wild ancestors. Take together these molecular data indicated a distinct history at the origin of the diversity of indigenous European, Asian and African livestock.

The legacy of these molecular studies is not only altering our current archaeozoological views on the origin of livestock farmers' societies but also the design of conservation and utilisation strategies of the diversity of farm animal genetic resources.

P579

Evolution Highway: large-scale analysis of vertebrate chromosomal evolution

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Although the major divergence among mammals occurred ~65 million years ago, mammalian chromosomes contain large blocks of homologous synteny (HSBs). This observation gave rise to the question as to whether breakages in mammalian chromosomes are random after divergence from an ancestral species. Comparison of the mouse and human genomes using sparse gene mapping information appeared to support the random chromosomal breakage hypothesis proposed by Nadeau and Taylor. However, on the basis of multi-species comparison of high resolution maps and genomic sequences, 20% of all evolutionary breakpoints in mammalian genomes appear to have been "reused," and HSBs spanning up to 50 Mbp were shown to be conserved for ~100 My (Murphy et al., *Science* 309:613, 2005). A key tool used for these discoveries was *Evolution Highway*, which provides a visual means for simultaneously comparing genomes at differing levels of chromosome organization. Features of *Evolution Highway* include selection of reference genomes for comparison, the alignment of HSBs in different species, assignment of centromere and telomere positions, overlay of chromosome HSBs from reconstructed ancestral genomes, and addition of user-defined custom tracks. We have used *Evolution Highway* to identify reuse breakpoints predating the evolution of mammals and large HSBs that have been conserved for ~300 million years of vertebrate evolution. To accomplish this, a human genome sequence was compared with sequence-based and RH maps of mammals (chimp, mouse, rat, dog, cattle, pig), a bird (chicken) and fishes (tetraodon, zebra fish). Nine breakpoint positions shared between mammalian genomes and chicken were identified. In species having the ancient reuse breakpoints, gene duplications and an abundance of genes encoding zinc finger proteins were found within and near the breakpoint sites. The presence of ancient sites for recurrent chromosomal breakpoints suggests that non-random chromosome breakage has occurred for ~300 My of vertebrate evolution.

P585
Identifying genes for endoparasite resistance using physiological genomics, linkage and association studies

ALLAN M. CRAWFORD

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Nematode parasite infections of the alimentary tract are the largest, most pervasive health problem for sheep and goats produced in a grazing environment. Over the last 40 years chemical drenches have been the major therapy for control of nematode parasite infections, however, drenches are now failing. Nematode parasites resistant to all three of the main chemical families used in drench manufacture are now found in all sheep producing countries. In parts of the tropics sheep production has halted because there are now no effective therapies for *Haemonchus contortus* control(*1*).

Drench rotation strategies to slow the build up of drench resistance, the use of nematode trapping fungi(*2*), diets high in condensed tannins(*3*), and other plant materials(*4*) as well as other nutritional approaches(*5*) have all been examined as possible ways to reduce the impact of nematode parasites in sheep. One of the most promising approaches has been to breed sheep resistant to parasite infection. In Australia and New Zealand specific extension programmes have been developed to help farmers measure parasite burdens in sheep and use that information in breeding programmes (*6, 7*).

Parasite resistance is a moderately heritable trait and the benefits of measuring parasite burden in lambs and including this information in a production index for breeding is now well documented(*8*). Measuring parasite burden involves collecting faeces from each animal and, after suitable dilution of the faecal material, directly counting parasite eggs using a light microscope. This is understandably an unpopular chore with sheep breeders, made doubly so by the low repeatability of each measurement necessitating duplicate or triplicate samples having to be taken over 3 days.

The appeal of a DNA test to identify those sheep resistant to parasites is obvious, especially as in addition to saving faecal sampling it would mean animals could be tested early in life and saved from a potentially debilitating parasite challenge.
So far 5 QTL searches have been published (*9-14*). Unfortunately 2 studies did not give location information, however, the early results are not encouraging in that only QTL with quite small effects have been identified. Chromosomes implicated so far by 3 of the studies (*9-11*) are 2, 3, 6, 8, 14, and 20.

Another gene discovery option which complements the QTL approach is to examine gene expression using arrays of cDNA sequences on glass slides. All the pedigrees used in the QTL searches have been derived from crossing resistant and susceptible breeds or selection lines of sheep and then either backcrossing or outcrossing the F1 sire. The same resistant and susceptible selection lines or breeds of sheep have been used to compare gene expression. The majority of this presentation concerns what we have learnt from gene expression studies in parasite resistance and susceptible Perendale sheep(*15, 16*) and how this compares with studies in other animal systems.

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Wokshops

W580

An STR population genetic database of cat breeds for genetic individualization of domestic cat (*Felis catus*) samples

MARILYN MENOTTI-RAYMOND[1], VICTOR A. DAVID[1], BRUCE S. WEIR[2], AND STEPHEN J. O'BRIEN[1]

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A simple tandem repeat (STR) PCR-based typing system developed for the genetic individualization of domestic cat samples, has been used to generate a population genetic database of domestic cat breeds. A panel of eleven tetranucleotide STR loci and a gender identifying sequence tagged site (STS), from the domestic cat Y-chromosome SRY gene, were co-amplified in genomic DNA isolated from a sample collection of 1243 domestic cats representing 38 cat breeds. A small sample set of outbred domestic cats was included for comparison to heterozygosity and allelic profiles of the cat breeds. The STR panel exhibits relatively high heterozygosity in cat breeds, with an average 11- locus heterozygosity of 0.68 (this number represents an average of 38 breed specific heterozygosities for the 11 member panel), with a range of 0.55 - 0.84. When the entire set of breed individuals (n=1043) was analyzed as a single unit, a heterozygosity of 0.87 was observed and an average of 24.6 alleles observed per locus. An average heterozygosity of 0.85 was observed for the 11-locus set in the sample set of outbred cats. Heterozygosities observed for the independent loci, averaged across the 38 breeds, range from 0.56 (FCA441) - 0.78 (FCA723), with an average 7.4 alleles observed per locus/breed. Breed specific differences were observed with regard to locus heterozygosities, distribution of alleles, size ranges and frequency of alleles. The power for genetic individualization of domestic cat samples of the multiplex is moderate to high within cat breeds, with a probability of match (Pm) range of 2.4E-06 – 1.3E-14. The potential for genetic individualization observed in the sample set of outbred cats (n=24), which represent approximately 97% of household cats, is high with a P_m of 7.8E-13.

W581

Resequencing microarray technology for nucleotide sequence analysis of the entire mitochondrial genome.

MATTHEW C. LORENCE

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Mitochondrial genomic DNA (mtDNA) sequence information, specifically the hypervariable segments (HVI and HVII) of the control region (D-loop), has been a standard tool in the study of diverse human populations. Subsequently, sequence analysis of the D-loop has been applied to forensic identification of human hair collected from crime scenes, and trace samples collected from mass disasters, especially when evidentiary DNA is limited in quantity. Recent studies have demonstrated enhancements in resolution by analyzing additional mtDNA sequences outside of the hypervariable segments, but the effort, time, and number of amplification reactions required to perform such additional analysis using conventional DNA sequencing techniques are significant. As an alternative, resequencing microarrays allow rapid and cost-effective sequence analysis of the entire mitochondrial genome in a single experiment. The Affymetrix GeneChip® Human Mitochondrial Resequencing Array 2.0 interrogates each nucleotide position in the entire 16.5 kb mitochondrial genome on both strands, and provided call rates exceeding 98% in a study of 14 individuals of varying ethnic origins. This resequencing microarray technology could also be applied to canine mitochondrial genomes isolated from dog hair. Shed hair is an additional source of evidence present at many crime scenes as a result of either direct transfer from the dog, or secondary transfer from a person at the scene.

W582
Development of STR assays for identification and forensic testing

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Short Tandem Repeat (STR) loci, i.e. microsatellites, are a class of nuclear DNA markers consisting of tandemly repeated sequence motifs of two to seven base pairs in length. Due to numerous technical and practical reasons, microsatellites have become widely used in the fields of human and animal identification and parentage testing, as well as in human forensics. The human identification and forensic testing communities have standardized and validated a set of STR loci encompassing mainly tetranucleotide repeat markers. However, ISAG's cattle and horse standing committees have chosen sets of dinucleotide STR loci for bovine and equine testing. The majority of animal forensic casework involves canine or feline DNA. Prior to selection of canine and feline loci for forensic use, the community should now try to learn from the experiences that the human, cattle and horse testing laboratories have had over the past decade. This talk aims to review the benefits and disadvantages of tetranucleotide and dinucleotide repeat loci in forensic testing, as well as to highlight some key differences that occurred in human versus cattle and horse identification assay development.

W583
Improved Genomic Typing for Animal Identification and Breed Estimation

PETER W. KESNERS[1], GEORGE SOFRONIDIS[2], CRAIG A. MCLURE[1], BRENT J. STEWART[1], JOE F. WILLIAMSON[1], GARY S. COBON[2], ROGER L. DAWKINS[1]

[1] *C.Y. O'Connor Village Foundation, Perth, Australia*
[2] *Genetic Technologies Pty. Ltd., Melbourne, Australia*
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Issue
Currently genomic DNA typing relies predominantly on data obtained from micro satellite analysis. A single micro satellite test may not be sufficiently informative to make useful deductive inferences. Improvements can be obtained by testing more sites and as a result analytical protocols have been developed for multiplex PCR reactions involving panels of 10 or more primer pairs. Frequently one such multiplexed panel will still have to be augmented by a second panel.

Approach
By contrast, rather than to expand existing micro satellite panels, we propose a new strategy focused on a well described region of particularly high genomic polymorphism, the Major Histocompatibility Complex (MHC). Although cost prohibitive in many scenarios, molecular sequence based typing (SBT) of the MHC DR and DQ genes has revealed this extensive polymorphism in the human and other species including the dog.

Results
Using the dog as an example we demonstrate that GMT captures the information content of the canine class II polymorphism in a most effective manner. To date, testing of 330 dogs from 37 breeds has revealed 43 different amplification products. The alleles correlate with SBT haplotyping results from Cafa DRB1 DQA1 and DQB1. Furthermore, the GMT technique has demonstrated additional specificity when compared to the SBT results i.e. several individuals typed as homozygous in DRB1, DQA1 and DQB1 in SBT were shown to be heterozygous using GMT.

Conclusions
The GMT assay is highly informative and offers a number of useful features:

1. Direct display of Gene Duplication
2. More polymorphic than a micro satellite
3. Possibility of breed estimation / exclusion
4. Demonstration of ancestral haplotypes
5. Improved haplotype differentiation compared to SBT
6. Applicability to related species such as the Australian Dingo

Importantly, the GMT assay offers a clear cost advantage due to the use of a single primer pair. The reduced complexity of data simplifies the interpretation. The general applicability of the assay independent of supplier/vendor has been demonstrated on three instrumental electrophoretic platforms.

W584
Wildlife DNA forensics in the UK: a partnership approach.

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Despite being perceived as a new discipline, 'wildlife DNA forensics' has been established, and used, in the United Kingdom (UK) since the late 1980's, benefiting from early academic research in multi-locus DNA profiling. However, the association between academia and forensics was often problematic resulting in compromised evidence and inaccurate forensic interpretation. Currently, wildlife DNA forensics is carried out by non-university organizations, although the partnership approach with academics in this field is maintained through the Forensic Working Group (FWG) a sub-group of the UK government's Partnership for action Against Wildlife crime (PAW). This sub-group brings together wildlife law enforcement officials, forensic scientists and academics with the aim of not only encouraging the use of forensic science in wildlife investigations, but also advising government policy, implementing best practice and providing a central contact point for interested parties. Current wildlife DNA forensic research being undertaken under the auspices of the FWG includes the development and validation of STR profiling systems for six captive-bred raptor species and for the European badger (*Meles meles*), and work on species identification of 'wild meat' and timber species entering the country in breach of CITES legislation. Each of these projects has involved multi-agency collaboration. Such a partnership approach has proved essential to ensure correct procedures are in place for effective wildlife prosecutions, particularly when resources are scarce due to the low priority of wildlife crime.

SECTION A
Polymorphism and Biodiversity

A023

Genetic polymorphism at the Calpastatin, K-Casein, Leptin and BoLA-DRB3 loci in Iranian Sistani cattle (*Bos Indicus*)

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The genotypes for Calpastatin, K-Casein, Leptin and BoLA-DRB3 Loci were determined by polymerase chain reaction and restriction enzyme digestion method in native Iranian breed cattle, Sistani. Blood samples were collected from Sistani breeding station located in Zehak, Zabol. The extraction of genomic DNA was based on Guanidin Thiocyanate-Silica gel method. After PCR reaction, amplicons were digested with restriction enzymes. The Calpastatin locus had 3 genotypes with frequencies of 0.62, 0.29 and 0.09 for MM, MN and NN, respectively; K-Casein and Leptin had 3 genotypes with frequencies of 0.27, 0.57 and 0.16 for K-Casein, 0.77, 0.22 and 0.01 for Leptin for AA, AB and BB genotypes, respectively. For *BoLA-DRB3* we identified 19 alleles and *DRB3.2*8* had the highest allelic frequency (22.4%). One of the 19 allele had a new pattern. Average heterozygosity value for all loci was low. χ^2 test did not confirm the Hardy-Weinberg equilibrium for Calpastatin in this population. These data provide evidence that Iranian's Sistani breed have a variability, which opens interesting prospects for future selection programs, especially marker-assistant selection.

A070

Novel Y chromosomal haplotypes reveal wild and domestic sheep diversity

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The development of commercial sheep flocks is a male mediated process that to date has been measured using autosomal microsatellites or variation in the maternally inherited mitochondrion. This study presents the first analysis of both domestic and wild sheep genetic diversity by analysing molecular markers residing on the ovine Y chromosome. Analysis of the SNP oY1 revealed allele A-oY1 was present in all wild bighorn sheep (*Ovis canadensis*), two subspecies of thinhorn sheep (*O. dalli*), European Mouflon (*O. musimon*) and Barbary (*Ammontragis lervia*). A-oY1 also had the highest frequency (71.4%) within 458 domestic sheep drawn from 65 breeds sampled from Africa, Asia, Australia, the Caribbean, Europe, the Middle East and Central Asia. Sequence analysis of a second locus, microsatellite SRYM18, revealed a compound repeat array displaying fixed differences that identified bighorn and thinhorn sheep as distinct from the European Mouflon and domestic animals. Combining genotypic data resulted in the identification of 11 male specific haplotypes that represent at least two separate lineages. Investigation of the geographic distribution of each haplotype revealed one (H6) was both very common and widespread in the global sample of domestic breeds. The remaining haplotypes each displayed more restricted and informative distributions such as H5 which was likely founded following the domestication of European breeds and was used to trace the recent transportation of animals to both the Caribbean and Australia. A high rate of Y chromosomal dispersal appears to have taken place during the development of domestic sheep as only 12.9% of the total observed variation was partitioned between major geographic regions.

A088

Cost-effective parentage verification with 17plex PCR for goat and 19plex PCR for sheep

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Today microsatellite markers are well established in routine typing services as markers of choice for verifying the parentage and identification of individual animals since they are numerous, highly polymorphic and relatively easy to score. Multiplex polymerase chain reaction (MPCR) is a powerful technique typically used in genotyping applications where the simultaneous analysis of multiple markers is required. In parentage testing and individual identification easy and cost-effective MPCR is routinely used. MPCRs with at least 10 microsatellite markers were developed for cattle, horses, dogs and cats; commercial kits are also available (Applied Biosystems, Finnzymes Diagnostics). In small ruminants multiplex systems are not generally known up to now. In this study we propose a set of 17 markers for goat (BM1258; BM1329; BOBT24A; CSRD247; ETH10; HSC; ILSTS005; INRA005; INRA040; INRA063; INRA231; OarFCB20; OarFCB128; SPS113; SPS115; SRCRSP01; SRCRSP08) and a set of 19 markers for sheep (CSRD247; ETH10; HSC; ILSTS005; ILSTS011; INRA040; INRA063; INRA231; MAF65; MAF209; McM527; OarCP49; OarFCB20; OarFCB128; OarFCB304; SPS113; SPS115; TCRGC4; TCRVB6) which can be co-amplified simultaneously and meet the needs for parentage testing and individual identification. Markers were amplified using the QIAGEN Multiplex PCR Kit, PCR products were separated on a ABI PRISM® 3100. A total of 165 and 249 alleles were found in 11 goat breeds (426 samples tested) and 13 sheep breeds respectively (344 samples). Assuming one known parent the individual exclusion power ranged between 0.20 and 0.84 for the 17 markers tested in goat and between 0.48 and 0.81 for 18 of the 19 makers tested in sheep. The cumulative exclusion probability reached 0.999993 in goat and 0.999999 in sheep. The range of gene differentiation coefficients of the markers (G_{ST}) was 0.10-0.26 for goats and 0.09-0.20 for sheep. Both MPCRs may therefore also be used to distinguish between breeds isolated for a longer time and without introgression. For breeds with a recent common history or living in the same region, more markers will be necessary in order to determine breed differences.

A095

Analysis of polymorphisms at candidate genes in chicken associated to genetic resistance in avian viral diseases.

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The objective of this study is to assess polymorphisms at candidate genes associated with genetic resistance to viral diseases in chicken, such as Marek's disease and avian influenza. Two candidate genes were shortlisted from literature and their sequences obtained from Ensembl. BLBII (class II) major gene is present in chicken within the MHC region on chromosome 16. It has been shown to be associated with resistance to avian viral infection, including Marek's disease. Mx gene on chromosome 1 is a putative candidate gene for genetic resistance to avian influenza. Analysis of the ChickVD database revealed polymorphism present in both genes. Species homologs of these two genes were obtained from the NCBI BLAST database and analysed with the PAML package for detection of signature of positive adaptive selection (dN/dS ratio). For the Mx gene, in chicken, the results indicated a dN/dS ratio of 0.64: a result in agreement with high level of sequence conservation observed in this gene in both mammalian and other bird species. PCR-sequencing was carried out for both genes to detect novel SNPs and to validate the SNPs in the ChickVD database in both indigenous and commercial chicken populations. Polymorphism analysis at the BLBII gene exon 2 revealed that this gene is highly polymorphic with new polymorphisms detected in indigenous chicken populations. Analysis of exon 13 at the Mx gene revealed two SNPs, one of which is present in codon 631. This SNP is a G/A transition which leads to a non-synonymous amino-acid substitution from serine to asparagine believed to be associated with avian influenza resistance. Analysis of this exon 13 Mx SNP in different chicken populations reveals distinct allelic frequencies among the indigenous rural and commercial chicken populations examined.

A096
Characterization of miniature Ohmini pig by mitochondoria DNA sequence

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Ohmini pig has been placed on the world watch list for domestic animal diversity by the Food and Agriculture Organization of the United Nations. About 35 years ago, a pure breed of miniature Ohmini pig was established from the Chinese pigs imported from northeast of China. Recently, the near-complete mtDNA genome was reported in 17 breeds of indigenous Chinese pigs (northern China breeds, south China breeds, central China types, lower changjiang river basin breeds, southwestern China, and western plateau types). We first report a partial mtDNA sequence including 11 proteins coding genes in Ohmini pig, representing a novel type of pig mtDNA. Ohmini pig was found to be very closely related to the Chinese breeds but did not correspond to published data on 17 breeds of Chinese pigs. Except for three genes (NADH1, COI, and COIII) of 11 genes, mtDNA sequences of Ohmini pig was identical to that of Jinhua (central China) and Rongchang breeds (southwestern China). While, NADH1 of Ohmini was only correspond to that of Yimenghei (northern China) among the 17 Chinese breeds. In further analysis, the determination of the near-complete mtDNA sequence of Ohmini pig and its comparison with Chinese pigs will be not only important for understanding of genetic characterization of this breed but also genetic diversity within the mtDNA genome of Chinese pigs.

A111
The traditional mating system of the cattle in Bhutan and necessity of its genetic verification.

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Mithun (*Bos gaurus var. frontalis*) is a species of domestic bovine and its original wild species is assumed to be Gaur (*Bos gaurus*) inhabited India, Bhutan, Myanmar, Bangladesh and a part of Southeast Asia. In Bhutan, Mithun is important for re-producing milk and draft cattle and a traditional mating system is conducted with Zebu cattle (*Bos taurus indicus*). From the mating of Mithun bulls with Zebu cows, the F_1s (female: *Jatsam* and male: *Jatsa*) are produced first. The F_1 cattle are larger than their parents in body size and exert exquisite performance as milk and draft cattle, but *Jatsa* are almost infertile. *Jatsam* are crossed with Zebu bulls and then the later offspring continue to be backcrossed with Zebu bulls. After 5 backcrosses, the last female offspring recognized to be Zebu cows and crossed with Mithun bulls again. Because the purpose of this mating system is improvement of milk production through use of heterosis, it is not desired the cows, which will be crossed with Mithun bulls first, have much of Mithun genes. If this mating system is repeated thoroughly, cows in the 5th backcross generation have very little Mithun genes. However, it is not sure this backcross mating is repeated thoroughly or not. So we need to categorize individuals according as backcross generation by hearing from owners and clarify their genotype by using genetic markers. It is significant to verify that their gene constitution dose not contradict the theory, and from the correlation of genotypes and performance of milk production in each mating generation we may find a toehold to more improvement of milk production in Bhutan.

A134
Assessment of genetic diversity and structure of indigenous goats of sub-Saharan Africa.

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The rapidly growing human population in Africa has led to indiscriminate crossbreeding and replacement of indigenous goats with exotic breeds in an effort to improve productivity, hence the loss of diversity of the indigenous counterparts. This study was undertaken to assess the genetic diversity and structure of 18 goat populations from sub-Saharan Africa using 15 microsatellite DNA markers. Grison Striped goat population from Switzerland was included as a reference population. We identify a total of 154 distinct alleles with a calibrated (31 samples) mean number of alleles per locus and population ranging between 4.5 ± 2.5 (Ujiji, Tanzania) and 5.9 ± 2.5 (Ugogo, Tanzania). Observed heterozygosity values range between 0.43 ± 0.02 (Pafuri, Mozambique) and 0.59 ± 0.02 (Sebei, Uganda), while the unbiased expected heterozygosity values range between 0.49 ± 0.06 (Landim, Mozambique) and 0.59 ± 0.05 (Borno White, Nigeria). The results obtain in this study also indicate that nearly half of the goat populations deviate significantly from Hardy-Weinberg equilibrium (heterozygote deficiency). Thirteen populations had a significant inbreeding coefficient (F_{IS}) values ($P<.05$). STRUCTURE analysis, $K = 3$, clusters the populations into three distinct groups in relation to their geographic distribution. Populations from Mozambique, Mali and Nigeria in Southern and Western Africa show a high level of genetic admixture between the groups.

A151
Genetic diversity of two French dog breeds analyzed by microsatellites and pedigree data

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Beauceron and Brittany breeds are the two dog breeds of French origin with the highest population size. The Beauceron breed is a traditional shepherd dog, nowadays occasionally used as a protection dog, whereas the Brittany breed is a continental pointing dog, mainly selected for hunting purposes. In comparison with other breeds, genealogical data are very well known for both breeds, with the equivalent of about 8 generations traced. This research seeks to compare the results found about genetic variation through pedigree data and molecular methods. In each breeds, buccal or blood samples of 50 non-related dogs are used and analyzed with the 2006 ISAG panel of 21 microsatellite markers. Inbreeding rate, efficient number of ancestors and mean number of efficient alleles per locus will be then evaluated and compared between the two breeds. First results show a mean inbreeding rate of 4.5% for the Brittany breed, and of 5.4% for the Beauceron breed. For the first samples analyzed, mean number of alleles is of 3.52 for the Brittany breed. It drops down to 2.74 for the mean number of efficient alleles. Results get with the two methods will be then discussed and compared.

A161

Analysis of genetic diversity and relationships of African male sheep using two Y chromosome specific markers.

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In livestock, evolutionary or population genetics studies at the male lineage are uncommon due to lack of informative markers on the *Y* chromosome. Recently, in sheep, two *Y* chromosome specific markers have been developed. A bi-allelic A/G single nucleotide polymorphism (SNP) and a multi-allelic microsatellite *SRYM18* marker. These two markers were used to analyze the genetic diversity and population structure of 448 thin-tailed and fat-tailed African and 44 non-African male sheep from 35 distinct populations (n = 14.06 ± 6.57). A total of five microsatellite alleles (131 bp, 139 bp, 141 bp, 143 bp and 145 bp) were observed. At *SRYM18,* the highest diversity was found in two populations from West Africa, Djallonke from Senegal and Maure of Mali, each with three alleles (139 bp, 141 bp and 143 bp). All the other populations had at least two different alleles, with the exception of Gumez, Sekota and Tekur, all from Ethiopia showing only one allele (143 bp). Continent-wide, the 143 bp allele was the most common, with a frequency of 72.8%, and it was distributed in all geographical regions. The 145 bp allele was only found in the fat-tailed sheep though at low frequency (9.23%). SNP screening detected only the *A* allele. A combination of results from the SNP and the microsatellite marker confirmed the presence of three haplotypes: 141/*A*; 143/*A* and 145/*A* with a respective frequency of 16.67%, 72.5% and 10.83% in the African populations. Haplotype 143/*A* had the highest distribution in the East and Southern Africa region (90.54% and 60% respectively) while West Africa was dominated by haplotype 141/*A* (87.5%). These results are in agreement with archeological information suggesting a distinct origin for the West African thin-tailed and the East and Southern African fat-tailed sheep, respectively.

A165

Genetic variability study in 19 bovine breeds of five candidate genes related with marbling (DGAT1, TG, GH, LP and SCD).

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The use of Simple Nucleotide Polymorphisms (SNPs) in genetic characterization and Marker Assisted Selection (MAS) has increased in the last years. Marbling is one of the most important traits that influence the meat international market price; this fact makes it suitable for MAS programs. In the present study, the polymorphisms of five SNPs of candidate genes related with marbling were analyzed: Diacilglicerol aciltransferase 1 (DGAT1), tyroglobulin (TG), leptin (LP), growth hormone (GH) and stearoyl-coa desaturase (SCD). Samples from more than 400 animals of 19 Bos taurus (European and Creole) and Bos indicus breeds were collected. The DNA was analyzed by PCR-RFLP or PCR-SSCP. The results identified one or two alleles present in each studied population. Statistically significant variation was observed in the allele frequencies and expected heterocigocity between them. The distribution of these frequencies could be explained by the breeds' phylogenetic origin. DGAT1 distribution shows European and Cebuine breeds in each extreme and Creole, Retinta, Jersey and Wagyu breeds in an intermediate position. The TG genes presented a distribution with Cebuine and beef European breeds in one extreme, Jersey and Wagyu in the other, and Creole and Holstein in the middle. The extremes of GH locus distribution were occupied by Cebuine breeds, Holstein and Normande in one side and Wagyu on the other, appearing the rest of European breeds in the middle and the Creole distributed all over. The monomorphism of SCD was present in most breeds. Finally, LP locus distribution did not allow the classification of breeds in separate groups.

A181

Genetic variation of wild musk shrew (*Suncus murinus*) populations in Asia.

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The musk shrew is a small mammalian species belonging to Insectivora. Wild musk shrews are distributed mainly in tropical and subtropical area of South and Southeast Asia, and have wide distributional area as far western as Mauritius in East Africa and as far north as Japan. Although its body size, coat color and chromosome number are largely differentiated according to locality, it is possible to produce offspring from the crosses of pairs from different localities in laboratory, so this animal is classified only as one species, *Suncus murinus*. To clarify the genetic relationship among wild musk shrew populations in Asia, shrews in Nepal, Bangladesh, Bhutan, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia, Philippines, Vietnam, Cambodia and Japan were analyzed by using 8 blood proteins/enzymes dominated by 10 loci as genetic marker. From principal component analysis using allele frequency, the populations in Asia were largely divided into South and Southeast Asian group bounded by Myanmar. The degree of divergence among populations within a group was small within South Asian group and large within Southeast Asian group that was contrary to results of mitochondrial DNA restriction fragment length polymorphism analysis. Furthermore, individuals that classified as South Asian group based on mtDNA and as Southeast Asian group based on blood proteins/enzymes variants were detected in Myanmar and Malay Peninsula.

A187

Accounting for concurrence between breeds in the derivation of conservation priorities based on Weitzman's diversity concept

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Weitzman's diversity concept is widely accepted as a framework to derive optimal conservation plans in farm animal conservation. Within this framework, the so-called conservation potential of a breed is the main quantity to rank breeds according to their conservation priority. The conservation potential of breed i reflects how much expected diversity can be saved if breed i was made completely safe. However, a crucial assumption is the independence of the extinction probabilities of the considered breeds. Breeds at risk may also be in concurrence, e.g. since they compete for the same resources like food or management capacity. In this contribution, a concurrence parameter is introduced which reduces the probability that two breeds in concurrence both are maintained, while the extinction probability of each breed alone is kept constant. A rigorous mathematical model is presented which allows to study the effects of concurrence on conservation priorities, reflected by the conservation potential of a breed. This model is illustrated with a small example data set, but the methods and results easily can be generalised for problems of practicable relevance. It is shown, that concurrence strictly reduces the conservation potential of all breeds in concurrence. Within the subset of concurring breeds, the ranking of breeds w.r.t. conservation priority may change with an increasing value of λ. Under strong concurrence, the conservation potential of a breed even may become negative, meaning that by conserving this breed, expected diversity of the total set of breeds will be reduced. This model opens new options for the design of conservation programs: besides reducing extinction probabilities of breeds with the highest conservation potential, it might be an alternative to reduce the degree of concurrence, e.g. by setting up separate *in situ* conservation schemes for the breeds in concurrence. The results indicate, that in presence of strong concurrence reducing the degree of concurrence in some cases will be a more cost-efficient conservation strategy than conserving single breeds.

A191

Discovery of a SNP in exon 7 of equine *OCA2* and its exclusion as a cause for Appaloosa spotting.

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Appaloosa spotting in horses is caused by a dominant gene previously mapped to ECA1 between *ASB8* and *1CA43*. Two genes, OCA2 and TRPM1 have been identified as candidate genes using genes mapping to homologous regions in the human genome and known functions in other mammals. Both of these genes have been *FISH* mapped to the region containing the *LP* locus. A SNP detected in exon 7 of equine *OCA2* (GenBank accession # DQ454071) has been identified as a missense mutation that could be responsible for appaloosa spotting. This mutation causes an amino acid change from threonine to alanine, which is a switch from a polar amino acid to a non-polar amino acid. Secondary structure analysis predicts a change from an alpha helix to a beta-pleated sheet. Genotyping results from 54 appaloosa horses demonstrated that this SNP is not associated with appaloosa color pattern and therefore cannot be the cause for appaloosa spotting. We investigated the frequency of this allele in three horse breeds: Appaloosa, Thoroughbred, and Standardbred. The frequency of this SNP allele in Appaloosas was 0.26, 0.19 in Thoroughbreds and 0.41 in Standardbreds. So far no phenotypic differences in eye color or hair color have been found associated with this SNP

A192

Genetic variability at seven codons of the prion protein gene in nine sheep breeds of Pakistan

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The genotypes of 284 sheep from six native (Buchi, Kachi, Kajli, Lohi, Sipli, Thalli), two crossbred (Hissardale, Pak-Karakul) and the imported Awassi breeds in the Punjab province of Pakistan were determined at seven codons of the prion protein (PrP) gene. The breeds were polymorphic at codons 112 (M,T), 154 (R,H), 171 (Q,R,H) and 231 (nucleotides A or C), and monomorphic at codons 136 (A), 141 (L) and 241 (P). Six haplotypes and 18 genotypes were detected. $M_{112}A_{136}L_{141}R_{154}Q_{171}Ra_{231}P_{241,}$ which is likely the ancestral haplotype of the PrP gene, was present in all breeds, and had high frequencies in the native breeds, ranging from 0.67 in Kajli to 0.90 in Kachi. Two rare haplotypes were detected, $M_{112}R_{154}H_{171}Rc_{231}$ and $M_{112}H_{154}Q_{171}Rc_{231,}$ each of which is the result of two mutations (H_{171} or H_{154} in combination with Rc_{231}) on the ancestral haplotype background. The former was present in all nine breeds at rather high frequencies. The $M_{112}A_{136}R_{154}R_{171}Ra_{231}$ haplotype, that confers resistance to the typical scrapie agent, was absent from the Buchi breed but present in all other breeds at low frequencies. Although the V_{136} allele, which is associated with the highest susceptibility to scrapie, was not present in any of the breeds, the low frequency of the highly resistant R_{171} allele puts the native breeds of Punjab at a moderate risk of infection by typical scrapie agents. As a result, strict import regulations need to be implemented to prevent scrapie outbreaks in Pakistan.

A200

Polymorphism and diversity of bovine major histocompatibility complex (BoLA) class II *DQA1* gene in four breeds of Japan.

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The major histocompatibility complex (MHC) class II genes in cattle are located within two distinct regions of chromosome 23. Class IIb contains the genes of undefined status such as *DYA*, *DYB*, *DMA*, *DMB*, *DOB*, *DOA*, *TAP1*, *TAP2*, *LAP2* and *LMP7*, while Class IIa contains functionally expressed *DR* and *DQ* genes, is the most polymorphic region in cattle genome and is associated in various diseases and economic trait. Fourteen *DRA*, *DRB (DRB1-DRB3)*, *DQA (DQA1-DQA5)* and *DQB (DQB1-DQB5)* genes have been identified in the class IIa region. In this study, we firstly developed a new method for sequence based typing (SBT) of alleles of *BoLA-DQA1* gene. Next, we sequenced exon 2 of *DQA1* gene from 333 individuals in four different Japanese populations of cattle (82 Japanese Black, 91 Holstein, 100 Japanese Shorthorn, and 60 Jersey cattle) using a new method for SBT. We identified the 17 previously reported alleles. These alleles were 83% to 100% identical at the amino acid level to the *BoLA-DQA1* genomic clone (W1). Among the 17 alleles, 4 alleles were found in only one breed in this study. However, these specific alleles did not form specific clusters on a phylogenetic tree of 234-base pairs (bp) nucleotide sequences. Furthermore, these breeds exhibits similar variations with respect to average frequencies of nucleotides and amino acids, as well as synonymous and non-synonymous substitutions, in all pairwise comparisons of the alleles found in this study. By contrast, analysis of the frequencies of the various *BoLA-DQA1* alleles in each breed indicated that *DQA1*10011* was the most frequent allele in Holstein cattle (30.2%), *DQA1*12021* was the most frequent allele in Jersey cattle (35.0%), *DQA1*0204* was the most frequent allele in Japanese Shorthorn cattle (23.5%) and *DQA1*10011* was the most frequent allele in Japanese Black cattle (35.4%), indicating that the frequencies of alleles were differed in each breeds. In addition, a population tree based on the frequency of alleles of *BoLA-DQA1* typed in this study and *DRB3* previously determined by Takeshima *et al*. (2003) in each breed suggested that Holstein and Japanese Black cattle were the most closely to related, and that Jersey cattle were more different from both these breeds than Japanese Shorthorns.

A201

Relationship between polymorphism of BoLA class IIa region and susceptibility or resistance for development of mastitis and bovine leukemia virus (BLV)-induced lymphoma

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Bovine major histocompatibility complex (MHC) (BoLA) class IIa region revealed to associate in various diseases and economic trait. This region contains *BoLA-DRA* gene, *-DRB* genes, *-DQA* genes and *-DQB* genes and shows a high degree of polymorphism. Recently, we developed new methods for sequence based typing (SBT) which allow identification of alleles of *BoLA-DQA1* gene and *BoLA-DRB3* gene of large numbers of animals with relative case and characterized the frequencies and the phylogenetic relationship of alleles of each gene in Japanese population of cattle. In this study, we analyzed the association between BoLA class II-*DQA1* allele and *-DRB3* allele and the susceptibility or resistance for mastitis and BLV-induced lymphoma in Japan. First, we sequenced exon 2 of *DQA1* gene and *DRB3* gene from 30 of BLV-infected but healthy cattle and 23 of BLV-infected cattle with lymphoma using a new method. Typing analysis revealed that, among the 32 haplotypes, the *DRB3*0701-DQA1*0101* and *DRB3*14012-DQA1*10012* might protect against development of BLV-induced lymphoma ($p=0.035$ and $p=0.018$, respectively). Moreover, the BLV-infected cattle with two resistance haplotypes gave the low viral load. Next, we determined the nucleotide sequences of exon 2 of *DQA1* alleles of 188 cattle with mastitis and 95 normal Holstein cattle using a new method and calculated each allele frequency in two groups. The *DQA1*1201* were apparently low frequency in mastitis group as compared with a normal group ($p=0.006663$), suggesting that this allele might be positively related to resistance to mastitis. Our results demonstrate the existence of alleles linked with resistance to development of BLV-induced lymphoma or mastitis. Collectively, the breeding by the marker selection using BoLA class II haplotype might probably contribute to the improvement of disease resistance traits in domestic cattle.

A205

Discrimination of Chicken Breed by DNA Microsatellite Polymorphism

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Recently in Japan, given the public demand for high quality meat such as Japanese native fowl, Japanese Black Cattle or black pork pig, false or misleading claims have been perpetrated at various times by the retail sector. Consumer safety and peace of mind are therefore being threatened. Consequently, increased surveillance and reinforcement of standards has been promoted. We have developed a unique prefectural chicken “Hyogo Ajidori (HA)” obtained from a 3-way cross between a White Plymouth Rock (WR) and a Hyogo, itself a 2-way cross between a Satsumadori (SD) male and a Nagoya (N) female. HA has been distributed across the nation. Unique chickens are being produced across the whole country. Therefore, it has become necessary to discriminate HA from other chickens. To achieve this end, discrimination at the DNA level is indispensable, although the relevant technology has yet to be established for chickens. We have investigated the possibility of identifying chicken breeds using DNA microsatellite polymorphism. Ten blood sample were collected each from 1,049 birds and genomic DNA was isolated. Bird samples included 2 main brands of broiler (104 Chunky and 102 Cobb), 96 SD, 135 N, 110 WR, 147 Japanese large game, 97 Rhode Island Red, 103 Barred Plymouth Rock, 88 White Leghorn and 67 Silky. Seventeen highly polymorphic DNA microsatellite markers (marker) were tested : ADL0019, ADL0192, LEI0043, LEI0062, LEI0064, LEI0071, LEI0073, LEI0098, LEI0103, LEI0161, LMU0013, MCW0184, MCW0193, MCW0207, MCW0250, MCW0255 and MCW0262. The number of alleles in all markers ranged from 4 to 17 and averaged 8.1. In comparison with HA and broiler, specific alleles for broiler were observed in 9 markers. In particular, the allele frequency of the 314bp and 316 bp fragments in MCW0193 was very high. Birds with 314bp or 316 bp fragments in MCW0193 accounted for 94.2 % of Chunky and 61.8 % of Cobb birds. Birds without these two alleles were completely discriminated using the other 16 alleles of the 8 markers. All chicken breeds in this study were discriminated using the plural marker alleles.

A206

Effects of artificial selection on domestic duck biodiversity based on multi-locus analysis

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The biodiversity conservation is very important for animal sustainable development. The main objective of the present study was to evaluate the effects of artificial selection on domestic duck biodiversity using 15 microsatellite markers. A population of random-mated Peking ducks (RMPK, 31 individuals) and a line of Peking ducks selected for growth rate and other important traits for over 20 years (ASPK, 62 individuals) were used. The gene diversity (H_O), inbreeding coefficient within populations (F_{IS}), allele richness were calculated. The gene diversity decreased in the ASPK (0.399) compared with RMPK (0.433). The F_{IS} was 0.150 and 0.286 for RMPK and ASPK, respectively, indicating that artificial selection also increased inbreeding within the selection population. Some SSR allele frequencies had been changed. The F_{IS} increased for CAU089, CAU068, CAU073 and CAU004 locus while F_{IS} decreased for CAU076, CAU017, CAU041 and CAU067 in the ASPK population. The Hardy-Weinberg equilibrium (HWE) exact test evaluated by F_{IS} for ASPK population showed that CAU089, CAU068 and CAU004 were not in HWE while these loci were in HWE for RMPK population. Some locus combinations (CAUD017*CAUD044, CAUD078*CAUD035, CAUD078*CAUD041, CAUD041* CAUD050) showed linkage disequilibrium in the ASPK population while all locus combinations in the RMPK population expressed genetic linkage equilibrium after the correction for *P*-value at the 5% significance level. The genetic relationship analysis results confirmed that three clusters could be defined by STRUCTURE under the no admixture for ancestral population assumption. The cluster I included 79.4% ASPK and 10.5% RMPK ducks, cluster II owned 16.2% ASPK and cluster III comprised 89.5% RMPK and 4.4% ASPK. The selection has driven divergence between conserved and artificially selected population.

A208

Phylogenetic studies on domestic water buffaloes based on the mtDNA Cyt*b* and SRY gene sequence analysis

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The domestic water buffaloes are classified into two major groups: the swamp and river buffaloes. This study was undertaken to clarify the phylogenetic relationships among water buffalo populations; through the analysis of 640bp of mtDNA Cyt*b* (in 225 animals) and 690bp of the open reading frame of SRY gene sequences (in 28 animals). A total of 38 different haplotypes were observed from the analysis of mtDNA Cyt*b* sequences. Using these 38 haplotypes, a phylogenetic tree (based on the NJ method) and a Median Joining network were constructed. Both the NJ tree and Median Joining network clearly distinguished the swamp buffaloes from the river buffaloes. Furthermore, the swamp buffalo populations were sub-divided into the water buffaloes distributed in Southeast Asia (Swamp group 1) and those distributed only in the Indochina Peninsula (Swamp group 2). The corrected pair-wise differences, and the F_{ST} value (0.887, P<0.001) indicated a clear genetic differentiation between the two sub-groups within swamp buffaloes. Moreover, Tajima’s test indicated Swamp group 2 was at neutral equilibrium, while the Swamp group 1 and the river buffaloes were not. From the results of the SRY gene sequence analysis a single nucleotide substitution was found at position 202. This substitution is related to the amino acid substitution. Therefore, the results of SRY gene show that the swamp and river buffaloes can be classified using this substitution as observed in mtDNA Cyt*b* sequence analysis. From these results of maternal and paternal inheritance information, it is strongly suggested that the swamp and river buffaloes have been domesticated from different origins. Furthermore, it is suggested that the swamp buffaloes are derived from multiple maternal origins.

A209

mtDNA sequences shows Japanese native chickens have multiple origins

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Japanese native chickens are valuable genetic resources in Japan. Seventeen breeds are currently classified as national natural monuments. However, the genetic origin and relationships between these breeds is not clear. In this study, we analyzed the mtDNA D-loop region of Japanese native chickens to clarify their phylogenetic relationships, possible origin and their routes of introduction into Japan. Two major types of D-loop sequence were identified in Japanese native chickens. One sequence was observed in chicken that originated from China and Korea (Primitive breeds of Japanese native chicken, that is Jidori, Shokoku and related breeds). The second sequence was observed in the chicken that came from Southeast Asia (Japanese fighting cock, that is Shamo and related breeds). Earlier studies indicated that the Jidori breed was first introduced into Japan via the Korean peninsula; while Shokoku breed was continuously introduced from China. Shamo breed was on the other hand introduced from Thailand. It is believed that the majority of today's Japanese native chickens were derived from these three main breeds. These results support historic findings. However, Tosa-Jidori breed, a Jidori type of chicken has close relationships with Indonesian native chickens and was included in a group of Southeast Asian origin. While majority of the Shamo breed are derived from Southeast Asia, some of them have close relationship with Chinese native chicken; and were included in a group made up of chicken from China and Korea. The results of this study suggest that some breeds were introduced into Japan via different routes. This contradicts past hypothesis. In the Median Joining Network, the Shamo breed (not related to their geographic origin) were derived from the Jidori and Shokoku breeds.

A211

Genetic relationship among chinese domestic ducks as revealed by microsatellite analysis

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Domestic duck (*Anas platyhynchos*) is one of the most important avian species in the world. China has the largest duck population and 31% of the domestic duck breeds worldwide, representing a rich genetic resource. The objective of the present study was to investigate the genetic relationship among China domestic ducks and estimate the divergence time between different breeds using 15 microsatellite markers. Blood samples from 806 individuals representing 26 domestic duck breeds throughout the country were used. Average number of alleles varied from 4.667 (Liancheng) to 8.600 (Jingjiang). The observed heterozygosity and expected heterozygosity ranged from 0.401 (Jinding) to 0.615 (Enshi) and from 0.498 (Jinding) to 0.707 (Jingjiang), respectively. The greatest distance between domestic ducks was found between Peking (meat-type) and Jingding (egg-type) duck (D_S=0.7638; D_A=0.4739), while the smallest between Chaohu and Huainan duck (D_S=0.0120; D_A=0.0965) and Hanzhong and Putian duck (DS=0.0170; DA=0.0836). The estimates of F_{IS} for 26 duck breeds varied from 0.039 (Jianchang) to 0.333 (Chaohu), suggesting nonrandom mating within populations (P<0.001). All populations were not in HWE using the Markov chain method implemented in GENEPOP. The divergence time calculated from the microsatellite mutation rate and D_S distance ranged from 43.64 years (Hanzong and Putian) to 2777.45 years (Peking and Jinding). The smallest F_{ST} value was 0.039 (Chaohu and Huainan) while the largest was 0.342 (Peking and Jinding). The F_{ST} analysis showed that the Chaohu and Huainan duck cannot be accurately defined as two different populations (P>0.05). A neighbor-joining tree constructed based on D_A distance and the divergence time analysis showed that the some traditional defined duck breeds should be reinvestigated.

A222

Phylogenetic position of the African forest hog (*Hylochoerus meinertzhageni*) within the Suidae family

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The extant Suidae family comprises five genera: *Babyrousa* restricted to Sulawesi in Indonesia; *Sus* from Eurasia; and *Potamochoerus, Phacochoerus* and *Hylochoerus* from Africa). Our recent DNA study showed that suids from Africa and Eurasia correspond to different monophyletic lineages using the *Babyrousa* as an outgroup but the position of the genus *Hylochoerus* (forest hog) within the Suidae family could not be determined as no samples were available. This study presents update phylogenetic analyses including sequences from three mitochondrial and two nuclear DNA markers of *Hylochoerus meinertzhageni* to assess its evolutionary position within the Suidae family. Combined mitochondrial sequences show that *Hylochoerus meinertzhageni* and *Phacochoerus africanus* cluster as a sister clade of *Potamochoerus larvatus* and *Potamochoerus porcus*, which is consistent with paleontological studies. Nuclear sequences confirm that *Hylochoerus meinertzhageni* clusters within African suids but not enable clear resolution of the position of *Hylochoerus* within Suidae from Africa. Overall, the present study shows that African suids comprise two major lineages, *Hylochoerus*/*Phacochoerus* and *Potamochoerus*, and confirms a monophyletic origin of African suids.

A229

Microsatellite diversity within the SLA region of homozygous and heterozygous samples

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The DNA sequence of the entire SLA region (2.4 Mb) of the H01 haplotype was completed recently and is available as a reference for developing new genetic markers. On the basis of this H01 haplotype sequence, we previously developed 40 polymorphic microsatellite (MS) markers with an average distance of 59 kb between markers. To clarify the microsatellite diversity and recombination hot spots in the SLA region, we have now analyzed genetic polymorphisms of these markers in 23 SLA homozygous/heterozygous swine with 12 SLA serological haplotypes and 28 NIH homozygous/heterozygous miniature pigs with five SLA serological haplotypes including two recombinant haplotypes. Haplotype-specific patterns and allelic variations at MS loci were observed in the comparison of these different haplotypes. Some of the haplotypes were found to share large haplotype blocks extended over 2-Mb, suggesting the existence of strong linkage disequilibrium (LD) in the entire SLA region. For example all of the polymorphic markers across 2 Mb of the SLA genomic region were matched between the pigs with the H04 haplotype and the NIH pigs with the 'd' haplotype. On the other hand, one of the pigs with the H10 haplotype shared alleles with the NIH 'a' haplotype across 1 Mb of genomic sequence located between the class II and I regions. In contrast, none of the first 12 SLA serological haplotypes correlated with the NIH 'c' haplotype. Crossing over within the class III and I regions was found within the NIH 'g' and 'h' haplotypes, respectively. The present haplotype comparison shows that this set of MS markers provides a fast, economical and alternative method to the direct determination of the SLA alleles based on sequencing or SNP typing for the characterization of SLA haplotypes.

A238

Estimation of genetic distances from two partly overlapping microsatellite marker data sets.

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Microsatellite markers are widely used to calculate genetic distances to construct phylogenetic trees. Studies that use this method to examine the phylogeography of animal breeds usually focus on regional or continental groups or may employ non-standard marker sets. The use of commercial marker sets leads to an increasing overlap of the studies in breeds and markers. Combining these data sets thus is very challenging. A new method was developed to estimate three different genetic distances, Nei's standard distance, Cavalli-Sforza's chord distance and Reynolds' distance using partly overlapping information. To test the methods, an existing data set has been split into two sets with randomly overlapping breeds and markers. It is demonstrated, that reliable estimates can be obtained with various degrees of overlapping. Accuracy of estimated genetic distances are high for Cavalli-Cforza's chord distance (average differences < 2%, mean squared error .084 - .085) and Reynolds' distance (average differences < 3%, mean squared error .12 - .13). Estimates for Nei's distance show lower accuracy for data sets with a limited degree of overlapping. Average differences are < 10 % and MSE < .15 for reasonable number of common breeds. For data sets with low number of markers (< 6), average differences increase up to 25 % and MSE to 0.2.
It is shown, that the estimated genetic distances are reliable for a reasonable amount of overlappings and the taxonomy of phylogenetic trees drawn from these estimates are similar to trees derived from the complete data set.

A240

A mitochondrial survey of South American goat breeds reveals the existence of an ancient Canarian genetic signature

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The colonization of South America by the Spanish and Portuguese in the 16th century involved the massive transport and dissemination of livestock from these two countries to the New World, providing the genetic basis for the foundation of novel creole breeds. Ships and galleys departing from Spain usually took supplies in several Atlantic islands, such as Cape Verde and the Canary archipelagos, located in the crossroads of both continents. We were interested in investigating if this old marine practice left a traceable and long-lasting signature in the genetic background of South American goat breeds. With this aim, we have sequenced 653 bp of the mitochondrial D-loop region in 47 native creole goats from Perú, Bolivia, Chile, Cuba and México; 33 Spanish Peninsular goats (Murciano-Granadina, Malagueña and Guadarrama); 20 Canarian goats (Majorera, Tinerfeña and Palmera breeds) and 9 Portuguese goats (Bravia breed). Bayesian and median joining network phylogenetic analyses of these sequences revealed a highly significant cluster formed by Canarian, Bolivian and Chilean goats. These results cannot be explained by the recent introgression of these two creole breeds with Canarian individuals. More likely, these Bolivian and Chilean populations descend from goats brought by the Spanish colonizers when provisioning food and supplies in the Canary Islands. These results suggest that livestock inhabiting the islands that formed part of the Atlantic transoceanic route to South America, the so called *Ruta de Indias*, had a relevant role in the genesis of modern South American domestic breeds.

A241

Characterization of 220 new polymorphic tri and tetra nucleotide microsatellites localized on 18 pig chromosomes.

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An usual bottleneck in microsatellite genotyping workflow is allele calls review and allele size standardization across samples. Tandem repeats of three and four nucleotide motifs offer significant advantages at this step of genotyping procedures, compared to dinucleotide repeats, owing to the larger size difference between successive alleles, and a lower proportion of stutter bands. For these reasons, they have been favored when available, in genetic identification, linkage studies and parentage testing applications for most species, although these classes of microsatellites are not as abundant as dinucleotide repeats. Most microsatellites currently available in pig genetic maps are dinucleotide repeats. We used pig genomic sequences available (577,000 pig BAC end sequences; ftp://ftp.ensembl.org/) to search for tandem repeats of ACA, ATT, GAAA, GATA and GGAA motifs using NCBI blast software (*blastn 2.2.1*; $e < 10^{-4}$). From 5021 hits (39% GAAA, 21 %GGAA, 11% GATA, 17% ACA, 11% ATT), we designed 424 non-redundant primer pairs using *Primer 3 1.0* (Tm 60°C). Using robotic PCR setup (6µl PCR volume), touch-down PCR profile, PAGE on LI-COR automatic sequencer and pig DNA templates from 10 unrelated crossbred pigs (FH016 line, Pietrain type x FH019 line, synthetic line), we assessed PCR efficiency, specificity and microsatellite allelic diversity for each of these primer pairs. 290 primer pairs generated clean and single product matching expected size range. 220 of these 290 were found polymorphic with 2 to 7 alleles observed on setup sample (n=10; 3,57 alleles/microsatellite in average; median of 3). Genomic position on RH contig maps were inferred for 132 polymorphic microsatellites from BAC localization available using WebChrom / WebFPC databases and tools (http://www.sanger.ac.uk/projects/S_Scrofa/WebFPC/). Polymorphic tri and tetra nucleotide repeats were localized on all but one (SSC10) pig autosomes and X chromosome, with 3 to 19 polymorphic microsatellites characterized for each chromosome.

A245

Detection of polymorphisms in porcine immune genes

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Modern pork production encounters problems on economic losses by opportunistic infection because of intensive farming. To solve these problems, breeding aiming at disease resistance should be considered. Single nucleotide polymorphisms (SNPs) and other polymorphisms in immune genes may be related to responsiveness to pathogenic microbes and immune reactions after infection. We performed EST analysis using 17 full-length cDNA libraries derived from porcine tissues and cells including those closely related to infectious diseases (immune tissues, blood cells, lung and trachea). Assembling of the EST reads generated more than 2,300 SNPs in CDSs of the porcine genes, about 90% of which were expressed in the disease-related tissues and cells. The detected SNPs were stored into a database of porcine ESTs with annotation and information whether they affect coding amino acids (http://pede.dna.affrc.go.jp/). The genes possessing the SNPs included many immune genes such as cytokines and their receptors. Among the immune genes, we focused on several TLR genes and intensively investigated existence of SNPs within their CDSs in 96 pigs from 11 breeds. The result clearly indicated biased distribution and high heterozygosity of nonsynonymous SNPs in the region encoding leucine-rich repeats, particularly in *TLR1* and *TLR6*. This may affect recognition of pathogen-associated molecules, which would influence disease resistance and, thus, be important factors in the survival of different pig populations. We also explored polymorphisms of MHC (SLA) sequences, and SNPs of immune genes in untranslated regions such as introns and 3′-UTRs using the ESTs and the full-length cDNAs that we had determined. We show here our current status of accumulated polymorphisms detected in porcine immune genes, which are useful for analyses of association between particular immune genes and disease resistance in pigs. (This study is supported by the Japan Racing Association and MAFF, Japan.)

A257

A new variant of the amelogenin gene (AMG-X) in the Polish Red cattle population kept under the national preservation programme

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The bovine amelogenin gene (AMG), usually typed for sex determination, is located on both the X- and Y-chromosomes as single copies in X and Y homologous regions. AMG plays a crucial role in enamel structure and mineralization, but the function of its various domains is far to be understood. The hydrophobic, central region of exon 6 (approx. 300bp), considered as a mutation hot spot for the mammalian amelogenin, is the most variable region compared to the other exons. The aim of this work was to search for a new sequence polymorphism in exon 6 of the AMG X-linked copy. The material covered 150 cows, 12 sires and 15 young bulls of Polish Red (PR) cattle kept under the National Rare Livestock Breeds Preservation Programme. The PCR products of about 300bp length were separated on ABI PRISM 377 DNA Sequencer (AB). Typical fragment size for X-linked copy (278bp) and on Y-linked copy (215bp) was observed in all animals accept 24 cows and 2 young bulls where new variant of 269bp length was detected for X chromosome. The only possible explanation for this new phenomenon could be a mutation (deletion of 9bp) within the annealing region of the primers. The Polish Red is the only cattle endemic to Poland. It is characterised by vitality, fertility, calving ease, high calving rate, resistance to diseases and low maintenance requirements. Despite the crossing with other breeds applied in the past, the present PR material does, to a considerable degree, remain genetically distinct. Thus, it is anticipated that basing on the existing preserved population the reconstruction of a pure, or almost pure Polish Red cattle can be achieved and the DNA markers specific to PR which can be useful in restitution of pure breed population are searched.

A259
Genetic diversity in aboriginal Yakut horses

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Conservation of genetic variation of domestic animal species is recognized as a crucial concern at the international level to preserve a basis for selection. This study was undertaken to analyze the polymorphic characteristics of a wide range of genetic markers in aboriginal Yakut horses. The Yakut was developed in Yakutia by unconscious and natural selection in the harsh conditions of northern and central Siberia, Russia. In a previous study of this breed genetic diversity was measured using blood group and biochemical polymorphisms. In this study same frozen blood samples (n=31) were used to access levels of genetic diversity at 12 microsattelite DNA loci. Also, genetic variation at the mitochondrial DNA (mtDNA) was investigated. Sequencing (n=6 random samples) was performed on 421bp of the mtDNA control region, which is known to be more variable than other sequences. Three unique haplotypes were identified with total of twelve nucleotide differences. For the blood typing loci Yakut horses showed one of the lowest levels of genetic variability, however at microsatellite loci they exhibited high heterozygosity values. Phylogenetic analysis demonstrated that Yakut horses exhibited the greatest genetic similarity to the breeds from the Central Asian cluster, such as Akhal Teke, Arabian, Yabou, and Caspian Pony.

A261
Genetic variability of Bardigiano horse

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The Bardigiano horse is an Italian autochthonous population, classified in the subgroup of South European ponies and considered by some Authors as a variety of the Maremmana breed. In the early 20th century the Bardigiano breed underwent introgression from the Haflinger and its population size decreased progressively, with 5 stallions and 150 mares at the end of the 2nd World War. In the 70s a conservation programme was started, with the establishment of the Studbook in 1977. At present the breed numbers about 3,400 head, with some subjects in Germany and Hungary. The selection aims at obtaining a taller and lighter horse, suitable for saddle and light draught. Crosses with the Arabian are under evaluation. The aim of this study is to describe the genetic structure of the Bardigiano and to compare it with breeds linked to its breeding history. A total of 453 animals were analysed (Bardigiano: 103; Arabian: 155; Haflinger 104; Maremmano: 91), for eleven microsatellites (AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HMS7, HTG4, HTG7, HTG10, VHL20). A total of 94 alleles were observed, with a mean of 8.5 alleles/locus. The mean effective allele number per locus was 4.7. The mean observed and expected heterozygosity for the analysed markers were 0.69 and 0.77 respectively. The allele frequencies of the four breeds are available on request (liliana.distasio@unito.it). Compared to the other breeds, Bardigiano horse showed quite a high genetic variability, as indicated by the mean number of alleles (7.0 *vs* 6.1 ÷ 7.6) and by the observed heterozygosity (0.72 *vs* 0.66 ÷ 0.71). The F_{IS} indicated an excess of heterozygotes in Bardigiano and a deficiency in the other three breeds, but not at a significant level. The PCA analysis significantly discriminated Arabian from the other breeds (P = 0.005), but not Bardigiano from Haflinger and Maremmano. In addition, the overall F_{ST} value showed that the genetic differences among breeds accounted for 7.8% of the total variation (P = 0.001), with the pairwise F_{ST} values all significant. These results indicate that Bardigiano breed has a within and between breed variability higher than expected on the basis of its past history.

A265
Phylogenetic analysis of avian species classically-classified in order Galliformes based on whole mitochondrial genome

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Domesticated fowls such as chicken, turkey, quail, and guineafowl are classically grouped in order Galliformes comprising 283 species. Galliformes consists of 5 families. The 283 species are grouped into 75 genera constituting 5 families. The current grouping has been performed on the basis of morphological features and genomic DNA-DNA hybridization. In the present study, in order to obtain a phylogenetic relationship of avian species grouped in Galliformes based on genome sequence, we have collected blood samples from 29 individuals belonging to 23 species (14 genera in 5 families in Galliformes), and determined the nucleotide sequence of their entire mitochondrial DNAs. Then, using the concatenated amino acid sequence deduced from 12 mitochondrial genes (excluding *ND6* gene), phylogenetic analyses were performed by maximum parsimony, and Bayesian analysis methods. The analyses with the two different methods gave one and the same phylogenetic tree for the 23 species. The outline of the tree is as follows: (((((Phasianidae, Odontophoridae), Numididae), Cracidae), Megapodidae), Anseriformes). In the present study, two crucial findings for species classification have been revealed: one is that the species classically grouped in the tribes Phasianini (pheasant) and Perdicinin (partridge) were not grouped in corresponding monophyletic clusters. More precisely, species in Phasianini and species in Perdicinin formed monophyletic clusters. The other one is that *Lagopus mutus* and *Melgaris Gallopavo* are evolutionally much closer to genera *Lophura* than ever reported. These findings would contribute to species conservation and breeding of domesticated fowls.

A268
Genetic analysis of the Timor pony

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Genetic variation of 21 horses from the island of Timor, Indonesia was examined by genotyping of 15 microsatellites and mitochondrial D-loop sequencing. Heterozygosity was near the average for domestic horses as was the mean number of alleles per locus. Two different analyses of genetic relationships were done using two different sets of 12 microsatellites (nine loci were common to both analyses). The reason for the two analyses were that one group of breeds used for comparison had been tested for one set of microsatellites while another group of breeds had been typed for the set of loci. In one analysis, the Timor Pony was divergent from all breeds it was compared to. In the other, the Timor Pony paired with the Garrano, a Portugese breed. Six Timor Ponies from the 21 sampled representing the different areas of the island sampled were sequenced for mtDNA. Two different haplotypes were found. One of the haplotypes was the same as one found in the Garrano. It is likely that horses from Portugal contributed to the origin of the Timor Pony.

A276

Phylogeography of three stocks of the Amazonian fish Peacock bass (*Cichla* - Pisces) introduced in Minas Gerais State, southeast Brazil

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The transference of species between basins may bring undesirable environmental consequences, such as: genetic modification, pathogen introduction and local extinction of native species. Therefore, the study and periodic control of the impact caused by well established exotic populations on native populations are very important. Thus, one of the exotic fishes species of Minas Gerais State that needs attention is the Tucunaré (peacock bass), a piscivorous cichlid specie from the Amazon basin , that grows quickly and occupies lotic environments such as hydroelectric reservoirs and marginal lakes. Their population expanded rapid through the lakes replacing the native populations. On the other hand, they are very much appreciated in sportive fishing and they are an import resource for fisheries. In the present study, DNA sequences of the mtDNA 16S (410bp) from three stocks of peacock bass introduced and well establish in Minas Gerais at Três Marias reservoir (Morada Nova district - São Francisco River), one marginal lake of São Francisco River (São Francisco district - north of Minas Gerais) and Itumbiara reservoir (Paranaíba River – Prata drainage) were analyzed. The phylogeographic cladogram obtained from these stocks showed that all of them came from the Tocantins River, at the north region of Brazil. At Itumbiara reservoir were identified two species, *Cichla monoculus* and *C. temensis*, one at Três Marias, *C. ocellaris* and one at the São Francisco marginal lake, identified just as *Cichla sp*. The molecular data shows an unexpected result: no hybridization was detected between the two species at Itumbiara. Moreover, the observation of just one type of haplotype per site, were helpful to determinate the dynamics of the introduction, showing that these stocks were derived from one single act of introduction.

Key words: *Cichla*, mtDNA, phylogeography, fishes.

A288

Sequence and haplotype analysis of SLA genes in Korean native pigs

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Swine major histocompatibility complex (MHC) is regarded as important research subjects for overcome immunological barriers in relation to xenotransplantation. The eight SLA loci, *SLA-1, SLA-2, SLA-3, SLA-6, DRA, DRB1, DQA, DQB1* (four SLA class I genes and four SLA class II genes) were investigated in three Korean native pigs (ID numbers 1205, 1119 and 1159). At least more than three individual clones have been sequenced for SLA genotyping and the identified SNPs were confirmed by re-sequencing of different clones. Results indicate that six new alleles in Class I genes and three new alleles in Class II genes have been identified in Korean native pigs. We also identified three SLA haplotypes in this breed. The SLA alleles identified in this study will be very important clues to understand the genetic backgrounds of the Korean native pigs and for the success of xenotransplantation.

Key words: Korean native pig, Major histocompatibility complex (MHC), SLA, haplotype

A290

Assessment of genetic diversity and relationships between Hanwoo (Korean cattle) and other cattle breeds by microsatellite loci

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For the genetic assessment of the cattle breeds including Hanwoo (Korean cattle), eleven microsatellite markers were genetically characterized for 618 individuals of nineteen cattle breeds; North Eastern Asian breeds (Hanwoo, Korean Black cattle, Japanese Black cattle, Japanese Brown cattle, Yanbian cattle), Chinese yellow cattle (Luxi cattle, Nanyang cattle), European Bos taurus (Angus, Hereford, Charolais, Holstein, Limousin), African *Bos taurus* (N'Dama, Baoule), African *Bos indicus* (Kavirondo Zebu, White Fulani), Asian *Bos indicus* (Sahiwal, Nelore) and one Bali cattle, *Bos banteng* as an outbreed-reference population. Allele frequencies derived from the genotyping data were used in estimating heterozygosities, gene diversities and genetic distances. The microsatellite loci were highly polymorphic, with a total of 162 different alleles observed across all loci. Variability in allele numbers and frequencies was observed among the breeds. The average expected heterozygosity of North Eastern Asian breeds was higher than those of European and African taurines, but lower than those of Asian and African indicines. Genetic distances were estimated using Nei's DA genetic distance and the resultant DA matrix was used in the construction of the phylogenetic trees. The genetic distances between North Eastern Asian cattle breeds and *Bos indicus* were similar with those between European *Bos taurus* and *Bos indicus*, and African *Bos taurus* and *Bos indicus*, respectively. The clusters were clearly classified into North Eastern Asian, European and African taurines groups as well as different cluster with Chinese mainland breeds, firstly out-grouping with *Bos indicus*. These results suggest that Korean cattle, Hanwoo, had not been originated from a crossbred between *Bos primigenius* of Europe and *Bos indicus* of India, and North Eastern Asian *Bos taurus* may be have separate domestication from European and African *Bos taurus*.

A301

Genetic characterization of the Ventasso Horse compared with other horse breeds reared in Italy by means of microsatellites.

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The Ventasso Horse (VH) is a native Italian breed selected for saddle, and its name comes from Mount Ventasso, located in the province of Reggio Emilia. According to the FAO, the small number of the VH population of about 300 individual, place the VH in the list of endangered breeds. Molecular characterization is an essential tool to developing an effective conservation program for VH. The evaluation of the genetic structure of VH was investigated using 12 microsatellites (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, LEX03, HMS2, ASB2) including the 9 markers recommended for parentage testing by the International Society of Animal Genetics. The analyses were carried out on 118 VH individuals. Comparison with 11 other breeds reared in Italy (64 Anglo-Arab – AA, 202 Arab – AR, 46 Bardigiano - BA, 73 Haflinger - HF, 128 Italian Trotter - IT, 36 Lipizzan - LP, 50 Maremmano - MA, 42 Murgese - MU, 15 Sanfratellano - SA, 82 Rapid Heavy Draft - RD, 53 Thoroughbreed – TB) was studied. The GENEPOP and DISPAN software packages were used to calculate allele frequencies, average heterozygosity, Ht, Hs, Gst, genetic distances and phylogenetic trees. All microsatellites were polymorphic in VH and in the other breeds. A total of 124 alleles (from 6 to 19 alleles per microsatellite) were detected. Average heterozygosity was 0.745 in VH and ranged from 0.642 to 0.762 in the other breeds. Gene differentiation coefficient (Gst) had an average value of 0.097. Genetic distances were calculated using Nei's standard genetic distance (Ds) and Nei's genetic distance corrected for small samples (Da) using all markers but the sex-linked microsatellites LEX03. Phylogenetic trees constructed using Neighbour-joining method showed two clear separate clusters: the first includes the VH, AA and TB, the second contains the BA, HF and RD. This result is consistent with the studbook registrations reporting the use of AA stallions to improve VH breed in the last twenty years.

A307
Molecular assessment of genetic diversity of Zimbabwe chicken eco-types

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Indigenous chickens of Zimbabwe are reared extensively across variable farming systems and climatic factors in the agro-ecological zones of the country. Genetic diversity of these chickens was assessed using 29 microsatellite markers typed for 234 individuals randomly selected from the five eco-zones in Zimbabwe. In addition the D-loop region of mtDNA was sequenced for 53 chickens from the 5 eco-populations. Number of alleles, observed and expected heterozygosity and F statistics were computed. Nei and Reynold's genetic distances were calculated and a phylogenetic tree reconstructed using the Zimbabwe, broiler and layer commercial lines. A model based structure analysis was used to identify possible clustering in a gene pool consisting of the Zimbabwe eco-populations and commercial lines. A total of 238 alleles with an average of 8.41 (SD = 4.729) alleles per locus were observed. Gene diversity averaged 0.66 (0.02) while observed heterozygosity was 0.60 (0.01). An average inbreeding coefficient (F_{IS}) of 0.077 (SE = 0.012) was observed across the five populations. Thirteen, 11, 12, 9 and 5 loci contributed to heterozygote deficiency in eco-zone 1 – 5 respectively. Overall population variation (F_{IT}) was 0.084 (SE = 0.0129), 9% of which was due to among population variation (F_{ST}). Phylogenetic analysis indicated the Zimbabwe population clustered as one population surrounded by white and brown egg layers whilst more aligned to broiler lines. Structure analysis with K = 2 – 11 indicated the existence of the Zimbabwe population as one cluster with indistinct sub-structuring. Similar to phylogenetic results, the whole population tended to cluster with the broiler sire and dam lines at low K values. Five haplotypes one of which was common to all five eco-zones were observed. Eight individuals in eco-zone five shared a unique haplotype. Results show that the Zimbabwe chicken population that have minimum sub-structures might share a considerable part of their genome with broiler lines.

A308
Phylogenetic relationships among wild boar (Sus scrofa) populations in Italy

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In Italy, the wild boar (Sus scrofa) is distributed throughout the Apennine territories, the North-Western pre-Alpine and Alpine regions, with groups scattered in almost all the Provinces. Mainly for hunting purposes to restock local populations large numbers of Central European wild boars, and likely some wild boar x swine hybrids, are used to be released. This paper aims to analyse the phylogenetic relationships among wild boar populations in Italy and to verify the existence of some hybridization with foreign or rural pigs. The genetic variability of 59 animals hunted in the wild has been studied collecting DNA samples from 3 areas: Lombardy (Province of Bergamo), Tuscany (Province of Arezzo) and Emilia Romagna (Province of Parma). A 466bp fragment of the mtDNA control region between sites 15619 and 16085 (reference sequence #AJ002189) was sequenced. The analyses showed 13 SNPs (single nucleotide polymorphisms) and 9 different mtDNA haplotypes. A phylogenetic analysis was performed on the basis of distance matrices using the Neighbor-Joining method in PHYLIP software package, combining our 59 sequences with 53 GenBank entries from European and Asian wild boars and domestic pigs. The warthog (Phacochoerus aethiopicus) sequence was used as an outgroup and bootstrap analyses (1000 replications) were used to assess the confidence of each node. To evaluate the consistency of the Italian wild boar clustering, additional analyses were performed using maximum likelihood in TREEPUZZLE program package, in which the support values were calculated using quartet puzzling. Our results suggest that hybridizations with domestic pigs occurred in all the populations considered. These findings represent an important tool for estimating the genetic effect of introgression in the wild boar and for the development of conservation and management strategies in this species.

A313
Polymorphism and gene organization of *Bubalus bubalis MHC-DQB* show homology to *BoLA* DQB region

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Major Histocompatibility Complex (MHC) is a region of the mammalian genome containing several genes involved in the immune response, including polymorphic genes coding for proteins related to peptide presentation to lymphocytes, such as DR, DQ and DP molecules. In cattle (*Bos*), MHC is called BoLA, and there is evidence of more than 50 alleles of *BoLA-DQB*, which codes for the beta chain of a DQ molecule, distributed in at least five DQB loci. In this study, *DQB* alleles were analyzed for the water buffalo (*Bubalus bubalis*), another bovine species of economic relevance. Twelve alleles for *Bubu-DQB* were determined by nucleotide sequence analysis. The phylogenetic arrangement showed numerous transspecies polymorphisms, revealing alleles from at least three different loci homologue to *BoLA-DQB1*, *BoLA-DQB3* and *BoLA-DQB4*. The alleged loci were analyzed for synonymous (d_S) and non-synonymous (d_N) substitution patterns in peptide binding sites (PBS), indicating that *Bubu-DQB1* (d_N=19.54±5.71; d_S=1.68±1.63) is under high selective pressure for the polymorphism, similar to *BoLA-DQB1* (d_N=17.91±5.00; d_S=4.76±3.94); other *Bubu-DQB* genes did not show striking selection for the polymorphism in PBS, although d_N was always higher than d_S on those sites. In addition, *Bubu-DQB* loci showed haplotypes similar to those found for *BoLA-DQB* loci (*DQB1*; *DQB1-DQB3*; *DQB4*), except for two *Bubu-DQB3* haplotypes, which seemed to be an indication of the DQB region conservation through common ancestry between those two bovine species.

A320
Microsatellite and chromosome Y sequence analysis of wild boar and autochthonous pig breeds from Asia, Europe, South America and Africa.

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Recently, the worldwide phylogeographic analysis of wild boars and pigs provided evidence that pig domestication took place at multiple geographical locations and that, unexpectedly, modern European pig breeds do not have a Fertile Crescent origin. Since mitochondrial DNA is exclusively inherited at the maternal level, it may offer a biased perspective of the events that led to pig domestication. Therefore, we aimed to reconstruct the history of present day European pig breeds by combining the analysis of genetic variability in five genes located at chromosome Y (SscY) and in 8 autosomal microsatellite loci in European, African, Near-East and East Asian pigs and wild boars. Microsatellite analysis showed a clear split between East-Asian and European pig breeds and confirmed the lack of a genetic relationship between the later ones and Turkish and Iranian wild boars. Sequencing of 8 kb corresponding to five Y-linked genes defined the existence of 6 SNP segregating in two main SscY1 and Y2 haplotypes. Whereas haplotype Y1 had a worldwide distribution, the Y2 one was confined to Korean and Japanese wild boars, Meishan and Korean native pigs and, surprisingly, to Mukota Zimbabwean pigs. The wide geographical distribution of a single pig chromosome Y1 haplotype demonstrates that the clear-cut mitochondrial DNA divergence between European and Asian pig breeds is not paralleled at the SscY level. This result may suggest that a reduced number of males contributed genetically to domestic pig, or it might be the result of the combined action of evolutionary forces erasing genetic variation at SscY. The absence of the Y2 haplotype in European pig breeds is consistent with an exclusively maternal introgression of Chinese breeds in the 18-19th centuries, whereas in Africa both Chinese sows and boars might have intervened in the formation of local breeds.

A325
Genetic and Protein Polymorphism at CSN1S1 Locus in Two Goat Breeds

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Genetic polymorphisms of casein have received a considerable research effort for many years because of its potential effect on milk composition. Milk composition, specifically protein content, differs quantitatively and qualitatively among species and also among breeds and individuals. Sicilian goat breeds seem to be an interesting model since they have never been under a selection program, and therefore may carry unique casein polymorphisms. Moreover, the importance of goat milk in infant diets is growing probably because goat milk in some cases is less allergenic than cow milk. It is important to evaluate the genetic polymorphisms at the CSN1S1 locus in the populations of interest, like the Girgentana or Maltese goat breeds, because caseins are considered as the main cause of allergic reactions. Moreover CSN1S1 polymorphisms have been associated to different levels of this protein expression in milk. The goal of the present research was twofold 1) to genotype CSN1S1 locus, and 2) to type casein protein polymorphisms in two Sicilian breeds, Maltese and Girgentana. Blood and milk samples were obtained from 400 animals (200 of each breed) located in four different flocks. DNA was extracted from leucocytes and CSN1S1 polymorphisms were detected by PCR followed by restriction fragment length polymorphism (PCR-RFLP), and by allele specific PCR (AS-PCR). PCR and digestion products were analyzed by electrophoresis in 3% agarose gel stained with ethidium bromide after exposure to ultraviolet light. Milk samples, from the same individuals, were analysed for protein expression by isoelectrofocusing (IEF). IEF was carried out over a 2.5-6.0 pH gradient. Protein separation was performed in a polyacrylamide gel, and stained with Coomassie Brilliant Blue G250. Results showed a high percentage of A and B alleles (considered strong alleles), but also the presence of weak (F) and null alleles (N) was found in both breeds. The results may have practical implications to plan a selection program given that animals with allele A and B might be useful for cheese production since these animals express more casein than those carrying F or N alleles, and animals that do not express casein might be beneficial for hypoallergenic milk production.

A326
From man to animal, lessons from human forensic genetics: the Portuguese experience

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Human forensic genetics has reached high levels of response to the needs of justice (such as crime scene investigation and paternity investigations), namely due to advances in PCR, DNA sequencers and the exponential knowledge of the human genome. Meanwhile questions regarding standardization (of markers and protocols), quality control and bioethical concerns became key priorities. In an animal forensic genetics, different challenges are faced: the variety of genomes involved; the scattering of client expectations (which puts problems at lab resources and knowledge) and insufficient casework experience and judicial expertise. In this work we discuss the animal forensic genetics practice in Portugal, illustrated by real casework: a set of 15 cases from two different labs, one from National Institute of Legal Medicine and the other from a Biology University Centre. Six of the presented cases are stain analyses for which a pre-screening for blood is performed, followed by amplification of the Amelogenin. These tests produces X and Y chromosome-specific PCR products of different sizes and typical patterns are displayed for human, pig, dog and cat (species so far studied for this marker in our labs). Possible human source for those questioned stains is also tested by the human specific Quantifiler kit. The remaining cases represent hair samples (seven cases) for which, at present, only macroscopic and microscopic characterization is achieved and finally two illegal game cases where comparisons by microsatellite profiling were attempted.
In Portugal the emerging requirements of game management and conservation (namely the wild rabbit, red partridge, wild boar and recently red deer) and protected species (like the Iberian lynx and the Iberian wolf) place new challenge that emphasize the need for synergies. On the other hand Portuguese forensic scientists have a strong commitment and experience of harmonization and standardization at European and international level and the lessons from it could benefit the emerging field of animal forensics.

A331
Qualitative animal species identification by means of "universal vertebrate primers"

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The qualitative identification of genetic material is a frequently inquired analysis in forensic medicine and in quality control of food and feedstuff. A pair of "universal PCR primers" flanking a conserved region of ~600bp in the mitochondrial 16S rRNA gene was designed. It was applied to DNA-samples of more than 100 classified animal species, covering all vertebrate classes. So far there were all vertebrate DNAs accessible to amplification. The nucleotide sequences obtained were submitted to the GeneBank.The PCR-fragments can be analysed by sequence analysis or by multiple restriction endonuclease digest, particulary in cases of compound analyses in food and feedstuff. The method is highly sensitive and suitable for analysing even poor trace material. Samples to be analysed include blood stains, single hairs, processed food (e.g. cheese or sausage), bone, teeth, horn, feathers etc. The application potentials and limitations are discussed.

A352
Diversity and performance of a standard set of 17 microsatellites for paternity testing in Brazilian Crioulo, Campolina and Thoroughbred horses

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DNA has become the international standard for individual identification and parentage verification in horse breeds. We have optimized the analysis of 17 microsatellites for horse genetic typing. The multiplex systems include all nine recommended loci for equine typing by the International Society of Animal Genetics (ISAG) and the Brazilian Ministry of Agriculture lab accreditation system. We have typed these loci in the last two comparison tests run biannually by ISAG, allowing the construction of virtual allelic ladders for precise allelic declaration in conformity to international standards. Allele frequency data have been collected from a total of 710 Crioulo and 174 Campolina horses. In both breeds multilocus inbreeding was not significant ($p<0.01$) although a few loci departed from HWE, especially locus AHT29 suggesting null alleles causing an excess of apparent homozygotes. An average of 7.8 alleles per locus were detected in the two breeds. In Crioulo, only 22 out of 143 alleles detected at the 17 loci had frequencies above 0,25; in Campolina, 24 out of 123. These data indicate that this set of loci is highly polymorphic and powerful for individual discrimination and parentage verification in these breeds. For the large majority of markers, the power of paternity exclusion (PE) ranged between 40 and 70%. The 17 loci combined yielded a PE of 99.9996% for Crioulo and 99.994% in Campolina. Recently we have also been collecting genetic data for over 7,000 Thoroughbred (TB) horses for the Brazilian Studbook who started the migration to a DNA-only based registry system. For TB we have observed a reduced number of alleles and a significant level of inbreeding as commonly observed in such horses in other countries. To this end we have typed the Tokyo panel of 15 microsatellites for the 2005-2006 ISAG comparison test and started using it as a complementary set of markers in more complex paternity queries involving related sires or when single exclusion are observed in the routine panel.

A358
Genetic population structure of feral pigs in Eastern Australia

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Feral pigs in Australia are significant pests, they are established in around 38 percent of the country and number estimates range from 3.5 to 23.5 million. Feral pigs have a huge impact on agricultural production, biodiversity and public health in Australia. Gaining an understanding of the spatial structure of the pig populations is useful in the development of effective management strategies. In this study a molecular approach was used to investigate the population structure of feral pigs in a 500,000-km^2 area in southern Queensland and northern New South Wales, Australia. DNA was extracted from ear tissue from 306 animals killed by professional hunters and deposited at "chillers" in 15 towns spread across this region, with about 20 samples from each locality. Genotypes were determined for each of the animals using 13 highly polymorphic microsatellite markers. Data were analysed using a wide variety of different population genetics software. Using this molecular approach, we inferred five pig populations within the area. Heterozygosity (H_E=0.604) was moderate over all the populations, with low to medium levels of population differentiation (F_{ST}=0.089; R_{ST}=0.1041). The samples from each locality showed a heterozygote deficiency, which may have been due to the Wahlund effect since hunters deposited samples from a wide area at particular chillers. In areas that were known to have undergone major control efforts, there was no evidence of a reduction in the effective population size. The results indicated that the feral pigs were not in isolated closed populations and have also allowed us to determine where movement between populations are likely to occur.

A366
Detection of DNA polymorphisms using RAPD and SSCP in Korean chicken.

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Characterization of genetic polymorphisms in chicken has important implications for the design of genetic improvement and conservation programmes. Random amplified polymorphic DNA (RAPD) and single stranded conformation polymorphism (SSCP) following PCR amplification of genomic DNA were used to identify genetic polymorphisms and to assess genetic diversity in different Korean chicken populations. DNA samples of six Korean indigenous breeds (Korean black chicken, KBC; Korean white chicken, KWC; Korean red chicken, KRC; Korean yellow chicken, KYC; Korean gray chicken, KGC; Korean black-boned chicken, Ogol) and three inbred lines (Cornish, Rhode Island, White Leghorn) were amplified using RAPD primer UBC028 (University of British Columbia). PCR products were analyzed on 1.2% agarose gel and 6% polyacrylamide gel. A total of 102 specific SSCP DNA bands were purified, re-amplified, cloned and sequenced. Nucleotide sequence alignments of SSCP allele-specific patterns were classified into 36 groups. Single nucleotide polymorphisms (SNPs) were detected in 120 sequences from 15 groups. Phylogenetic tree constructed using nucleotide variations from sequenced RAPD fragments (KJ023) suggests three clusters of chickens (Ogol 3 and KGC; KYC and Ogol 1; Cornish and Rhode Island) with an estimation of 1.4% mean nucleotide divergence. This study demonstrates the usefulness of the RAPD and the SSCP methodologies for the identification of new SNPs in chicken.

A374
Generation of species specific probes by Suppression Subtraction Hybridisation method for various avian species

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Species identification is a growing field of interest for the study of prokaryotes and eukaroytes due to taxonomic, diagnostic and conservation purposes. Many molecular biology techniques have been developed so far to explore the differences between individual species. We report the use of Suppression Subtraction Hybridisation (SSH) to generate unique sequences for the identification of various avian species namely *Gallus gallus*, *Anas platyrhynchos*, *Chlamydotis undulata* and *Numida meleagris*. SSH was carried out between chicken/duck, chicken/ houbara and chicken/ guinea fowl species using each pair as tester and driver. This generated specific fragments of varying sizes. These fragments were confirmed for their specificity by using as probes for Southern blotting and *Fluorescent in situ hybridisation* (FISH). Southern blotting showed that the fragments were highly specific for the species they are generated from, with some cross hybridisation to DNA of other species but not the driver. FISH on interphase cells showed that these probes preferentially labelled the nuclei of the species they are generated from. To identify individual fragments, we cloned the generated fragments into pGEM-T vector and sequenced them. The sequences were searched for homology in chicken genome database and against other sequences deposited in NCBI using the NCBI BLAST server. Some of these sequences that do not show any homology to the chicken genome can be used for species identification in chimeras by FISH or for designing primers for PCR based species identification.

A375
Equine genotyping: minimizing the risk of parentage errors.

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Since 1st January 2005, we have increased the number of microsatellite markers that are routinely typed for each horse that is received in the laboratory. Recently, we have implemented robotic procedures for the PCR and set-up for the capillary electrophoresis.
These procedures have been implemented to improve the overall standard of equine genotyping in our laboratory.

Separately from the automation in the laboratory procedures, we have recently implemented new, automated, procedures to minimize the risk of geno-typing errors and parentage verification mistakes. We needed to further improve standards because of the increase in the number of routine samples from horses and other species as well as the growing number of cases that routinely end up in court.
Procedures supplementary to our existing systems include

- Independent, duplicate interpretation of genotypes,
- Automated comparison of the duplicate readings of genotypes,
- Addition of Quality-values to DNA-profiles,
- Automated parentage verification without manual control,
- Updated procedures for the use of control samples.

A flow diagram will be shown on the poster, in which these procedures are explained in more detail.

A378

The genetic structure of different horse breeds in the Czech Republic inferred from microsatellite markers.

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The main objective of this study was to evaluate genetic variability, parentage analysis and genetic diversity within some horse breeds in the Czech Republic. Populations of eight horse breeds were typed at 17 microsatellite loci (AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10, VHL20, HTG6, HMS2, HTG7, ASB17, ASB23, CA425, HMS1, LEX3). Breeds included were Czech Warmblood (n=100), Slovak Warmblood (n=100), Quarter Horse (n=69), Paint Horse (n=67), Shetland Pony (n=39), Haflinger (n=50), Kladruben (n=93), Arabian (n=46). The set of 564 animals was genotyped at each loci in one multiplex reaction and detected on ABI 310 DNA Sequencer. A total number of 157 distinct alleles were obtained from the whole set of animals. The number of distinct alleles at individual loci ranged from 5 (HTG7) to 16 (ASB17). Allele frequencies for the most frequently allele were as follows: AHT4 (H=0.28), HMS7 (L=0.27), HTG4 (M=0.46), VHL20 (I=0.23), AHT5 (N=0.28), ASB2 (K=0.26), ASB23 (J=0.25), HMS6 (P=0.43), HTG6 (O=0.42), ASB17 (N=0.30), CA425 (N=0.44), HMS1 (M=0.50), LEX3 (L=0.26), HMS2 (L=0.29), HMS3 (P=0.28), HTG10 (O=0.31), HTG7 (O=0.56). The highest heterozygosity (above 90%) was observed for locus HMS3 in Arabian (96%), Paint Horse (94%), Kladruben (92%), ASB17 in Haflinger (92%). The highest polymorphism information content (above 80%) was determined for locus LEX3 in Paint Horse (84%), Czech Warmblood (82%), Quarter Horse (84%), HTG10 in Paint Horse (81%), Czech Warmblood (82%), Quarter Horse (82%), VHL20 in Paint Horse (82%), Haflinger (84%), ASB2 in Paint Horse (82%), Quarter Horse (82%), ASB17 in Haflinger (84%). The average probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion estimated for this panel were 99.99%/99.98%/99.99% for the group of all animals. We evaluated genetic distance between breeds on the base of the dataset using three methods available in PowerMarker. We obtained that couples Shetland Pony-Kladruben, Shetland Pony-Haflinger are the most different from all other breeds combinations. Supported by Ministry of Agriculture of the Czech Republic (1G58073) and Czech Science Foundation (523/03/H076).

A380

Microsatellites and Y-chromosomal haplotypes of European and Middle-Eastern cattle, sheep and goats: geographic clines, clusters, male introgression and conflicting views on diversity.

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Concerns about the reduction of genetic diversity in domestic animals have motivated several studies of molecular diversity within and across breeds. However, this has not yet led to any consensus on the use of these data for support of decisions on conservation priorities. Supported by two EU projects, we have compared the microsatellite and Y-chromosomal diversity of European and Middle-Eastern breeds of cattle, sheep and goat. For all three species, regional clusters and genetic clines with a decrease in the number of alleles from South-East to North-West may reflect the Neolithic migration of livestock into Europe along different routes. Microsatellites as well Y-chromosomal haplotypes from cattle and goat show a clear geographical partitioning, indicating a geographical restriction of gene flow. The diversity pattern of cattle is proposed to be shaped also by (1) male auroch introgression in all northwestern dairy and in several northern Spanish breeds and (2) expansion of popular breeds. However, gradients of cattle genetic diversity still coincide with historic boundaries that divide the northern and southern parts of France and Germany, respectively. For the central and northwestern sheep a poor correlation between geography, genetic distances and Y-chromosomal haplotypes indicate a relatively frequent outcrossing with rams from distant regions. Despite a low number of alleles in central and northwestern Europe, breeds from this region have a higher level of distinctness than southeastern and Asian breeds. We propose that molecular diversity and breed distinctness are to be considered as separate and potentially conflicting criteria for conservation.

A384

Genetic characterization of Esperia horse breed by genetic markers.

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Conservation of genetic variability is an important objective of animal breeding. The Esperia horse is an autochthonous small population living in semi-feral conditions on the Ausoni Mountains near Frosinone (Central Italy). The morphology traits of this breed are the result of a rigid natural selection for the complete adaptation to the local environment and climate. In this study Esperia horses were analysed in order to evaluate breed genetic variability. Blood samples of 39 horses were collected and blood group markers, electrophoretic protein polymorphism markers and DNA microsatellite markers were analysed. Allele frequencies, genetic equilibrium according to Hardy-Weinberg and inbreeding coefficient ($F_{IS} \equiv f$) were estimated using GENEPOP program. The Principal Component Analysis (PCA) was performed using SAS statistical procedure. The unbiased coefficient of gene differentiation G_{ST} was computed using GENETIX software. Based on genotypes at marker loci (traditional marker and DNA microsatellite markers), individuals were clustered into a given number of populations and assigned probabilistically to a cluster using STRUCTURE genetic software. Allele frequencies were used to estimate Nei's genetic standard D distances, in order to evaluate genetic relationships among Esperia horse breed, some Italian horse breed, Akhal-Tekè and the Spanish Pure Breed. Based on genetic distances a phylogenetic dendrogram was constructed using UPGMA algorithm implemented in PHYLIP package. In Esperia sampled horses all genetic markers showed high polymorphism. The population was in genetic equilibrium according to Hardy-Weinberg and the inbreeding coefficient was close to zero. The results suggests that a good genetic variability in this breed is still conserved. Esperia horse population genetic management should be developed to avoid the risk of loss of genetic variability and consequent loss of genetic resources.

A387

A new DNA based method to assess MC1R mutations in wild boar and domestic pig

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In Italy, the wild boar is the only wild animal belonging to the *Suidae* family, where we find also its domestic homologous, the pig. Both are pertaining to the *Sus* genus and the *Sus scrofa* species. The belongings to the same species are verified from the traditional system of discrimination that defines two different species when their offspring is sterile. Such sterility does not happen in offspring, called "hybrids", derived from crossbreed between swine and wild boar, even if the chromosome number is different (38 *vs* 36). In Italy, the wild boar breeding is outside of the law, instead the breeding of hybrids is allowed.
Methods to distinguish between wild boar and domestic pig to prevent cheating are required in the interest of wholesalers, retailers and consumers. Since wild boar and domestic pig are so closely related, traditional methods and also method based on the mitochondrial DNA, do not give any clear discrimination. From several studies, a method based on the melanocortin receptor 1 (MC1R), which expressed coat colour, was used for the discrimination of the species domestic pig and wild boar. This method is also useful to distinguish hybrid animals from domestic pig and wild boar, in the first generation. The MC1R plays a central role in regulation of eumelanin and phaeomelanin synthesis within the mammalian melanocyte and is encoded by the classical *Extension* (E) coat color *locus*. Sequence analysis of MC1R from most porcine breeds revealed different allelic variants corresponding to different E alleles. After PCR with corresponding primers, we analysed two SNPs, both G/A mutations, on MC1R gene corresponding respectively to D121N and A240T codons (Kijas et al., 1998). The technique used for the SNPs discrimination, is the Minisequencing by ddNTPs single base extension (SNaPshot) realized on ABI 3100 Sequencer. Data analysis was performed using Genescan 3.7 software. We use samples from blood and muscle tissue, from wild boars, pigs and hybrids. The results of the analysis showed that wild boar samples are homozygous GG for both two mutations, meaning they were real wild boar. The hybrid samples are in part heterozygous GA and in part homozygous GG wheter for the D121N mutation, or for the A240T mutation. These results confirm the previous studies. The technique used identify clearly wild boar and domestic pig and discriminated clearly the hybrid subjects when they are heterozygous for at least one mutation described above.

A409

Prevention plan and resolution of livestock stealing cases in Buenos Aires Province, Argentina.

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Livestock robbery is a frequent illegality in Argentina that cause lost of several millions a years and is a serious risk to the public and animals health. To prevent and solve this type of illegal action, we designed and implemented the official plan "Prevention and Resolution of Livestock Stealing Cases in Buenos Aires Province". This project included different stages: (i) solving court cases using genetic information; (ii) genetic characterization of the bovine and equine population bred in the Pampa region; (iii) creation of a biological samples reservoir that could be used as reference samples in future robbery; (iv) A database with DNA sample information was created, and a sample management and analysis system is under development. Biological samples (N=227) from 84 court caseworks of livestock illegal hunting (78 cases) or robbery (4 cases) were analyzed. The remaining cases (N=2) corresponded with specific specie identification of the evidence samples. Eleven STRs were used for individual identification, while the mitochondrial cytochrome *b* was genotyped for specie identification. Match probabilities between evidence and reference samples were calculated from different cattle breed databases from the Buenos Aires Province. Because of the relatively high grade of putrefaction of the forensic biological materials, we can not obtained good quality DNA in 16 caseworks. Within the remaining samples, the number of successfully genotyping locus by sample varied from 4 to 11. In 16 caseworks, the genotypes of evidence and reference did not matched, while in 50 caseworks, the evidence profile match with the reference genotypes. In those cases, the likelihood ratio ranged from 68,000 to 1.2×10^{13}. In conclusion, herein we described a plan where DNA profiling was used as evidence for the detection of cattle and equine illegal hunting or robbery, supporting the prosecutor accusation in court.

A411

A New Commercial System Development for Ostrich (*Struthio camelus)* DNA Analysis.

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Recent interest in ratite farming, especially ostrich (*Struthio camelus*) breeds have been significantly increasing in the world, which has nearly four million commercial birds. The commercial interest in the ostrich was raised by the exploration of its feathers, nowadays, meat and leather, which are popular at international markets. The meat is red and soft, it resembles, in flavor and texture, to the best cuts of bovine meat. Presently, the genetic marker information on ostriches is very deficient; moreover, there is not an efficient system to assist in the designing of ostrich breeding programs that would be useful tools for parentage testing. Therefore, we have developed the first, the newest and the most powerful DNA test for ostriches. The "Ostrich System" are two multiplex STR systems to be used in DNA typing, including paternity testing, forensic DNA analysis and ostrich identity testing. Both of the multiplex systems allow the co-amplification and the three-color detection of eleven high polymorphic STR dinucleotide repeat *loci*, plus the female sexual marker. The first multiplex system has seven STR *loci* (**Multiplex1**: DCO1,DCO2,DCO3,DCO4,DCO5,DCO6 and DCO7) and the second multiplex system has four STR *loci* plus DCO5 against testing and female sexual markers (**Multiplex2:** DCO11,DCO12,DCO13,DCO5,DCO14 and OSTSEX). The two multiplex are separated amplified and analyzed at MegaBACE1000–GE Healthcare. The polymorphisms were detected by the genomic DNA in 143 unrelated individuals (African Black) at ostrich Brazilian farms. The total number of alleles found by the two systems was 114, which demonstrated that the DCO4 presented the lesser allele number (6), and the DCO12, the biggest allele number (16). The polymorphism information content (PIC) was PIC>50% and the probability of paternity exclusion (PE) was 0.9999. These results suggested that the eleven STR *loci* are powerful tolls to accomplish linkage analysis, to establish DNA databases for individual identification, and to perform ostrich paternity tests. Suported By: FAPESP

A414

RAPD variation in fecal and blood samples from Black and White rhinoceroses from South Africa

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A long-term goal of conservation is the retention of genetic diversity so that future adaptations, successful expansions, or re-establishment in natural populations are possible. Genetic variation within and between species can be assessed from blood and other tissue samples. The current techniques for obtaining samples from many wild animals require immobilization of the animals, which is dangerous and expensive. At the Hluhluwe-Umfolozi Reserve (HUR) in South Africa the conservation of the endangered white (Ceratotherium simum) and black (Diceros bicornis) rhinoceroses are a priority. The development of non-invasive techniques to obtain samples for genetic monitoring of these species would help. Both species practice the behavior of defecating at fixed dung heaps helping researchers obtain fecal samples for DNA extraction. Accordingly, the specific aims of this study were to (1) isolate and compare the quality of DNA extracted from blood and fecal samples of rhinos, and (2) determine if non-invasive (fecal) sampling technique provides equivalent genetic information using RAPD technique and can substitute for invasive (blood) sampling techniques. It was possible to collect samples using invasive and non-invasive techniques from rhinos at the HUR. The samples were run through RAPD protocols using 6 different primers and results indicated that (1) good quality DNA was obtained from most of the blood and fecal samples collected, and (2) blood and fecal DNA from the same animal did not reveal comparable RAPD fingerprinting patterns. The levels of RAPD diversity among and within the rhino species was then examined using only blood DNA with 5 oligonucelotide primers (Operon Primers A07, A08, Z03, B04, and B05). Preliminary results using 5 RAPD primers in 6 black and 6 white rhino samples showed that black rhinos appear to be less polymorphic than white rhinos and some RAPD bands were species-specific. These results need to be confirmed with additional samples and primers, and both feces and blood samples will be analyzed with co-dominant markers such as microsatellites.

A416

Allelic frequencies of two SNPs in the CAPN1 gene of Nellore cattle (*Bos indicus*)

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The CAPN1 gene (micromolar calcium-activated neutral protease) has been suggested as an important gene for meat tenderness in cattle. Several single nucleotide polymorphisms (SNPs) were identified in this gene but only three of them cause amino acid changes. Two SNPs, at positions 316 (exon 9) and 530 (exon 14), code for glycine/alanine and isoleucine/valine, respectively. In some beef breeds of *Bos taurus* and *Bos indicus* cattle, it was reported an association of alleles encoding glycine at position 316 and isoleucine at position 530 with decreased meat tenderness (increased shear force values) relative to the alleles encoding alanine at position 316 and valine at position 530. In this work we report analyses of these two SNPs in Nellore cattle, the biggest brazilian herd. Samples of genomic DNA were amplified in a fast and accurate strategy based on PCR amplification, using allele specific primers to amplify each SNP. A control primer was used in every reaction to amplify a 266 bp fragment. Amplification with allele specific primers for $316^{Gly/Ala}$ and $530^{Iso/Val}$ resulted in fragments of 523 bp and 387 bp, respectively. A total of 100 purebred Nellore bulls were analysed. The following allelic frequencies were found: 316^{Gly}: 1.0 and 316^{Ala}:0.0; 530^{Ile} :0.74 and 530^{Val}: 0.26. The allele 316^{Ala} , not found in our sample, has been reported in other beef breeds. This finding indicates the importance of amino acid variability at position 316 for meat tenderness in beef cattle.

A417
Microsatellite DNA polymorphism in two Zebu breeds raised in Brazil

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Nellore and Guzerat (*Bos indicus*) are two of the main Zebu breeds in Brazil. Breed improvement and selection programs require accurate pedigree information for estimating individual breeding values. Microsatellites are widely used for pedigree control and population genetics studies in domestic animals. The aim of this study was to analyse the genetic variability of ten microsatellite markers in Nellore (N=200) and Guzerat (N=150) cattle. The animals were genotyped for the following loci : TGLA227, BM2113, TGLA53, ETH225, ETH3, ETH10, BM1824, TGLA126, TGLA122 and INRA023. Amplified PCR products were separated by automated sequencer ABI 377. The number of alleles, heterozygosity, polymorphism information content (PIC) and exclusion probability (EP) were calculated. The number of alleles ranged from 6 (BM2113) to 16 (TGLA122) in Nellore, and from 6 (ETH10) to 15 (TGLA122) in Guzerat. The mean expected heterozygosity (He) and mean PIC observed in Nellore and Guzerat were He=0.679; PIC=0.640 and He=0.728; PIC:=0.695, respectively. The combined exclusion probability (CEP) was 0.9989 in Nellore and 0.9998 in Guzerat. These results demonstrate that the ten microsatellites are highly polymorphic and effective for parentage testing in these Zebu breeds.

A426
Genetic diversity between Corsican, Sardinian and Maltese breeds using microsatellite markers

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Dairy goat farming plays an important role in the Mediterranean area. It is based on local breeds well adapted to specific environments in marginal areas. Three different local breeds are present in the main Mediterranean islands: Sardinian, Corsican and Maltese in Sicily. The Sardinian population partially derives from the crossbreeding of the local type with the Maltese. It is likely that the Corsican breed had been crossed with Alpine. The aim of this study was to investigate the genetic diversity between these three populations. The genotype at 22 microsatellites, chosen from the ECONOGENE-project, was determined on 95 bucks: 35 from a selection nucleus of the Corsican breed, 11 from 5 Maltese flocks from Sardinia and 49 from 30 Sardinian flocks. First, the index of genetic differentiation (Gst), the Nei's Standard and Reynold's genetic distances were calculated. The significance level was assessed by 10,000 permutations. Second, the proportion of genome from the three populations of each animal was assessed by using the Structure software (admixture model, k=3). The percentage of animals admitted to the correct breed cluster was calculated using a genome proportion threshold of 80%. Gst was 0.069. Corsican and Maltese were always the most distant breeds while the Sardinian resulted intermediate. Both Gst and genetic distances were highly significant. Concerning the Structure results, 91% of Maltese, 80% of Corsican and 51% of Sardinian bucks fell in the correct breed cluster. 14% of Sardinian bucks were in the Maltese cluster and 35% were not assigned showing 17% of Corsican and 38% of Maltese genome on average. In conclusion, the Corsican breed was well differentiated from the Sardinian and Maltese. Moreover, the high presence of Maltese blood in the Sardinian population was showed. Further molecular researches are being conducted to highlight the genetic similarities between the Corsican and Sardinian populations and the influence of the Alpine on the Corsican breed.
This work was funded by the Community Initiative Programme INTERREG III A Italy-France, Islands "Sardinia-Corsica-Tuscany".

A433
Genetic variability of the Sardinian goat population using multilocus genotypes

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The Sardinian goat population (209.000 head) has been constituted by crossbreeding autochthonous animals with exotic improved breeds, mainly Maltese. The aim of this study was to analyse the genetic variability of this population basing on molecular data. The genotype at 22 no linked microsatellites, chosen from the ECONOGENE-Project, was determined on 330 individuals (35 flocks). First, all the individuals of one flock were assigned to Maltese (M), Crossbred (C) or Sardinian (S) groups basing on the overall morphology and the breeding history of the flocks. The index of genetic differentiation (Gst), the Nei's Standard and the Reynold's distances were calculated. The significance level was assessed by permuting only C and S flocks (10.000 permutations). Second, the proportion of genome derived from two hypothesized ancestral populations (native Sardinian and Maltese) was assessed by using the Structure software (admixture model, correlated allele frequencies and prior information for the M population). Similarly to previous approach, individuals were assigned to three groups on the basis of the genome proportion: more than 80%, between 80% and 20% and less than 20% of Maltese blood. These assignments were compared with those of the previous approach. Gst resulted 0.035. S and M groups were always the most distant and C group resulted intermediate. Both Gst and genetic distances were highly significant. The Maltese group issued from Structure included 0% of S, 13% of C and 96% of M individuals. The Sardinian group issued from Structure included 0% of M, 32% of C and 57% of S animals. No flock of S included animals assigned to the Maltese group issued from Structure results, while approximately 50% of the C flocks showed at least one animal with more than 80% of Maltese genome. In conclusion, the strategy of assigning animals to a genetic group basing on morphology of the whole flock and its breeding history was supported by the objective genetic based approaches. This strategy allowed us to clearly discriminate a group of flocks for starting a conservation program for the native Sardinian breed.
This work was supported by the Community Initiative Program INTERREG III A Italy- France, Islands "Sardinia-Corsica-Tuscany"

A435
Maternal lineages of Ventasso Horse detected by mitochondrial D-loop sequence analysis.

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The Ventasso Horse (VH) is a native Italian breed selected for saddle, and its name comes from Mount Ventasso, located in the province of Reggio Emilia. According to FAO, the small number of the VH population, of about 300 individuals, place the VH in the list of endangered breeds. During the XX Century, Maremmano and Lipizzan stallions were used to improve VH but no information about the introduction of mares is reported. The sequence analysis of hypervariable region of mitochondrial DNA D-loop is the ideal tools to detect maternal lineages present in this breed today. Samples of 46 VH individuals having different mothers were collected. Total DNA was extracted from hair roots. A 752 nt fragment of the D-loop region was amplified (nucleotides 15,339-16,090 of the reference sequence X79547). The amplified product was sequenced on both strands obtaining a 667 nt sequence (15,372-16,039). The sequences were aligned with MultAlin software. A total of 43 mutation spots, of which 20 showed high frequency, were detected. On the whole, 21 haplotypes were observed. VH sequences were compared with 480 horse mitochondrial D-loop DNA sequences retrieved from GenBank, representing a subset of 1,249 sequences found (March, 2006). Sequences longer than 300 nt where chosen. We also decided to include sequences belonging to all breeds present in the database. Furthermore, an Equus asinus sequence (accession No X97337) was included as outgroup for phylogeny studies. On the whole 99 horse breeds were compared with VH, using a sequence of 254 nt (15,487-15,740) common to all considered samples. With this shorter sequence only 14 out of 20 of the most represented mutation spots were considered. Multiple alignments and phylogeny construction were carried out with Mega 3.1 software. Dendrograms were obtained using Neighbour-Joining procedure with 1,000 bootstrap resampling. This analysis reveals a group of 19 out of 46 VH samples showing an unique haplotype not found in the other sequences considered.

A436
Analysis of bovine polymorphic markers (STR) in zebu animals from South Brazil.

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Zebu cattle (*Bos indicus*) are the widespread cattle of the world. At the moment, beef and milk cattle in Brazil is estimated to cover approximately 170 million animals 80% are zebu or crossbreed zebu animals. The aims of this study were (1) to analyze the potential application of previously defined bovine molecular markers to analyze zebuine animals, and (2) to establish preliminary frequencies of these markers in the studied population. Population consists of 65 zebus, from 4 different breeds (Gir, Tabapuã, Nelore and Brahman). From these animals, DNA was isolated from bloodspot using the DNA IQ™ SYSTEM kit (Promega®). Multiplex PCR was performed using StockMarks® kit (Applied Biosystems®), which amplify 11 different loci (BM1824, BM2113, ETH10, ETH225, ETH3, INRA023, SPS115, TGLA122, TGLA126, TGLA227 e TGLA53). Amplified fragments were analyzed by capillary electrophoresis using ABI Prism® 3100 Genetic Analyzer (Applied Biosystems®), and GeneScan® v.3.7 and Genotyper® v.2.5 (Applied Biosystems®) software. Frequencies and genetic variability of markers were calculated using Microsatellite Toolkit v.3.1, and Hardy-Weinberg equilibrium was determinate by GENEPOP v.3.4. We have demonstrated that these molecular markers included in this study can be also applied to zebuine genetic identification. We have also established the allelic frequency for the different molecular markers in four breeds. The expected heterozygosity (He) was relatively high in the analyzed population and by each breed considering a total of 100 different alleles identified in the 11 STR (Short Tandem Repeats) analyzed. We have also observed that Brahman breed presents the lowest diversity (0.60), and the Tabapuã breed the highest (0.69). Finally, we are aware that further studies are essential to confirm the reported allelic frequency due to the limited number of animals included in this sample. However, this is the first study to report the application of this specific set of bovine markers in animals of Zebu breed.

A437
Genetic characterization of Sardinian pig population using microsatellites markers

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The Sardinian pig industry is mainly based on two types of farming system: i) a sort of semi-intensive farms of small size where animals have limited access to grazing and are kept indoor only in certain periods of the year; ii) traditional extensive farms of large size where animals are kept mainly in a wild grazing system on bushland and forest. Since XIX century several improved breeds have been introduced in Sardinia either for terminal crossing or to improve the local breed by crossbreeding. However, in the extensive system farms pigs are rustic and show morphology close to the native population. The aim of this study was to analyse the genetic variability of the Sardinian pig population by microsatellite markers. 116 head (40 farms) were genotyped at 24 microsatellites from the ISAG-FAO panel. The actual (Na) and the effective (Ne) number of alleles, the expected (He) and observed (Ho) heterozygosity were calculated. The genetic structure of the population was analysed with the Structure software assuming a mixed ancestry (admixture model) and inferring from one to ten populations. Most microsatellites were highly polymorphic. Na ranged from 4 to 24 and Ne varied between 1.2 and 16.3. The lack of heterozygosity ranged from 0.03 to 0.36 (0.2 on average). The best number of populations inferred by Structure software was 8. Animals with more than 50% of genome from one population were assigned to that specific cluster (81 individuals). The cluster size ranged between 7 and 18 animals. Some particular associations between the identified clusters and the geographical location of animals were detected. On the whole, the results of the genetic analysis showed a large variability of the Sardinian pig population. This fact is confirmed by the large morphological heterogeneity due to the various and unplanned crosses realised in the past. Researches are being conducted to detect a nucleus of pigs to start a conservation programme of the native Sardinian pig. This work was supported by the Community Initiative Programme INTERREG III A Italy France, Islands "Sardinia-Corsica-Tuscany"

A440
Single nucleotide polymorphisms in myostatin (GDF8) gene in Nellore cattle (*Bos primigenius indicus*)

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Myostatin, also known as GDF8 (Growth Differentiation Factor 8), is located at chromosome 2 from bovine (BTA2) and composed by three exons and two introns. Myostatin is specifically expressed during embryo development and in the adult skeletal muscle, functioning as a negative regulatory protein. Several cattle breeds (Piedmontese, Belgian Blue, Blond'Aquitaine, among others) show polymorphisms in this gene, which are directly related with double muscling phenotype. The objective of this work was to identify potential polymorphisms in myostatin gene in Nellore cattle and compare them with others previously described in taurine and indicine breeds. Seven sub-regions, covering the three exons of this gene were PCR amplified and had their DNA sequence determined. The untranslated region (UTR) from exon 3 was included. DNA samples from thirty adult Nellore cattle were utilized, and DNA sequencing revealed 3, 7 and 4 polymorphisms in exons 1, 2 and 3 respectively. In UTR segment, 34 polymorphisms were found and at introns 1 and 2, 1 and 3 polymorphisms were found respectively. Several inedited polymorphisms were found and others, previously reported, were also found in this study. The degree of allelic heterogeneity in myostatin gene could be related to its high mutation rate. This heterogeneity could be result of a long history of artificial selection for meat production, which has probably favored such modifications and maintained them in cattle populations. The polymorphisms identified in Nellore cattle could be useful in further studies for phenotypic correlations.

A443
Mitochondrial DNA sequences reveal a putative East Asian ancestry for old Chilean chickens.

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European chickens were introduced into the American continent by the Spanish after their arrival in the 15th century. However, there is ongoing debate about whether Amerindians might already have introduced chickens into South America before the Spanish arrived, particularly in relation to stocks in Chile. The Araucana chicken breed has been suggested to be descended from such Amerindian chickens. The Araucana chickens lay blue/green shelled eggs and have been grouped into three variants: ketros (ear-tufted and normal tailed), kolloncas (non-tufted and rumpless) and kollonca de aretes (ear-tufted and rumpless). Alternatively these chickens could have been introduced from Asia via the Pacific, possibly by early Chinese or Polynesian expeditions or as a result of the early presence of Dutch corsairs and traders in the Pacific coast of South America. Another ancient Chilean breed, the Passion Fowl may also have a similar origin. This study presents phylogenetic analyses of mitochondrial control region sequences from 42 Chilean ketro, kollonca, kollonka de aretes and Passion Fowl chickens. The results show that Chilean chicken sequences cluster into clades corresponding to the West Asian and the East Asian centres of domestication. The West Asian sequence is consistent with Spanish introductions, while the sequences of East Asian origin could be explained by early pre-Columbian introductions. However, there are also suggestions that these Chilean chicken breeds may have had introgression from native Asian and modern breeds introduced since the 1930s. Definitive proof of pre-Columbian introduction will require analyses of ancient DNA from apparent chicken bones found in a pre-Columbian archaeological excavation in Chile.

A445

Haplotypes of Single Nucleotide Polymorphisms (Snps) in Casein Loci of Girgentana Goats Explain Isoelectrofocusing Results

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Goat milk, cheese and yoghurt offer an alternative to cow milk products. In Sicily one of the important goat breeds reared is the Girgentana. Traditionally the milk from Girgentana goats was used for nourishment of infants and elderly. Recently, the Girgentana breed has been in decline. This of concern, as the Girgentana may carry unique milk protein genetic variants. If health benefits of Girgentana goat milk is proven, then increasing production from this population will be of great interest. The most abundant proteins in goat milk, as in other milks, are the caseins, α_{S1}-, β-, α_{S2}- and κ-casein, coded by the loci CSN1S1, CSN2, CSN1S2 and CSN3 respectively. In other goat breeds, the casein loci have been found to be highly polymorphic, and a number of genetic variants of the casein genes that affect milk production traits have been described. The aim of this study was to quantify the variation of casein loci within the Girgentana breed, and additionally to determine if there was any agreement between DNA polymorphism and isoelectrofocusing (IEF) protein expression of α_{S1}. Forty individuals including goats and bucks were genotyped for 9 SNPs and one deletion (exon 9) in CSN1S1, 7 SNPs in CSN2, 3 SNPs in CSN1S2 and 13 SNPs in CSN3. Genotypes for the deletion in exon 9 were in very good agreement with the IEF, results; goats that were homozygous for this deletion had no or very low levels of α_{S1} expression, goats that were heterozygous had intermediate levels of expression, and goats that were homozygous for the non deletion allele had full α_{S1} expression. For each casein, haplotypes of the SNP genotypes were constructed. There were a limited number of haplotypes within each casein locus; 6 for CSN1S1 and CSN2, 3 for CSN1S2, and 7 for CSN3. The limited number of haplotypes indicates strong linkage disequilibrium between SNPs within each locus. Future work will determine if the haplotypes have significant effects on health and production traits, and their potential for use in marker assisted selection.

A458

Wide-response variation to *Babesia bovis* infection can be used to select babesiosis-resistant cattle

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Bovine babesiosis is a tick-borne disease caused by *Babesia* spp haemoprotozoans. The disease is of great importance at tick enzootic unstable areas and hampers cattle production in several developing countries. Babesiosis causes heavy losses in weight gain and abortion, it also causes cattle deaths during disease outbreaks. Immunisation alternatives such as pre-immunition and attenuated vaccines are unsafe because they use cattle blood cells as innoculum and may spread other diseases. Identifying cattle genetically resistant to *Babesia bovis* infection will allow selection for increased resistance and help in babesiosis control. The objective of this work was to measure cattle response against a challenge of *Babesia bovis* infection. One-hundred and twenty half-sib Hereford and Aberdeen Angus 12-18 months old naïve heifers, originated from a tick-free area in Southern Brazil (32ºS), were used in the experiment. All animals tested sorologically negative for *B. bovis*, *B. bigemina* and *Anaplasma marginale*. After an innoculation of $1x10^7$ *B. bovis* parasitised erithocytes, all individuals had their responses monitored. Results showed three different phenotypes: 1 - susceptible, animals with babesiosis clinical signs that had to receive treatment to avoid death; 2 - reactive, animals without babesiosis clinical signs with no need of specific babesiosis treatment, however these animals showed parasitaemia, >20% reduction in packed cell volume (PCV) and 1ºC increase in body temperature when compared to their pre-challenge physiological parameters, and 3 - resistant, animals without babesiosis clinical signs that showed parasitaemia, <20% PCV reductions, with little or no increase in body temperature and no need for babesiosis treatment. The frequencies for each phenotype were: 57.5%, 17.5%, and 25%, respectively, showing that naïve Hereford and Aberdeen Angus cattle, cattle breeds predominantly raised in Southern Brazil, may vary in genetic response to *Babesia bovis* infection. We have demonstrated for the first time that phenotypic variation for *B. bovis* does exist and these phenotypes could allow animal selection for resistant phenotypes. At the moment we are screening DNA from these animals for microsatellite markers aiming to identify molecular markers associated to these phenotypes.

A459

Identification and characterization of Endogenous Retrovirus family in Cattle.

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Endogenous retroviruses (ERV) are copies of exogenous retroviral genomes integrated into the host genomes and they are transmitted vertically to the offspring. ERV are classified into the retroviral β (B/D-type) and γ (C-type) genera and present multiple copies in all mammals. The potential application of ERV includes understanding of retroviral distribution in the genomes, genome evolution and retroviral pathogenesis. The aim of this study was to characterize the bovine endogenous retroviruses (BERV) sequences by analysis of the highly conserved retroviral *pro/pol* sequences. Here we have amplified and sequenced 108 clones of the 0.9kb conserved region of BERV using degenerate PCR. Sequence analysis revealed that 71 of the amplified clones had retroviral origin and all of them were divided into 3 distinct groups (γ4, γ9, β3) according the similarity of the nucleotide sequences (>90%). γ4, γ9 and β3 family contain 55, 1 and 15 different clones respectively. Sequence comparison between BERV and OERV families demonstrated that γ4 family of BERV had high homology with OERV γ4 family, and β3 was homologous to the OERV β3 family. In addition sequence of γ9 exhibited high homology with OERV γ9 and murine leukemia virus-related retrovirus. Hybrid BERV due to recombination events was not detected. In conclusion, we report for the first time the results from identification and characterization of the endogenous retroviruses in cattle.

A462

Comparison of microsatellite variation in Portuguese and South American horse breeds for ECA20.

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Assessing the levels of variability underlying the genetic legacy of domestic animals, such as the horse, is important to establish a conservation strategy that allows the retention of maximum genetic diversity. Variability levels at four microsatellite loci (HMS42, HTG5, LEX52 and UM11) mapping to the same chromosome as the MHC (ECA20) were surveyed in three Portuguese and six South American horse breeds considered to descend from horses of the Iberian Peninsula. Results demonstrated high levels of variation, except for the Portuguese native horse breed Sorraia. Interbreed *F*ST comparisons (*FST* > 0.110) also emphasize the high degree of isolation of this breed to the remaining ones, supporting the uniqueness of Sorraia's genetic lineage. The Ewens-Watterson homozygosity test of neutrality revealed that, for three out of the four analyzed *loci*, 11.1% of the surveyed populations rejected neutrality assumptions. This result, along with the ratios of observed to expected homozygosity (*F*obs/*F*exp) below 1 detected for these loci, suggest that balancing selection may be having extended effects over the otherwise neutral evolution of those microsatellite markers. However, the effect of other evolutionary forces cannot be disregarded.

A463
Polymorphism on the Mitochondrial D-loop and Cytochrome b Gene in Porcine

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Mitochondrial d-loop and cytochrome b gene were sequenced to investigate the difference among the four different pig breeds (Duroc, Landrace, Large Yorkshire and Korean Native). Twelve animals per breed were used to define their nucleotide sequences. Twenty four variations were found on the d-loop region and classified into 8 haplotypes (DLT1~8), and seventeen ones on the cytochrome b gene and 5 haplotypes (CBT1~5). Conjunctive haplotypes in Korean Natives were grouped into 4 kinds (DLT1-CBT1, DLT2-CBT1, DLT3-CBT1 and DLT4-CBT5), and in Large Yorkshire into 3 kinds (DLT1-CBT2, DLT1-CBT3 and DLT7-CBT4). in Duroc haplotypes were grouped into 2 kinds (DLT6-CBT4 and DLT8-CBT4), and in Landrace into 2 kinds (DLT5-CBT4 and DLT6-CBT4). Fifteen out of the 17 variations on the cytochrome b gene were deciphered as silent mutations, but 2 as missense ones. As the consequence, it was presumed that Large Yorkshire and Korean Native would take 2 types of cytocrome b enzyme that were different in the composition of amino acids.

A467
Genetic variation at 23 STRs loci in five Brazilian populations of Santa Inês hair sheep breed

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Santa Inês is a naturalized sheep breed, known to be highly adapted to the most diverse and harsh Brazilian ecosystems. As part of a concerted effort to increase its production potential through selective breeding it became imperative to adopt DNA marker typing to verify animal parentage and origin. In this study, a database of allele frequencies of Santa Inês hair sheeps at 23 STRs loci is described. The loci genotyped were INRA23, OARFCB304, MAF214, INRA63, OARHH35, INRA35, OMHC1, ILSTS87, ILSTS05, ILSTS11, MAF65, BM827, OARFCB20, OARCP20, OARAE129, INRA172, HUJ616, SRCRS05, BM6526, OARCP49, OARFCB11, D5S2 and SPSP113, 13 of which have been recommended as part of ISAG comparison tests marker panel for sheep. Data were collected from a total of 285 animals from five populations distributed along the Central-Western and North-Eastern states of Brazil. Overall, the set of microsatellites typed exhibited an average of 10 alleles, PIC value of 0.712 and average expected heterozygosity of 0.745. The cumulative probabilities of paternity exclusion, with and without one parent already known (PE1 and PE2 respectively) were 0.999999 and 0.999989. Among all 23 loci, 11 did not depart from Hardy-Weinberg Equilibrium expectations ($P \leq 0.001$) and three of the five populations studied displayed significant heterozygote deficit ($P \leq 0.001$). The database reported in this work can be directly applied for verifying parentage in sheep breed registers and analyses of genetic diversity of herds.

A474
Identification of Brown Bears (*Ursus arctos*) involved in damages in the Cantabrian Mountains.

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The population of Brown Bear (*Ursus arctos*) in the Cantabrian Mountains is distributed in two highly endangered subpopulations, the western Cantabrian subpopulation with around 100 individuals and the eastern one with 25-30. Population distribution range overlap with human settlements which in some cases lead to economical damage of human properties (attacks to crops, beehives and/or livestock). This enforce the design of a plan for bear-human conflict management within the program of conservation and recovering of the species in Spain. Genetic individualization analysis can help to understand this kind of behaviour showing whether or not there are individuals more prone than others to wander around human settlements and/or if the cubs learn this comportment form their mothers. To do this, remains left by the bear in the area of the damage such as faeces or hairs, are collected and used as starting material for DNA extraction. A set of 16 microsatellites loci, which allow the individualization in these populations, have been optimised. Loci microsatellites have been chosen from the ones available on the bibliography and then tested on individuals from both subpopulations. The more informative ones have been selected and several multiplex reactions have been developed. Genotyping was made in two steps (a first conventional PCR followed by a second PCR using an internal marked primer). Validation of the genotypes was made using a multi-tube approach. Some practical examples are shown.
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A480
Unexpected Electrophoretic Mobility Complicates Parentage Analysis By Microsatellite Genotyping

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Using differing electrophoretic mobility to identify allelic variants within a microsatellite is the basis for parentage analysis by microsatellite genotyping. The insertion or deletion of di-nucleotide bases, predominantly within the repeated region of the microsatellite, and the resultant differences in length of the subsequent PCR product, produce allelic variants (after electrophoretic separation) a minimum of one base apart. Alleles separated by a distance less than the minimum expected one base, were observed in the bovine parentage microsatellite AGLA293. These alleles were shown to be inherited. AGLA293 is a complex microsatellite consisting of adjacent di-nucleotide repeat regions. Sequencing of these non-conforming alleles indicated that the unexpected mobility was due to same size fragments with different relative proportions of these adjacent repeat regions.

A481

Genome-wide SNP analyses of Holstein Friesian cattle reveal new insights into Australian and global population variability.

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Past breeding strategies for dairy cattle have been very effective in producing rapid genetic gain to achieve industry targets and raise profitability. Such gains have been largely facilitated by intense selection of sires combined with the use of artificial insemination. However, this practice can potentially limit the level of genetic diversity through inbreeding and selection plateaus. The rate of inbreeding in Australia has approximately tripled from 1979 to 1999, primarily as a result of semen importation from a small number of prominent bulls from the USA. The effect of this genetic influx in the Australian dairy cattle population is poorly understood both in terms of diversity and local adaptation / divergence. This study uses 845 genome-wide SNP genetic markers and 431 bulls to characterize the level of genetic diversity and genetic divergence within the Australian and international Holstein Friesian (HF) dairy population. These 431 bulls represent an average of 23% genetic contribution to the population, and hence represent a significant proportion of the total genetic diversity. It is the first published comprehensive analysis of a livestock species with genome-wide SNP loci investigating the effects of intense selection, international importation and inbreeding over time in Australia. No significant differences in genetic diversity (*Ho* and *A*) were observed over a 25 year time frame (1975 to 1999) for HF bulls used in Australia. The impact of semen importation from the USA had the effect of increasing *Ne* (77 to 125) until the Australian population was in effect a sub-sample of the global population. Our data suggests that most HF individuals are equally closely related to one another, regardless of country of origin and year of birth, with the global net genetic difference (1-PSA) of only 0.004. In effect, the global population can be considered as one single population unit. These results indicate that inbreeding, genetic drift and selection has had little effect at reducing genetic diversity and differentiating the Australian HF population at a genome-wide level.

A482

Genetic basis of the Australian merino.

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The Australian Merino is an economically important breed which is likely a hybrid of European, Asian and African origin. Individuals from European Merino (n = 18), the Indian Garole (n = 18), African Namaqua Afrikaner (n = 14), Ronderib Afrikaner (n = 15) and the Macarthur Merino (n = 78) were each collected based on anecdotal evidence or breeding records suggesting a role in the development of the modern Australian Merino (n = 82). Sequence analysis of 1060 bp of the mitochondrial DNA control region revealed haplotypes belonging to two of the three known ovine maternal lineages (clades A and B). Shared haplotypes were identified between a number of population combinations including Macarthur Merinos, the Garole and commercial animals. Examination of two Y chromosomal markers revealed all male Macarthur Merinos (n = 36) and commercial Merinos tested (n = 24) shared a single haplotype (H6) while higher diversity was observed within male European Merinos (n = 18) and rams from Africa (n = 25). The findings provide insight into the foundation and genetic basis of the Australia Merino and suggest it is a deep hybrid intercross which likely carries genomic components derived from independent domestication events.

A494

Polymorphisms within the cattle myostatin gene

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Myostatin (MSTN), a gene known to affect muscle growth in cattle, is located at 0 cM on BTA2 and is an obvious candidate gene for retail beef yield. The MSTN gene (promoter, 5' and 3' untranslated regions, exons, splice junctions and most introns) was sequenced for the 6 Limousin-Jersey sires. A number of polymorphisms were found, including several previously undescribed. Ten of these polymorphisms were within introns and 2 polymorphisms were within exons. The only potentially functional polymorphism was a C to A transversion at nt282, which substitutes a leucine for phenylanine94 in the inhibitory domain of the myostatin protein. The frequency of the "A" allele in the Australian Limousin, Belgian Blue, Angus, Hereford and Jersey populations was estimated to be 83%, 3%, 0.6%, 0% and 0%, respectively. Thus, the results indicate the A-allele is present in the Australian Angus and Belgian Blue, although the frequency of the A-allele in these breeds is much less than Limousin. The polymorphism is being investigated for any association with beef yield.

A497

Use of SNP markers for individual identification and genetic traceability in the pig based on the SNPlex™ Genotyping System

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Traceability of meat has become an important issue for consumers. Different markers such as microsatellites and single nucleotide polymorphisms (SNPs) are being used to obtain DNA fingerprinting. SNPs are biallelic markers and their information content is consequently lower than microsatellites although, as a result of their abundance in the genome, reduced genotyping costs and simplicity of analysis, they represent an alternative of interest for individual identification. Therefore, their lower informativity can be compensated by using a higher number of markers. In the present work, we have selected a panel of 46 SNPs whose segregation had previously been described within different pig populations. A genotyping assay was performed on individuals belonging to commercial populations from 6 pig purebred lines (Duroc, Iberian, Landrace, Large White, Piétrain, and Meishan) and European wild boar samples using the SNPlex Genotyping System based on the oligonucleotide ligation/PCR assay. Considering the genotype frequencies of SNPs for each population, we estimated the probability that two unrelated individuals selected at random would share identical multilocus genotypes (probability of identity, P_I). In our study, P_I values ranged from 5.12×10^{-9} in the Piétrain population to 7.93×10^{-4} in the Meishan population (included in the analysis by the unusually high inbreeding level of the samples). In order to increase power for individual identification in populations with a high consanguinity level a second set of 48 SNPs is currently under validation. These two panels of 46 and 48 SNPs, respectively, complemented with the SNPlex™ methodology of genotyping will allow us to analyze a large number of pig samples for traceability in less time and at lower cost than microsatellites. Additional breeds and populations are actually under analysis in order to validate the universality of the designed panels.

A506

Genetics characterization of testudo populations for reintroduction purposes into the wild in Spain

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Tortoises (*Testudinidae*) are a clade of terrestrial turtles which face serious threats to survival throughout their distribution range. Among all *Testudo* specimens, only *T. graeca* and *T. hermanni* can be considered as native inhabitants of Spain. Both species are included in the list of species protected by CITES Appendix II besides to be protected at European level by a still more restrictive trade regulation (CE 1332/2005). However, the number of wild specimens is steadily diminishing due to illegal capture and uncontrolled release of exotic specimens which entail risk of genetic contamination and disease transmission. The main goal of the present study is to analyse the genetic variation between *T. graeca* and *T. hermanni* with the aim to reintroduce captive pure specimens into the wild. We have addressed this issue by sequencing two mitochondrial DNA regions (12s rRNA and cytocrome b) and by genotyping seventeen nuclear microsatellite loci in 98 samples of different origins: 48 *T. graeca* and 50 *T. hermanni*. Two specimens of *Agryonemis horsfieldii* were used as an outgroup. We have characterized four new haplotypes of the 12s rRNA and seven of the cytochrome b gene. The joint analysis of both mitochondrial genes allowed us to identify 16 different haplotypes, of which 10 were exclusively found in *T. graeca* specimens and 6 in *T. hermanni*. Microsatellite markers were highly polymorphic (13.76 alleles per locus as an average) and informative (the mean number of non-shared alleles was 8.94). In summary, clear differences between *T. graeca* and *T. hermanni* have been found using either mitochondrial DNA and microsatellite markers. This study substantiates the use of molecular markers for the genetic management of populations of these two closely related *Testudo* species in Spain.

A511

Isolation and characterization of the bovine microsatellite loci

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Isolation of microsatellite loci was conducted with five repetitive probes for Korean native cattle. Eleven microsatellite loci were developed based on a biotin hybrid capture method, and the enrichment of the genomic libraries (AAAT, TG, AG, T and TGC repeats) was performed using Sau 3AI adapters. The isolated markers were tested in two half-sib Korean cattle families, and 4 imported breeds such as Angus, Limousine, Holstein, and Shorthorn. Nine informative microsatellite loci were observed, and two microsatellite loci were revealed monomorphic loci in Korean cattle breed. However, all of the markers were informative in imported breeds. In total, 213 alleles were obtained at the 11 loci across 5 breeds, and the average number of alleles found by locus, considering all populations, was 4.26. The expected and observed heterozygosities were 0.71 and 0.57, respectively. The ranges of the polymorphic information content for the markers in all cattle populations were 0.43 to 0.69. Total 11% of genetic variation contributed to the differentiation between populations by the mean F_{ST} values, the remaining 89% corresponding to differences among individuals. The isolated markers may be used to share in identification and classifying the local breeds based on molecular classification.

A515

In silico discovery, mapping, and genotyping of 1,039 cattle SNPs on a panel of eighteen breeds

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To contribute to cattle haplotype map construction and the fine mapping of quantitative trait loci, we discovered ~3,000 putative single nucleotide polymorphisms (SNPs) by comparison of repeat-masked BAC-end sequences (BESs) from the cattle RPCI-42 BAC library with the cattle whole-genome shotgun (WGS) contigs (Btau_1.0). For the sequence alignment, the TimeLogic tera-BLASTn was used with minimum e-value of E-50. Alignments were parsed for identification of putative SNP positions. For SNP-containing contigs, COMPASS, an *in silico* comparative mapping tool, was used to assign SNPs to positions on the cattle chromosomes. In total, 2,914 SNPs were assigned to cattle chromosomes, with 1 SNPs every ~1.2 Mbp on average. Genotyping a subset of these SNPs was performed using the Illumina platform and a DNA panel containing 186 samples from 18 cattle breeds, including 43 trios. Of 1,039 SNPs confirmed as polymorphic in the panel, 998 had minor allele frequency $\geq .25$ in individuals of at least one breed. Frequency of scorable genotypes was 95.5% and heritability in sample trios was 99.5%, thus demonstrating high accuracy of the genotyping. We reanalyzed the SNPs against the second build of the cattle genome (Btau_2.0) and found 1,034/1,039 sequences flanking the SNPs that were initially identified in Btau_1.0 WGS contigs. Of these, 932 SNP positions had nucleotide bases identical to bases found in Btau_1.0 contigs, whereas 102 bases were changed, resulting in the loss of the *in silico* SNP. As these SNPs were all directly confirmed by genotyping on the multi-breed panel, it is likely that the original polymorphisms were correctly identified and that the animal sequenced was heterozygous at these sites, but the base was changed in Btau_2.0 due to the process used for assembling the contigs. All genotyped SNPs were submitted to the NCBI dbSNP database and will become a part of bovine SNP panel to be used by the Bovine Genome Sequencing Consortium and the International Bovine HapMap Consortium.

A517

Microsatellite variability of chamois subspecies *cartusiana*, *tatrica* and *ornata* and evolutionary position within species phylogeny

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The genus *Rupicapra* is occupying most of the high to medium altitude ranges across Southern Eurasia, from the Caucasus to the Cantabrian Mountains. Populations occupying each mountain range have been recognised as subspecies and grouped into two species: *R. pyrenaica*, with the subspecies *parva*, *pyrenaica* and *ornata*, and *R. rupicapra*, with the subspecies *cartusiana*, *rupicapra*, *tatrica*, *carpatica*, *balcanica*, *asiatica* and *caucasica*. Most of the chamois populations are medium to large in size and represent a cinegetic recourse but populations from the Apennines (*ornata*), the massif of Chartreuse (*cartusiana*), the Tatra (*tatrica*) and the Balkans (*balcanica*) have been drastically reduced in size during the last century and are object of conservation plans. The evolutionary relationships among several populations of the two species were analysed in a previous study on the basis of variation at 20 microsatellite loci. Here we include the subspecies *cartusiana* as well as additional samples of *ornata* and *tatrica* (previously only represented by 1 and 2 individuals, respectively). The subspecies *cartusiana* and *tatrica* show diversities of 40% and 32%, respectively, in the lowest range of the values. Both group within the species *rupicapra*, being *cartusiana* very close to the alpine chamois. The population of the Apennines show the extremely low expected heterozygosity of 3%, possibly the lowest diversity reported in the literature for microsatellites in a population of non-selfing diploid organisms. Six out of 12 individuals were homozygous for the 20 loci and only 3 loci presented more than one allele. This low heterozygosity for the Apennines population is a reflection of its recent past with two extreme bottlenecks during the last century. Phylogenetic analysis places the *ornata* subspecies in the middle between the two recognised species as a third phylogenetic group clearly differentiated from the *rupicapra* and *pyrenaica* clades.

A527

Analysis of genetic variability related to microsatellite markers IDVGA-51, LEP-2, NRDIKM009, GHRm e BMS1216 in beef cattle.

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Genetic variability related to five microsatellite markers (IDVGA-51, LEP-2, NRDIKM009, GHRm and BMS1216) was investigated in 240 beef cattle individuals from ten different breeds raised commercially in Brazil, being four *Bos taurus* (Angus, Limousin, Simmental and Braunvieh), four *Bos indicus* (Nelore, Guzera, Tabapuã and Brahman) and two composite breeds (Canchim and Montana). PCR analysis was followed by capillary electrophoresis using fluorescent markers. Heterozygosity (H) and polymorphism information content (PIC) were calculated and compared among these breeds, generating allelic and genotypic frequencies for each marker. The average heterozigozity was higher than 0,60, except for the marker GHRm (0,23) which was the only showing PIC value lower than 0,55 (0,20). The most informative marker was the BMS1216 with average H of 0,73 and PIC of 0,72. The genetic distance was determined using Nei´s proposed method (1972). These markers were able to differentiate the main genetic groups clustering breeds with similar origin. Among *Bos taurus* breeds, Angus was the one showing higher genetic distance to indicine breeds and the Guzera has the higher value (F_{st}=0,4121). The analysis also shown that among indicus breeds, Nelore and Guzera were the closest (F_{st} =0,0192) and among taurine breeds the Simmental were equally closer to Braunvieh and Limousin (F_{st} =0,0455). The coefficient of genetic distance found to Canchim and Montana breeds had intermediate values when compared to indicine and taurine pure breeds, confirming the composite origin of those breeds. The present data has indicated the viability of the use of those markers to access the genetic variability and distance between those breed populations.

A528

SNP analysis in fatty acid synthase and adiponectin genes in Italian pig breeds.

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We investigated polymorphisms in pig Fatty Acid Synthase (*FASN*) and Adiponectin (*ADN* or *ACDC*, Adipocyte C1q and collagene-domain-like) genes, candidates for meat and carcass quality, analysing DNA samples of main and local pig breeds reared in Italy. FASN is an enzyme that plays a central role in fatty acid biosynthesis catalysing the production of long-chain saturated fatty acids. *FASN* gene has been assigned to SSC 12p1.5 and a T>C polymorphism in the fourth exon was found at position 265 of sequence AY183428. Adiponectin is a fat derived hormone involved in insulin sensitivity, in lipid and glucose metabolism. The gene was mapped on SSC 13 at 53.6 cM, in a region containing a QTL for intramuscolar fat. In this gene a G>A missense mutation within the 60th codon, determining a Val-Ile substitution in the protein, has been reported in literature. The T>C polymorphisms of *FASN*, and the G>A mutation of *ADN* genes were studied by PCR-RFLP. All analysed breeds showed the polymorphisms, except for Hampshire samples that presented only the G allele at *AND* locus. In *FASN* locus the allele C was the most frequent in the analysed breeds and its value was higher than 0.80 in Duroc, Belgian Landrace, Pietrain and Cinta Senese samples.Two groups of Italian Large White pigs with extreme divergent genetic index (50 highest and 50 lowest) for backfat thickness were genotyped for *FASN* polymorphism. Significant differences of allele frequencies between the groups were found (P=0.016). In *ADN* locus G allele was the most frequent in the examined breeds with values ranging from 0.85 (Duroc) to 0.99 (Italian Large White). The sequencing of PCR products obtained using three different primer pairs allowed to determine the sequence of part of intron 1, exon 2, intron 2 and almost complete encoding region of 3th and last exon of *AND* gene. Additional SNPs were detected in the introns of the gene by sequencing the DNA of pigs of six different breeds.

A535

Genetic structure of hair sheep breeds in Brazil by microsatellites markers

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The objective of this work was to characterize the genetic structure within and among ten naturalized and commercial sheep breeds in Brazil, using 19 microsatellite loci. The analyses included 383 individuals using data from loci INRA23, OarFCB304, MAF214, INRA63, OarHH35, INRA35, OMHC1, OarFCB48, ILSTS87, ILSTS05, ILSTS11, MAF65, BM827, OarfCB20, OarAE129, INRA05, INRA172, HUJ616, SRCRS05, and BM62526. The AMOVA analysis indicated that 11.76% ($p<0.001$) of total variation occurred among breeds. A Bayesian analysis conducted with the Structure software (1,000 iterations for a tested K value) suggested that only the naturalized breeds Santa Inês and Morada Nova showed significant substructuring. In the Santa Inês breed, samples from DF, GO and SE states (central-western and northeastern Brazil) clustered separately from the samples from MA and CE states (northeastern Brazil). This pattern is consistent with the "Old" vs. "New" Santa Inês breeds. In the Morada Nova, substructuring occurred between the red and the white varieties of fur color. Since the two populations effective numbers of Morada Nova herds are low, this pattern may be explained by directional selection and random loss of alleles. These results allowed for a better understanding of the distribution of genetic variability among Brazilian sheep and for its application on management and conservation programs of naturalized sheep in Brazil.

A536

Molecular and evolutionary study of genetic variability in Equids DRA gene.

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The α chain of most classical Class II genes at the Mayor Histocompatibility Complex are essentially monomorphic in mammals. Several reports indicate that the DRA locus is moderately polymorphic in equids, where eleven DRA exón 2 alleles were reported, four have been found widely distributed among horse breeds and others are almost exclusive in a particular *Equidae* species. To analyze the genetic variation of the ELA-DRA locus, we screened polymorphism in different domestic horse breeds by PCR-SSCP and DNA sequencing in 121 horses of the Argentine Creole, Thoroughbred, Peruvian Paso, Arab, Spanish and Silla Argentino breeds. In addition, to evaluate the evolutionary pattern of this gene, we compared DRA exón 2 nucleotide sequences from several species of different mammalian orders. The obtained sequences and those retrieved from the database were analyzed by using the MEGA program. For the evolutionary purpose, DRA exón 2 sequences from horses and other mammalian species were analyzed for amino acid sequence variability and the pattern of synonymous and non synonymous substitutions was compared and phylogenetic trees were constructed. Three alleles were detected in the analyzed sample which corresponded to DRA*0101, 0201 and 0301 horse alleles. Allele profiles were significantly different among breeds, while estimated heterozigosity ranged from 0.90 to 0.50. Sequence analysis of the exón 2 showed that equid species presented six amino acid residues that are exclusive for this order. In addition, amino acidic variability among orders was concentrated into 6 well defined amino acidic motifs distributed trough all exón 2 sequence. As expected, this region exhibited high ratios of nonsynonymous to synonymous substitutions in the Antigen Binding Sites (ABS), but in equine species the non synonymous substitutions were only present in ABS sites. Phylogenetic analysis showed that DRA sequences were grouped in agreement with their evolutionary origin. The present study could contribute to increased our knowledge of the patterns of molecular evolution that determine the unusual ELA-DRA polymorphism.

A539

Genetic characterization of 6 Portuguese goat populations using 25 microsatellite loci reveals reduced levels of breed differentiation

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This work studies the genetic relationships between goat populations from Portugal (6 populations, 193 animals) using 25 microsatellites. Concerning this objective, characterization of the genetic structure of Portuguese goats and the study of their relationships was performed, through different specific statistical methods. Besides the five Portuguese goat breeds (*Bravia*, *Serrana*, *Charnequeira*, *Serpentina* and *Algarvia*), this study involved a population not officially recognized: *Bravia do Montesinho* (BM). Twenty two of the 25 markers used, detected high levels of polymorphism (global expected heterozygosity of 0.754; mean number of alleles 7.5) witch make them very useful in goat genetic characterization studies. Besides the high within breed genetic variability, a reduced level of differentiation between breeds was detected (0.031 and 0.039 for the F_{ST} and G_{ST} values, respectively), in agreement with other studies developed with goats, namely from the Iberian Peninsula. In general, the results of the phylogenetic analysis agree with the geographical distribution of the Portuguese goat populations, with a higher genetic distance observed between the Bravia and Algarvia breeds. Algarvia and Serpentina breeds were always in the same independent cluster, in all of the Neighbor-joining tree representations. Although Bravia do Montesinho population shows some differentiation from the other breeds, both through genetic distance and Factorial Correspondence Analysis, the genetic analysis revealed a close relationship to the Serrana breed. It was also possible to assign half of the individuals (BM) to its population of origin. These results emphasize the importance of combining this information with that obtained from other types of molecular markers as well as morphological data, attempting to identify animals with the more indicative genotypes among the original population.

A540

Characterization of a highly informative genome-wide bovine SNP assay.

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Advances in genomic resources and tools have aided in SNP identification, RH and, LD map construction, and QTL studies. Allele frequencies of a SNP, within a given population will be a key factor determining its usefulness as a marker. We have examined the performance and minor allele frequencies (MAF) of 6738 SNPs in 464 *bos taurus* cattle. These steers comprised 28 families which were produced from mating Angus (10), Charolais (4), and University of Alberta hybrid bulls (14) with University of Alberta hybrid dams. In addition the map locations for 5036 SNPs were determined using the Roslin-Cambridge 3000 Rad bovine hamster whole genome radiation hybrid panel. Position and allele frequency information was used to develop two optimized multiplex assays containing 3072 SNPs, which span the entire genome with an average MAF of 0.29. The optimized assays were then used to examine allele frequencies in eight breeds of cattle (Angus (A), Brahman (B), Charolais (C), Dutch Black and White (DBW), Holstein (H), Japanese Black (JB), Limousin (L), Nelore (NEL)). The number of SNPs with MAFs above 0.2 ranged from 67% in Angus to 25% in Nelore. The ratio of polymorphic SNPs ranged from 97.0% in Angus to 68.3% in Nelore. In addition we examined the polymorphic information content (PIC) of the two optimized multiplex assays. The resulting high density SNP set is highly informative with known map locations, making the optimized SNPs powerful tools for building LD maps and for QTL studies.

A543

SNP and insertions/deletions in LEI0258 microsatellite marker further define MHC haplotypes in the chicken

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The microsatellite marker LEI0258 has been shown to be useful in determining MHC haplotype in chickens. Sequence information was obtained for the regions defined by this marker for 51 serologically defined haplotypes. The cause of the large size difference in alleles of this marker is primarily due to changes in the number of two internal tandem repeats, with repeat numbers ranging from 1 to 28 for the first repeat and 3-20 for the second repeat. This results in allele sizes ranging from 182-552 bp. In addition, four insertion/deletion polymorphisms and 5 SNP were also detected in the sequences flanking the two repeats. These variations in sequence of LEI0258 allows for
further distinction of MHC haplotypes.

A547

Detection of single nucleotide polymorphisms (SNPs) in bovine immune-response candidate genes for mediating resistance to infestations with the cattle tick, *Rhipicephalus* (*Boophilus) microplus*.

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Ticks are hematophagous arthropods that cause serious losses in animal productivity and health, especially in tropical environments. Alternatives to acaricides, the current method of control, are needed because of the resulting contamination of the environment and food products, and loss of effectiveness ensuing from development of resistance by the ticks. Bovine hosts express breed-specific, heritable, contrasting phenotypes (susceptible: S or resistant: R) when exposed to larvae of *Rhipicephalus microplus*. *Bos indicus* breeds are significantly more resistant than *Bos taurus* breeds, while animals in segregating populations derived from crosses between these groups show varying levels of resistance. This suggests a polygenic basis for the trait and offers an opportunity to identify specific genes/mutations associated with tick resistance. In addition, different breeds develop specific, albeit qualitatively different, immune responses against ticks, indicating that many of genes involved in conferring resistance to ticks may code for molecules of the immune system. We looked for breed-specific SNPs by comparing genomic sequences from Nelore (N = 16) and Holstein (N = 16) for candidate genes (TGF-β, IFN-γ, IP-10, TNF-α and MIP-1α) we had previously determined to be differentially expressed in the tick-infested skin of resistant hosts. Genomic DNA from each animal was PCR-amplified with specific primers designed to generate an amplicon anchored on two exons and spanning at least one intron. The PCR fragments were purified with ExoSapit™ and sequenced in an ABI 3100 DNA analyzer. A total of 26 SNPs were found- at least five SNPs per gene and several alleles were found to be breed-specific. The results provide information that will allow for association studies in composite/segregating populations resulting from crosses of *B. taurus* and *B. indicus* breeds to ascertain if these markers are associated with causal mutations which confer tick resistance to *B. indicus* cattle.

A549
Genetic diversity in Nellore, a Zebu cattle breed, using ten ISAG/FAO recommended DNA microsatellites

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Nellore cattle is the main commercial breed in Brazil and was formed as a result of continued importings from Indian breeds, specially Ongole, being submitted to extensive selection and artificial insemination. A lack of studies at genetic diversity within this breed using standardized genetic markers is observed. We genotyped a set of ten ISAG/FAO microsatellites (ETH225, ETH10, ETH3, BM1824, BM2113, TGLA53, TGLA122, TGLA126, SPS115, INRA23) in four multiplex PCR for semi-automated fluorescence genotyping. All microsatellites were polymorphic, with a number of alleles between 4 (ETH10) and 13 (TGLA53), with an average of 8.6 alleles per locus. Allele frequencies, effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and polymorphism information content (PIC), as well as Hardy-Weinberg genetic equilibrium (including heterozygotes deficit test) were calculated for each locus. Mean Ho was 0.51 (ranging from 0.22 for ETH10 to 0.92 for TGLA122), lower than expected at nine of the loci. PIC values exceeded 0.5 at all loci (mean of 0.68). A significant deficit (<0.01) of heterozygotes was observed for the global test (all loci) and for six loci. This study contributes to the knowledge of the genetic characteristics of the cattle breeds raised in Brazil, and indicates that reduced levels of heterozygosity may be a result of the intensive selection and inbreeding, as applied in this animal. Granted by: CNPq, SECTAM, FINEP.

A550
Bovine haemoglobin polymorphisms and allelic frequencies in Bonsmara cattle in Brazil

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The first reports on bovine haemoglobin electrophoresis were made towards 1955. At present, alleles A (slower) and B (faster) are the most frequent. Less frequent alleles are C, F, Killary, D, G e I. Bovine haemoglobin alleles vary accross different breeds. The current work aims to evaluate bovine haemoglobin polymorphisms and allelic frequencies in Bonsmara cattle, recently introduced in Brazil. 137 purebred animals aged from 3 months to 5 years were sampled. Samples were analysed in the "Laboratorio de Imunogenética" of UNOPAR through 10% polyacrilamide vertical gels electrophoresis. Phenotypes and genotypes were established according to the ISAG international accepted nomenclature. 90 out of 137 (65,7%) animals were AA, 22 (16,05%) were AB, 15 (10,95) were AI, 6 (4,38%) were II, 4 (2,92%) were IB and none BB. Allelic frequencies were calculated: A (0,7920), B (0,0948) and I (0,1131). It is evident that Bonsamara breed is polymorphic for the Haemoglobin locus and that the I allele, not reported until now in the Brazilian herd, will be shortly introduced through Bonsmara crosses.

A552
A supplemental panel for use in cattle theft casework

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In an effort to combat the rising incidence of cattle theft, law enforcement agencies have turned to DNA analysis to establish ownership and to prosecute perpetrators. Since most stolen cattle are unbranded or have had their brands altered, parentage verification is increasingly used to identify individuals and return them to their rightful owners. The cattle parentage panels used by laboratories worldwide are robust and adequate for verifying parentage in proposed parent-offspring scenarios. However, when considering a large number of potential parents for an individual, a greater power of discrimination is required. It is imperative that when comparing calves against a pool of prospective dams or sires, there are sufficient markers to resolve complex inbred relationships. To augment the strength of our standard 14-marker parentage test, we developed and validated a 12-marker panel for use on cattle theft cases. The supplemental panel contains 11 unlinked autosomal dinucleotide repeat markers and one marker that is duplicated from the primary panel for continuity. All markers are in the public domain. The implementation of this test has led to an enhanced ability to exclude an incorrect parent in single-parent analyses commonly encountered in forensic casework. The single parent probability of exclusion (SPPE) has increased from 0.9987 for 14 markers to 0.99999 for 25 markers. The probability of identity (PI), which is an estimate of the probability that two randomly chosen individuals will have the same genotype, is less than 1×10^{-10} for each of the fourteen breeds tested with this panel. Furthermore, PI can also be calculated for small numbers of individuals and for populations comprised only of siblings, both of which may occur in cattle theft cases. The enhanced statistical power of the combined panels has increased confidence in our results and strengthened their ability to withstand challenges in court.

A556
Evaluation of the bay monotonous phenomenon in Japanese native horse: KISO pony by the coat color gene polymorphism

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The progenitor of KISO pony which is one kind of Japanese native horses came from Mongolia grassland horse at the second century. And this pony has been used as an employment animal for a long term. In the present, this pony is precious as genetic resource for horse therapy. However, in recent years, the number of habitation of KISO pony decreases rapidly. Furthermore, in this pony, hereditary homozygosis of many traits occurs by inbreeding. In particular, in the past, the various coat colors such as black, bay, chestnut, gray, etc. were observed in KISO pony, but the most ponies are the bay or the dark bay coat color currently. We evaluated the monotonous phenomenon of coat color of KISO pony by the polymorphisms of MC1R gene on Extension locus and ASIP gene on Agouti locus which regulated the amount of melanin granule composition (phaeomelanin and eumelanin) of equine coat color. Fifty-five KISO ponies were used for the observation of coat color and the blood collection for DNA extraction. The primer synthesis of MC1R and ASIP genes were based on the reports of Marklund et al.(1996) and Ueda et al.(2003). The polymorphism analysis of MC1R gene and the ASIP gene were performed by PCR-RFLP method and by PCR-AFLP method, respectively.The phenotype frequencies of coat color of KISO pony were bay:83%, dark bay:4% and other:13%. MC1R genotype frequencies were E/E:58%, E/e:42% and e/e:0% (E gene freq.:0.79, e gene freq.:0.21) , and then ASIP genotype frequencies were A/A:73%, A/a:23% and a/a:4% (A gene freq.:0.85, a gene freq.:0.15). In the family analysis of KISO pony, coat color genes of the THIRD HARUYAMA of a stallion of only survival were the homozygote to E and A genes. Furthermore, the broodmares and the foals had similar genotypes, and the inbreeding was done between those ponies. Accordingly, as for the coat color of the next generation in KISO pony group, the increase of bay and dark bay is suggested. For the biodiversity continuance of Kiso pony, it is necessary to prevent the bay monotonous phenomenon of this pony by the random mating which utilized coat color gene polymorphism in future.

A558
Parentage analyses in Nelore cattle using blood groups and haemoglobin polymorphism

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Since the beginning of the 1940s, blood groups and biochemical polymorphisms have been used in parentage analyses for high value cattle, especially for pedigree registration. The antigens of the erythrocytes surface are genetically defined by sire and dam inheritance. There are rare cases of genetic permutation and they are highly complex, especially in the B system. The present work had the objective of evaluating the probability of parentage exclusion in Nelore cattle by blood groups and hemoglobin biochemical polymorphism. Blood samples of 547 Nelore indidivudals from several Brazilian regions were collected and tested for 40 blood factors, distributed within eight blood systems, and for hemoglobin polymorphism by electrophoretic separation. The B system was responsible for the greatest exclusion rate (86.92 %), followed by the C (46.04 %), S (45.67 %), A (22.11 %), F (21.97 %), Hb (16.30 %), J (13.98 %), L (2.46 %) e Z (1.60 %) systems. The system with the smallest exclusion power (Z) presented 97.25 % of positive animals for the Z factor which that justifies the low exclusion rate. The overall exclusion probability (for all systems) was equal to 98.39 %. This demonstrates the high efficiency of blood test for parentage analyses in cattle from Nelore Breed.

A562
Benefits of additional markers in routine Bovine Genotyping

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We have implemented various measures in the laboratory Standard Operating Procedures to improve the overall standard of bovine genotyping in our laboratory. This was necessary to perform bovine genotyping for forensic purposes and because customers nowadays demand higher laboratory standards. ISAG members need to reconsider the minimum set of markers in routine testing to raise the quality of parentage analysis. Since 1st March 2006, we have increased the number of microsatellite markers that are routinely typed for each animal that is received in the laboratory. Simultaneously, we have implemented robotic procedures for the PCR and set-up for the capillary electrophoresis. Furthermore we have implemented new, automated, procedures to minimize the risk of genotyping errors and mistakes in parentage verification. Currently, the following markers are used routinely: BM 1818, BM 1824, BM 2113, CSRM 60, CSSM 66, ETH 3, ETH 10, ETH 225, HAUT 27, ILSTS 006, INRA 23, SPS 115, TGLA 53, TGLA 122, TGLA 126, TGLA 227. The sixteen markers are amplified in two PCR-reactions, one 11-plex and one 5-plex. The products of these reactions can be loaded together on a sequencer with the capability to identify five fluorescent dyes. Allele frequencies of the markers will be presented. Case studies will be shown to demonstrate the benefits of increasing the number of markers in routine testing. The use of sixteen markers leaves room for the addition of a limited number of extra markers, which would be recommended in many (routine) cases.

A563
Populations structure of buffalos in Brazil using microsatellite markers

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The objective of this work was to characterize the genetic structure within and between buffalo populations in Brazil (Baio, Carabao, Jaffarabadi, Mediterranean and Murrah). The analyses included a total of 382 animals, from twelve herds distributed in different Brazilian regions. The microsatellite markers utilized were the following: CSSM06, CSSM19, CSSM33, CSSM42, INRA05, INRA23, INRA35, CSSM08, CSSM09, CSSM66, ETH152, ILSTS05, OARFCB20 and INRA172. The number of alleles/loci by genetic group varied from 4 to 13, with an average of 10.07. The AMOVA indicated that 11.91% ($p<0.001$) of the total variation, occurred between genetic groups. The differentiation of the groups was studied using the Structure Program (80,000 iterations to test K value). A number of six populations was fixed (K=6) as the best value for K. The certification probability of the five genetic groups sampled on the six suggested populations showed a direct correlation among the populations (2, 4, 5 e 6) and the analyzed genetic groups: Carabao (0.869), Mediterraneo (0.769), Murrah (0.781) and Baio (0.756), respectively. These values show the proportion of animals in each genetic group, confirming their genetic identity. Even though it was observed the occurrence of shared gene introgression events on the five genetic groups, the only substructure was identified on the Jaffarabadi, fragmented in the subpopulations 1 and 3, containing 25.7% and 54.3% of the individuals, respectively. This suggests that there are differences among the populations from the state of Pará and the populations from the states of Goiás and São Paulo. The set of molecular markers utilized was efficient to evaluate the structure of the populations as well as to identify the existence of a substructure within the analyzed buffalo genetic groups.

A568
Using Microsatellite markers to estimate genetic variability in the Caspian horse breed.

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In this study genetic variability within Caspian horse population was assessed using 10 Microsatellite markers **(**HTG4, HTG6, HTG10, HMS2, HMS6, HMS7, AHT5, ASB2, VHL20 and TKY335**)**. Blood samples were collected from 100 horses randomly and their DNA was extracted by modified Salting out procedure. Polymerase chain reactions (PCR) were performed with all primers and products were separated by electrophoresis through 8% denaturing Urea-polyacrylamid gels and results were analyzed by POPGENE software. All loci were polymorphic and they fit with Hardy-Weinberg equilibrium ($P<0.005$). There were Maximum observed alleles (10) at AHT5 and HMS7 while a minimum was observed for VHL20, ASB2 and TKY335 (7alleles). Maximum effective allele number was observed at UMO26 for about 7.14 according to Kimura and Crow formula. Observed heterozygosity for each locus varied from 0.3 to 0.5 and Observed mean heterozygosity for all loci was about 0.42. Most frequent allele was observed for C allele of ASB2 (0.35%) and minimum allele frequency was observed for all Loci (0.05). On the base of findings of this research we can conclude that microsatellites could be a useful tool for screening of biodiversity. In contrast with previous studies reporting a restricted gene pool for Caspian horse breeds, it appears that there is a reliable genetic variability in its population for future breeding project.
Keywords: Caspian horse, Microsatellite markers, Genetic variation, Heterozygosity, Polymorphism.

A573
Microsatellite DNA polymorphism of Lipizzan horses in Hungary

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In this work we typed a random sample of Hungarian Lipizzan horse (n=630) with a set of 17 microsatellites (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1, CA425). Lipizzan was compared to Thoroughbred. DNA extracted from blood was amplified in one multiplex PCR (StockMarks for Horse, AB). Amplified PCR products were separated by ABI PRISM^{TM} 310 Genetic sequencer. Statistical testing was carried out by GENEPOP, FSTAT, and GENECLASS. Microsatellites were highly polymorphic. The alleles and allele frequencies of the two investigated breeds (Lipizzan, Thoroughbred) were significant different ($P<0.05$; $P<0.01$; $P<0.001$). In some cases different microsatellites were found in the samples of the investigated breeds (ASB2, HTG10). In the case of some microsatellites (HTG7, AHT5) the number of alleles was the same, but the frequencies were different in both breeds. DNA markers tested by us, due to their extremely large number and the more definite polymorphism of some loci, can be used on higher level and more efficiently in numerous fields of animal breeding, e.g. examination of genetic structure of populations, test of homozygosity, estimation of the degree of inbreeding of populations, maintenance of autochthonous populations (gene-reserve), parentage control, estimation of genetic distance between populations and breeds, planning of crossing programs (heterosis breeding).

A574
Weitzman´s approach and the diversity within and between chicken populations

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Human and chicken common history formed an indeterminable variety of breeds. This variety represents a basis for future breeding work forming an allele reservoir out of which it will be possible to select. For preservation it is necessary to find criteria to identify the breeds to be included in conservation projects. We accomplished an analysis on the basis of Weitzman's diversity concept for the set of 20 non-commercial chicken breeds and eight commercial broiler and layer parental lines. However such an analysis is based on the between breed diversity only. Eding (2002, Diss. Wageningen) and others argue that the selection of breeds on phylogenetic criteria involves the danger to prefer inbred lines with small genetic diversity. In this contribution we suggest to account for Weitzman's diversity between individuals. For the considered set of breeds, Reynolds' distances based on 29 microsatellites was calculated between 9 to 50 animals in each breed. The Weitzman diversity between breeds and additional parameters like marginal diversities and conservation potential for all breeds were calculated using an exact algorithm. The within breed diversity was calculated with an approximate Weitzman algorithm. For the computation of the total diversity samples were generated by randomly sampling N = 10, 20, 40, 60, to 80 or 100 animals from all breeds. The expected Weitzman diversity is a linear function of the sample size, and so a relative diversity index (RDI) was defined which accounts for this systematic effect. It is shown that although the diversity within breeds covers more than 90 % of the entire diversity, within breed diversity is slightly positively correlated with the conservation potential of the breeds. Hence, Eding's concern cannot be confirmed in this study. A further calculation on individuals from 8 populations in each case revealed the diversity among fancy breeds to be up to more than twice the diversity among commercial lines, reflecting the contribution of the former lines to the chicken gene pool.

A575
Genetic variability of milk proteins (κ - Casein, β-Lactoglobulin and α-Lactoalbumin) in "Hartón Del Valle" Creole cattle

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About 276 animals of Creole cattle Hartón del Valle (GCHDV) into 11 herds at the Valle del Cauca (Colombia) were sampled in this study of the genetic variability of three milk proteins-coding loci: κ-Casein (κ-CN), β-Lactoglobulin (β-LG) and α-Lactalbumin (α-LA). PCR-SSCP and PCR-RFLP analysis permitted to discriminate the allelic variants of κ-CN, β-LG y α-LA. Six allelic variants were observed for κ-CN and those correspond to: κ-CN A, κ-CN B, κ-CN A_1, κ-CN E, also two variants which weren't possible to identify and were denominated: HV^1 y HV^2 whit the purpose to describe them in this document. Allelic frequencies of the two common genetic variants were: κ-CN A = 0,454±0,021 and κ-CN B = 0,296±0,020. Every one of κ-CN allelic variants was observed in every herd evaluated except κ-CN E y κ-CN A_1. In addition, two variants were identified in both β-LG and α-LA and have a frequency similar to Iberian and Creole cattle breeds. Frequency allelic of β-LG A and β-LG B were: 0.382±0.021 and 0.618±0.022 respectively. While the genetics variants of α-LA A and α-LA B were: 0.144±0.016 y 0,856±0,016 respectively. The average gene diversity (H_e) estimated to GCHDV was: 0,473±0,014 which together whit the number of detected alleles suggest that yet this breed is a valuable reservoir of genetic diversity about milk protein loci. A little but significant value of genetic differentiation was observed between herds of GCHDV (F_{ST}=0,0403) and this breed differed to Holstein an Zebu cattle.

A577
***ShrimpTest12* – A panel of microsatellite genetic markers for pedigree tracking in breeding programs, forensic analysis and genetic diversity of *Penaeus vannamei* shrimp**

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We are compiling a reference database of unique molecular genetic tags that differentiate among and within various marine shrimp species. Information will be presented on a panel of 12 microsatellite genetic markers (*ShrimpTest12*) that was developed to examine allele diversity in wild ad cultured shrimp, trace the pedigree of breeding programs, identify missing pedigree data caused by loss of physical tags, monitor individual or family performance, and begin association studies with disease susceptibility status. The marker panel allows for differentiation among the different lines of *Penaeus vannami* shrimp maintained by the US Marine Shrimp Farming Program (USMSFP) at the Oceanic Institute in Hawaii. The markers (TUMXLv5.66, TUMXLv5.79, TUMXLv8.106, TUMXLv8.205, TUMXLv9.28, TUMXLv9.77, TUMXLv10.62, TUMXLv10.204, TUMXLv10.312, TUMXLv10.359, M1, and TUGAPv1-3.132) correspond to various linkage groups in *ShrimpMap* and were used for genotyping in 40 broodstock and 20 offspring each of 20 families of the Taura Syndrome Virus-Resistant (TSV-R) Line of the USMSFP. Cervus 2.0 software was used to calculate mean allele number, heterozygosity, polymorphism information content (PIC), and the total exclusionary power for each of the parents. Allele frequency statistics indicated that the mean number of alleles per locus was 10.5 (range 2-14), the mean expected heterozygosity was 0.72 (range 0.324-0.879) and the mean PIC value was 0.66 (range 0.271-0.865). The total exclusionary power for each of the parents was 0.99-1.00 which allows for determination of potential pedigree management errors. Some of these markers also identified (a) unique alleles among and within families from other USMSFP's lines, (b) unique alleles that differentiate between wild and USMSFP germplasm, (c) unique alleles that differentiated USMSFP stocks from a batch of cultured *P. vannamei* stocked in shrimp farms in Thailand, and (d) unique alleles that differentiate among wild penaeid species throughout their natural range. In summary, *ShrimpTest12* is the first microsatellite-based genetic marker kit developed for cultured and wild *P. vannamei*. It provides information on allele variation of loci located in over ~25% of the shrimp genome. The marker panel can be used by shrimp producers to reduce labor costs associated with tagging in hatchery and grow-out conditions, to develop good quality breeding stock to produce improved shrimp lines of good meat quality and to increase profitability. The panel is also being used for forensic analysis to determine potential source of postlarvae used during disease outbreaks and trace the origin of imported shrimp.

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SECTION B
Structural Genomics

B049

Fine mapping the silkiness locus of the chicken with microsatellite molecular markers to chromosome 3

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The silky feather is inherited as an autosomal complete recessive characteristic and has been studied for almost 100 years. The fluff type of feathers is due to the defective microscopic hooklets and can be found on the whole body of Silky fowl. The silkiness (*H*) locus was shown to be linked to the naked neck (*NA*) locus, which had been assigned to chromosome 1 (or classical group III) in the latest update classical linkage map. But in 2000, the *NA* locus was located on the chromosome 3 with microsatellite molecular markers. It was presumed that the map position of the *H* locus possibly could be on chromosome 3 due to linking to *NA*. The main objective of this study was to confirm it and locate the silkiness (*H*) locus exactly. An informative family was produced in China Agricultural University, by derived from reciprocal crosses between Silky fowl and White Plymouth Rock with strategy of F_2 design. There were 26 males and 160 females in F_0 population. The F_1 progeny consisted of 33 males and 165 females. Over 3,000 F_2 offspring were hatched. The F_2 generation showed a variation in the feather structure, normal and silkiness. The observed ration between normal and silkiness F_2 progeny (2404 : 826) did not deviate significantly from the expected 3 : 1 ration (χ^2 =0.296, d.f. = 1; P = 0.586 > 0.05). Twelve microsatellite markers on chromosome 3 were chosen from ARKdb chicken database and eight new microsatellite markers were developed. The genotype derived from the phenotype was also introduced and the linkage analysis was performed using *CRIMAP* version 2.4 software. Data of genotypes from 15 full-sib families including 238 F_2 individuals on these microsatellite markers and *H* locus were analyzed. There were four markers linking to *H* locus based on a LOD score threshold of 10.0, *CAU0001, CAU0002, CAU0006* and *CAU0008*. These four markers were all located on the middle region of chromosome 3. The sex-averaged map distances (in Kosambi centimorgans) for them: *CAU0008*—4.4—*CAU0006*—0.0—*H*—7.0—*CAU0002*—2.1—*CAU0001*. There was no recombination between *CAU0006* and *H* supported by a LOD score = 32.21. It is groundwork for identifying the causative gene of the silky feather.

B062

A radiation hybrid map of bovine Y chromosome using a 5000 Rad bovine WG-RH panel .

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There is a considerable interest in the identification of molecular markers related to improve cattle production, because cattle are a major economic resource worldwide. Although an international effort has been made to create genetic and physical maps of the bovine genome, comparisons between these maps show regions of the Y chromosomes that have sparse marker coverage. Radiation hybrid mapping can be used in combination with Somatic Cell genetics to produce ordered maps. Radiation hybrid cell panels also allow the construction of genetic maps with different types of markers, including non polymorphic ones. The goal of this study was to build a framework map of bovine Y chromosome integrating linkage maps of microsatellites, ESTs and evolutionary conserved genes into an ordered map. We have genotyped five genes, AMELY (Amelogenin Y gene), SMCY (Histocompatibility Y antigen gene), SRY-HMC (sex determining region of Y gene), TSPY (testis specific protein, Y-linked gene) and ZFY (*Ovis aries* zinc finger protein gene), one EST, EIF1AY (Eukaryotic translation Initiation Factor 1A-EST) and 14 microsatellites BC1.2, BM861, BRY.1, BYM1, BY39, HEL26, INRA30, INRA57, INRA124, INRA 189, MAF45, TGLA325, XBM451, UMN3008. Then, twenty markers were mapped to bovine Y chromosome using the 5000-rad whole genome cattle-hamster radiation hybrid cell panel. (This study is supported by grants from FAPESP N° 04/08768-6, 04/15641-2 and 04/15605-6).

B180

A high-resolution, integrated, comprehensive, and comparative radiation hybrid map of sheep chromosome 23.

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An ovine 5000 rad whole genome radiation hybrid (RH) panel has recently been created. The usefulness of this panel for generating physically ordered maps of individual ovine chromosomes was tested by typing 69 markers on sheep chromosome 23 (OAR23). Statistical analysis was done using the Carthagene software package. The resulting RH map included 11 EST and 40 markers derived from ovine BAC end sequences (BES) of the CHORI-243 library BAC clones with high and unique BLAST hits to sequences of the orthologous human chromosome 18 (HSA18). Relevant BES markers were FISH mapped to align the RH and cytogenetic maps. In addition, 18 microsatellite markers that span ovine chromosome 23, and had been mapped on the International Mapping Flock (IMF) linkage map, were typed on the RH panel to integrate the two maps. Comparison of the ovine chromosome 23 RH map with the HSA18 map identified and localized three breakpoints for intrachromosomal rearrangements between HSA18 and OAR23. The positions of these breakpoints are equivalent to those that have previously been shown for the syntenic cattle chromosome 24 and HSA18. In conclusion, this study presents the first comprehensive map for a sheep chromosome that integrates all available mapping data and adds new information that spans the entire chromosome. The comparative analysis confirms and refines current knowledge about conservation and rearrangements between sheep, cattle and human. The constructed RH map demonstrates the resolution and utility of the ovine RH panel.

B207

Towards a microsatellite-based linkage map for the saltwater crocodile (*Crocodylus porosus*)

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Constructing high-density genetic maps to identify quantitative trait loci (QTL) requires many polymorphic markers spaced evenly across the genome. We describe the development of 585 microsatellite markers to construct the first dense genetic map in the saltwater crocodile (*Crocodylus porosus*). These markers will be used to identify QTL for a variety of economically important traits in farmed *C. porosus*. Small insert libraries enriched for microsatellite repeats were constructed using a refined hybridization capture approach. Briefly, the genomic DNA was digested with *Rsa* I, ligated to SuperSNX linkers, independently hybridized to mixes of biotinylated oligonucleotides [$(AACC)_5$, $(ATCC)_5$, $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, $(ACTG)_6$, $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(ACAT)_8$, $(AGAT)_8$, $(AAG)_8$, $(ATC)_8$, $(AAC)_8$, $(AAT)_{12}$, $(ACT)_8$, $(AG)_{12}$, $(TG)_{12}$,] and captured on Streptavidin beads. From 4128 clones sequenced, 372 tetranucleotide, 79 trinucleotide and 62 dinucleotide microsatellite markers were developed. In addition, 60 markers were developed from repeat-enriched libraries derived from 768 fosmid clones with inserts of approximately 40kb. These fosmids will be mapped by fluorescent *in situ* hybridization to physically anchor the genetic linkage groups to chromosomes in *C. porosus*. Currently, all microsatellite markers are being characterized with respect to allele size and heterozygosity and we anticipate having the first dense linkage maps completed by early 2007.

B210
Phylogenetic analysis and genetic diversity of the Korean wild boars using mitochondrial DNA D-loop sequence polymorphisms

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We investigated the genetic diversity and phylogenetic relationships between 47 Korean wild boars (*Sus scrofa* coreanus) using the complete mitochondrial DNA (mtDNA) D-loop region sequence. Based on polymorphisms at 88 nucleotide positions, we found 38 haplotypes, and 74.5 % of the Korean wild boars had a T at position 282, compared with 0.73 % in the worldwide population. The phylogenetic tree grouped all wild boar into four main geographical clusters (India, Islands of Southeast Asia (ISEA), Asia and Europe) the Korean population formed four distinct subclusters (K1-4) within the Asian cluster. Contrary to our expectations, subclusters K1 and K2 were found to relate more closely to groups from Myanmar and Thailand. Subcluster K3 and the Japanese wild boar (*S. scrofa* leucomystax) were genetically close, suggesting that they derived from a common progenitor but specific sequences (T) at position 282 were not found between the K3 cluster and subcluster K4 represented an indigenous Korean population. In conclusion, our analysis indicates that the extant Korean wild boars have derived from at least four groups of progenitors.

B212
A comparative radiation hybrid map of sheep chromosome OAR26

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The physical gene map of the sheep (*Ovis aries*) is not as developed as the maps of cattle, horse, pig, honey bee, and chicken. In contrast, the ovine genetic map includes more than 1500 informative markers. This powerful tool has been effectively used for linkage analyses in sheep reference families to identify genome regions associated with economical important traits including health, wool, growth, reproduction, meat and milk. However, without genes assigned along the chromosome, positional cloning of candidate genes or the identification of causal mutations is difficult. Radiation hybrid (RH) mapping will greatly enhance the integration of genes and informative markers into a single map. Using an RH mapping strategy, we performed PCR typing of a 5000-rad sheep-hamster whole genome radiation hybrid panel and generated a comparative high-resolution gene and marker map for ovine chromosome 26. The map contains orthologous gene sequences of human, as well as informative cattle and sheep microsatellite markers. This RH map of OAR26 is one example of the ovine RH framework map, which is under development.

B220
Radiation hybrid maps of sheep chromosomes 2 and 6

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In this study, radiation hybrid (RH) maps of ovine chromosomes 2 (OAR2) and 6 (OAR6) were constructed using a 5000 rad sheep x hamster RH panel. Of 118 microsatellite markers originally selected from OAR2 and OAR6 linkage maps, 60 markers (50.8%) were typed across the full RH panel. The other 58 markers had poor amplification on the panel or the ovine and hamster bands could not be differentiated. The average marker retention frequencies were 0.172 across 39 markers on OAR2 and 0.218 across 21 markers on OAR6. Using two-point analysis in RHMap3.0, the markers on OAR2 were divided into three linkage groups at LOD > 4.0, with two, ten and 25 markers in each linkage group respectively. Two markers (*BMS1864* at 137.1 cM and *MCMA4* at 288.3 cM) could not be assigned to a linkage group at this LOD score. As for OAR6, the markers were assigned at LOD > 4.0 to three linkage groups of two, two and 16 markers respectively. One marker (*OARCP125* at 2.6 cM) was not assigned. The overall order of the loci was in agreement with the available linkage maps for OAR2 and OAR6 respectively. These markers will be included in the ovine RH framework map that is under development.

B223
First generation linkage disequilibrium map of X-chromosome of cattle.

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Linkage disequilibrium (LD) plays an important role in the planning and success of association studies. The X-chromosome is a unique resource to study the extent and pattern of linkage disequilibrium in mammals. There is only one copy of the X-chromosome present in males and hence it provides unambiguous haplotypes. We genotyped 1551 Australian Holstein bulls for 226 X-linked SNPs whose positions were determined in the bovine Location DataBase (LDB) integrated map. SNPs located in the pseudo-autosomal region were excluded from this analysis. SNPs with minor allelic frequency (MAF) less than 0.05 were also excluded. With the remaining 118 SNPS, we constructed an LDMAP of the X-chromosome based using the LDMAP software (http://cedar.genetics.soton.ac.uk/pub/ PROGRAMS/LDMAP). The average MAF for these SNPs is 0.283 ± 0.012. Mean spacing between SNPs is 1310 ± 244 kb. The total length of the LDMAP is 4.22 linkage disequilibrium units (LDUs) which span over 153 Mb of the X-chromosome. This map shows a variable pattern of LD over the chromosome when plotted on the physical distance derived from the integrated map. The LD map is defined in term of blocks and steps at different levels of stringency of LDU. The whole of the X-chromosome can be delimited into just five LD blocks at a loose stringency of one LDU. The average swept radius computed from this map is 36.3 Mb, which is smaller than the swept radius for bovine autosomes of equivalent physical length. The LDMAP map length of the bovine X-chromosome reported here is many-fold smaller than in humans. A detailed comparison of LDMAPs of the X-chromosome with bovine autosomes is in progress and will delineate a number of properties of LD structure in cattle.

B228
Assignment of 117 genes localized on HSA5 to a porcine RH (IMpRH) map to generate a dense new human-pig comparative map

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Several significant quantitative trait loci for average daily gain have been located on porcine chromosome (SSC) 2, which was shown to correspond to human chromosome (HSA) 5 by chromosome painting. In order to provide gene map information for efficient selection of trait candidate gene(s), we have, therefore, assigned 117 genes localized on HSA5 to a porcine radiation hybrid (IMpRH) map, and generated a comprehensive comparative map between HSA5 and SSCs in the present study. Sixty-six genes were assigned to SSC2 and 48 to SSC16. One gene was suggested to link to SSC2 markers and another to SSC6. The remaining gene did not link to any genes/expressed sequence tags/markers registered, including those in the present study. The correspondences between HSA5q13-q35 and SSC2q21-q28, and between HSA5p-q13 and SSC16, were essentially consistent with the observations obtained from bi/uni-directional chromosome painting or other mapping results. This study further demonstrated as follows: 1) The conserved synteny between HSA5q13-q35 and SSC2q21-q28 is interrupted by at least two sites; and that between HSA5p-q13 and SSC16, by at least two sites. 2) The region ranging from 150.4 Mb to 169.1 Mb in HSA5q32-q35 were assigned to SSC16q22-23.

B232
A gene-based high-resolution radiation hybrid map as a framework for genome sequence assembly of BTA6 associated with QTL for growth, body composition, and milk production traits

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QTL for various phenotypic traits, including milk production, functional, and conformation traits in dairy cattle as well as QTL for growth and body composition traits in meat cattle, have been consistently localized on bovine chromosome 6 (BTA6). Dense genetic and physical maps and, ultimately, a fully annotated genome sequence as well as their mutual connections are required to efficiently identify genes and gene variants responsible for genetic variation of the indicated phenotypic traits. Based on the whole-genome cattle-hamster 12,000 rad panel, we constructed a high-resolution radiation hybrid (RH) map for the QTL containing chromosomal region of BTA6 including a total of 234 loci. The respective chromosomal region of BTA6 we focused in this study includes the segment between *BM1329* and *BM2320* as the most proximal and distal anchor markers. Associating densely spaced markers with 115 genes and ESTs, and cross-referencing them to the human and bovine genome sequences, our RH map achieved a high gene-anchored resolution for the targeted region of BTA6. One cR on our high-resolution RH map covers about 19.8 kb on the sequence contig map of BTA6. This RH map, which integrates 1 locus per 350 kb proved to be a valuable platform to guide high-quality assembling and annotation of the currently existing bovine genome sequence in order to establish the final architecture of BTA6. A sequence-based map of BTA6 will provide a key resource to facilitate prospective efforts for the identification of relevant positional and functional candidate genes and the validation of trait-associated mutations underlying the QTL mapped on BTA6 and on similar chromosomal regions from evolutionary closely related species like sheep and goat. Furthermore, the high-resolution sequence-referenced BTA6 map will enable precise identification of multi-species conserved chromosome segments and evolutionary breakpoints in mammalian phylogenetic studies.

B233
Development of a BAC-based physical map of the horse genome

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The genome analysis of several livestock species is progressing rapidly. Recently the horse was included in the list of species for which a whole genome database sequence will become available. High-resolution BAC-based physical maps have the potential to complement the assembly of whole genome sequences, which will lead to an improved long-range contiguity of the assembled genome sequence. In a Lower Saxonian study a physical map of the horse genome will be created. The horse physical map will be based upon a combination of BAC fluorescent fingerprinting and BAC end sequencing of the CHORI-241 library. Fluorescent fingerprints of 150,000 BAC clones (~10x genome coverage) will be obtained by using the 4-restriction enzyme 4-color technique and separation of the resulting fragments in capillary sequencers. The fingerprinted clones will also be end sequenced. The BAC end sequences (BESs) will enable the anchoring of the emerging BAC contigs to the equine RH map as well as the comparative analysis with the human genome. So far ~29,000 BESs have been produced and submitted to the public databases.

B234
Characterization of the shadow of prion protein locus in sheep.

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Transmissible spongiform encephalopathies (TSEs) are a group of fatal, neurodegenerative diseases, including Creutzfeldt-Jakob disease, bovine spongiform encephalopathy (BSE) and scrapie. They are caused by prions, 'alternatively' folded forms of the body-own prion protein, encoded by the prion protein gene (*PRNP*). There are indications that other genes might be involved in the pathogenesis of TSE. Shadow of prion protein (*SPRN*), a gene coding for a protein with remarkable similarities with prion protein, is such a candidate gene. Until now, *SPRN* is described in man, mouse, rat, and fish. Here we describe the *SPRN* sequence in sheep, a highly relevant species in prion matters. A BAC minicontig was built around *SPRN*. The BAC containing *SPRN* was mapped by FISH to sheep chromosome 22q24. Comparative mapping with other genes from the human *SPRN* region (10q26) suggests conserved linkage between sheep and man in this region.

B242
Genomic characterization of a defensin gene cluster on ECA 27q17

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Defensins are a family of evolutionary ancient antimicrobial peptides consisting of three sub-families: α-, β- and θ-defensins. This investigation was focused on the genomic characterization of equine β-defensins and the investigation of the potential clustering of β-defensin genes in the equine genome. Six genomic BAC clones were isolated from the CHORI-241 library and one of these was mapped by FISH to ECA 27q17. This location was confirmed by RH-mapping. The contiguous 212 kb sequence of this clone was determined. Sequence analysis revealed the identification of ten pseudogenes and nine genes, six of which were highly homologous to human β-defensin *DEFB4*. Clustering of β-defensin genes was confirmed and the order of the genes on the analyzed BAC was related to the corresponding defensin cluster on HSA 8. The knowledge about sequence and genomic structure of the equine β-defensin genes will improve the classification of different paralogous defensin genes and is a prerequisite for subsequent functional studies. Additionally, the first α-defensin-like sequence outside the groups of primates, lagomorphs and rodents (glires) was identified.

B247
Linkage map construction for the ParAllele 10K SNP Panel

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High-density linkage maps are required to optimize the use of linkage disequilibrium (LD) in quantitative trait loci (QTL) fine mapping. Advances in technology have made it possible to cost-effectively genotype a large number of single nucleotide polymorphisms (SNPs) on animals that are part of an experiment identifying QTL and genes affecting dairy production. The Affymetrix GeneChip® Bovine Mapping 10K SNP Kit was used to genotype a Holstein x Jersey F2 experiment for a total of 1679 animals. SNPs were assigned to physical positions along different bovine chromosomes based on a comparative alignment between the human genome and a dense bovine genetic map. Specifically, SNPs harboring bovine scaffolds were mapped to the human genome by BLAST alignment, then by combination of the position of the bovine SNPs on the human genome and the human-bovine comparative alignment, the relative order and distance of SNPs on the bovine genome was estimated by extrapolation. Before undertaking map construction, SNPs were screened for segregation distortion by Hardy-Weinberg Equilibrium (HWE), and non-inheritance. Approximately 400 SNPs were removed based on departure from HWE and/or a large number of parentage errors. 1189 SNPs were initially unassigned to a chromosome from the physical mapping. Using previously 297 mapped microsatellites and CRIMAP (TWOPOINT option), 1053 SNPs were mapped to a chromosome using the criteria of a Likelihood of odds (LOD) threshold greater than 15 with at least two microsatellites belonging to the same linkage group and no other significant linkage to an alternative chromosome. MULTIMAP was used to make linkage maps that combined the existing microsatellites and SNPs. SNPs with a recombination fraction greater than 20 centi Morgans (cM) with other markers were removed from map. Several options for MULTIMAP input parameters were used to obtain different linkage maps for each bovine chromosome in resolving cases of SNP order. Linkage results were compared to physical maps and any incongruence resolved. Preliminary high-density linkage maps will be presented.

B269
A comprehensive genetic and physical map of the bovine genome

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A composite map of the 29 bovine autosomes and X chromosome was constructed. Two linkage and four radiation hybrid (RH) data sets were utilized. Pedigree and marker genotypes from the Shirakawa-USDA linkage data and an eight-breed reference population (MU) were merged with RH vectors from the third-generation Illinois/Texas, SUNbRH, and BovGen maps. The fourth RH data set (UA) represents SNP from the bovine genome sequencing project, which were scored on the BovGen panel. Markers in the MU data set are a subset of the UA SNP. The individual data sets represent a total of 26,076 markers. Twopoint analysis to assign markers to chromosomes resulted in removal of 494 markers not meeting LOD score and distance criteria. The combined data include 17,254 distinct markers, 6,716 of these are shared by two or more data sets. Sequences for 10,329 mapped markers were aligned with sequence from 25,712 BAC clones on the International Bovine BAC Consortium fingerprint map; 14,382 markers were matched to 12,160 whole-genome shotgun contig sequences from the second bovine assembly. Consensus marker orders for each chromosome were computed using maximum likelihood procedures in CarthaGene. Marker positions were projected onto common cM and kbp scales using LDB. Overall agreement between consensus marker order, fingerprint map clone order, and arrangement of contig sequence on whole-chromosome scaffolds is high, although discrepancies exist. The composite map, with markers connected to the BAC fingerprint map and bovine sequence, provides a comprehensive resource, supporting investigations into relationships between genomic and phenotypic variation in cattle.

B270
The Cytochrome P4501A5 gene in the turkey (*Meleagris gallopavo*)

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The Cytochrome P450 gene (CYP1A5) encodes the primary protein responsible for epoxidation of the mycotoxin aflatoxin B_1 (AFB_1) in the turkey. Hypersensitivity of turkeys to AFB_1 was associated with "Turkey X Disease" which caused widespread deaths of turkeys and other poultry throughout Europe in the 1960s. Comparative genomic approaches were used to selectively amplify and sequence the introns and 3'flanking region of CYP1A5. A single nucleotide polymorphism in the 3'UTR was used to genetically map CYP1A5 to turkey linkage group M16. The results of this study provide the framework for identifying allelic variants of this biochemically important P450 gene in poultry.

B271

A water buffalo whole-genome radiation hybrid panel for gene mapping

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Brazil is the largest buffalo breeding center outside the Asian continent, the origin of the domestic buffalo. According with the "Brazilian Institute of Geography and Statistics" (IBGE - http://www.ibge.gov.br) the national population of buffalo in 2004 exceeded one million head. The extensive use of buffalo in agriculture world wide, and especially in developing countries, begs for genetic resources to evaluate and improve traits important to local and regional economies. A 5000-rad whole genome radiation hybrid panel was constructed for river buffalo and used to build a preliminary RH maps from two chromosomes (BBU 3 and BBU 10). The preliminary maps contain about 64 markers, including coding genes and anonymous microsatellites loci. The RH maps presented here are the starting point for mapping additional loci, in particular, genes and expressed sequence tags that will allow detailed comparative maps between buffalo, cattle and other species to be constructed. A large quantity of DNA has been prepared from the cell lines forming the RH panel reported here and will be made available to the international community both for the study of chromosome evolution and for the improvement of traits important to the role of buffalo in animal agriculture.
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B280

Mapping and sequencing of PERV positive clones from a Korean native pig BAC library

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As an ideal donor for xenotransplantation, pigs are the possible solution for the shortage of human organs. Porcine endogenous retroviruses (PERVs) are one of the possible obstacles for using porcine organs regardless of the immunological barriers. Three main types of the PERVs, A, B and C, have been previously investigated in diverse pig breeds. To examine the copy numbers of PERVs and their integration sites in Korean native pig genome, we screened BAC (Bacterial Artificial Chromosome) library with PERV specific protease primers for initial screening of PERV positive clones and three envelope specific primers for the identification of PERV types. Total 45 PERVs positive clones, 9 PERV-A and 36 PERV-B, have been identified from the library screening. The SCH and IMpRH map information indicates 45 PERV clones belong to 8 contigs and a singleton. The sequence analyses of these clones will greatly help a general strategy to generate PERV-free lines of pigs suitable for xenotransplantation.

Key words: PERVs, BAC library, Xenotransplantation.

B283

Construction of pig gene catalog: determination and application of porcine full-length cDNA sequences

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Collection of nucleotide sequences of expressed genes in various tissues and determination of full length of their cDNA sequences is prerequisite for investigation of gene functions. We performed large-scale EST analysis from the 5′-ends in pigs by using full-length-enriched cDNA libraries derived from 12 kinds of tissues and cells. We have obtained more than 160,000 ESTs, which have been assembled into more than 9,000 contigs and 48,000 singlets, which can be viewed through the web database with annotation by BLAST similarity search and distribution of SNPs (http://pede.dna.affrc.go.jp/). In order to construct gene catalog in pigs, we picked up more than 20,000 cDNA clones, which represent all of the contigs, and singlets that possessed marked similarity to genes known in humans, from the cDNA libraries. The 5′- and 3′-ends of these cDNA clones were sequenced, and the full-length inserts of these clones have been subjected to determination by the primer walking and transposon shotgun sequencing methods. We have already determined sequences of approximately 7,000 full-length inserts of the cDNA clones. The information about combination of large-scale collection of 5′- and 3′-end sequences of cDNA clones, as well as full-length cDNA sequences, are valuable not only for utilization for expression analysis such as microarray studies, but also for precise assembly and annotation in pig genome sequencing, which are now being performed by the Swine Genome Sequencing Consortium. We also discuss utilization of these full-length cDNA sequences for development of SNPs, which are useful for construction of a reliable comparative map between pigs and other mammals. (This study is supported by the Animal Genome Research Project by MAFF, Japan).

B284

Construction of a radiation hybrid map for the QTL region for fat deposition traits

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A radiation hybrid (RH) map was constructed using the IMNpRH2$_{12,000}$ panel for the SSC6q32 related to quantitative trait loci (QTL) for fat deposition traits defined from an Iberian x Landrace intercross. Primer sets for 22 genes (*AK5, ST6GAKNAC5, PIGK, ST6GALNAC3, ASB17, MSH4, RABGGTB, ACADM, SLC44A5, LHX8, C1orf171, CRYZ, FPGT, TNNI3K, LRRC44, NEGR1, ZNF265, PTGER3, CTH, ANKRD13C, SFRS11* and *LRRC40*), located on the corresponding human region (HSA1p31), were designed. The RHMAP 3.0 software was applied to estimate two point distances, LOD scores, frame markers and multipoint distances. Two linkage groups were identified at LOD score criterion 4. Two 1000:1 (LOD score greater than 3.0) framework maps were constructed for each linkage group. On the basis of the order of markers in the framework maps, comprehensive maps for 22 markers were produced. The estimated order and the distance of markers were AK5 - 29.3 cR - ST6GALNAC5/PIGK - 36.3 - ST6GALNAC3 - 62.5 - ASB17 - 2.6 - MSH4 - 5.2 – RABGGTB - 8.1 - ACADM - 28.9 - SLC44A5 - 15.1 - LHX8 - 31.0 - C1orf171 - 11.5 - CRYZ - 46.8 - FPGT - 5.5 - TNNI3K - 5.2 - LRRC44 and NEGR1 - 49.0 cR - ZNF265 - 12.8 - PTGER3 - 48.8 - CTH - 26.0 - ANKRD13C - 10.2 - SFRS11 - 17.9 - LRRC40 in linkage group I and group II, respectively. The estimated order of genes was conserved with those of human and mouse excepting an inversion between FRGT and TNNI3K. The total map length of linkage group I and group II was 467.6 and 164.7 cR$_{12,000}$. Using the known human physical distances of 3.1Mb (*AK5-LRRC44*) and 1.26 Mb (*NEGR1-LRRC40*), the ratio between cR$_{12,000}$ and the physical distance was estimated to be 10.7 and 7.6 kb/cR$_{12000}$, respectively. These results are useful for the selection of genes of interest involved in the QTL. For example, *PIGK* and *ACADM* could be suggested as interesting genes for fat deposition traits, based on their functions related to transamidation in the endoplasmic reticulum and beta-oxidation in the mitochondria, respectively.

B316

Two novel mutations in the canine bestrophin gene are associated with autosomal recessive multifocal retinopathy.

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Canine multifocal retinopathy (CMR) is an autosomal recessive eye disorder occurring in multiple dog breeds, including Great Pyrenees, Coton de Tulear, and English Mastiff. The phenotype typically presents as multifocal serous retinal and retinal pigment epithelium (RPE) detachments with multiple gray-tan pink subretinal patches in the tapetal and nontapetal fundus. The disease generally develops in young dogs before 6 month of age and progresses slowly. We observed that CMR shares phenotypic features with diverse types of human maculopathies and tested the involvement of four candidate genes, *ABCA4*, *VMD2*, *PRPH2*, and *ELOVL4*, by haplotype analysis or exon scanning. The analysis of the canine *VMD2* gene resulted in identification of two novel independent disease-specific mutations: C73G in Great Pyrenees and English Mastiff, and G482A in Coton de Tulear dogs. This is the first report revealing the mutations in the canine bestrophin gene, and identifying the genetic cause of multifocal retinopathy in dogs. Based on phenotypic similarities observed in RPE and retinal lesions among human and dogs, we propose CMR as a relevant animal model for human retinal degenerative diseases.

B318

Use of autozygosity mapping in dogs: Mapping the gene for Trapped Neutrophil Syndrome, an immune dysfunction in Border collies.

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Trapped Neutrophil Syndrome (TNS) is an autosomal recessive disease which has spread through the Border collie population due to extensive breeding from champion dogs and subsequent inbreeding. To attempt to identify the mutation responsible for TNS the Functional Candidate Gene (FCG) approach was used in conjunction with autozygosity analysis to perform exclusion mapping of candidate genes. The candidate genes were identified based on four diseases: myelokathexis, Hermansky Pudlak Syndrome 2 (HSP-2) and Warts, Hypogammaglobulinemia, Immunodeficiency and Myelokathexis (WHIM) in humans, and cyclic neutropenia in dogs. Identification of the mutation responsible would allow for dogs to be used as a model organism for similar diseases in humans and elimination of the disease through DNA testing and selective breeding in dogs. Sequencing of one candidate, *CXCR4* involved in WHIM, revealed no differences between affected and control dogs. To eliminate mutations from other regions of this gene as the cause of TNS, potentially polymorphic microsatellites, close to *CXCR4* were developed for use in autozygosity analysis (homozygosity for a haplotype due to Identity By Descent). A lack of homozygosity allowed this gene to be excluded as the cause of TNS. Autozygosity analysis has similarly been used for each of the other candidate genes using both known and newly developed microsatellites. Each of the candidate genes tested has been excluded as the TNS gene. Further candidate genes will be examined before a whole genome scan, to look for regions of homozygosity in affected dogs and linkage analysis in large pedigrees using regularly spaced microsatellite markers, is undertaken. Such mapping studies will reveal candidate regions of the genome for fine mapping and candidate gene sequencing.

B333

A genomic analysis of the turkey major histocompatibility chromosome.

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The Major Histocompatibility locus (MHC) contains many of the genes responsible for peptide presentation an essential component for both innate and adaptive immunity. MHC class I and class II molecules present endogenous and exogenous peptides to $CD8^+$ and $CD4^+$ T cells, respectively. NK cells are thought to monitor MHC molecule expression preventing viral or tumor cells from evading immune surveillance. As part of the University of Minnesota's Turkey Genome Mapping Project we have begun to characterize this region in the turkey (*Meleagris gallopavo*). Mammals typically have an MHC region spanning 2-4 Mbps containing genes encoding MHC class I and class II molecules, as well as genes responsible for peptide antigen generation and loading onto their respective MHCs. Remarkably, the chicken MHC (B-locus) is a fraction of the size compared to mammals, spanning ~90 Kbps and only contains about 20 genes. This "minimal essential" MHC has strong linkage to disease susceptibility and resistance. In our studies we have sequenced turkey MHC regions and mapped regions physically and genetically. Comparisons between the chicken and turkey reinforces the high degree of homology between these two species.

B344

Pursuing the genetic origin of polydactyl and associated reproductive phenotypes in pigs

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Purebred Yorkshire pigs and crossbred pigs have been identified that variably express polydactyl phenotypes. The phenotypes were found on the front feet and include pigs having either an extra dewclaw, an extra "foot" with the normal number of dewclaws, or an extra "foot" missing one dewclaw. Pedigree analysis of isolated individuals indicated that common ancestry does exist. Planned matings using purebred Yorkshire individuals created full and half sibs of the original affected individuals, generating a larger study population. Additional matings were designed between a founding sire and all female progeny (n=6) in the original population. Thirteen litters have been produced to date totaling 87 live pigs and 53 stillborn and mummies totaling 12 affected (6 live and 6 stillborn) individuals. Two females expressing a polydactyl phenotype identified outside of the original population have produced zero offspring expressing any polydactyl phenotype. These results compiled to date suggest the polydactyl phenotype is under genetic control and is recessive in nature. Comparative mapping suggests multiple genes associated with polydactyl phenotypes in other species should be located on pig chromosome 18 (SSC18). Primers were designed for candidate genes on SSC18 along with additional genes used for markers. PCR-RFLP tests were designed to genotype any sequence identified SNP. Genotypes from the first ten litters were used to calculate LOD scores by the Elston-Stewart algorithm, testing for linkage between markers and phenotype. Though the causative mutation has yet to be identified, a 2.6 Mb region between the genes SPAM1 and WNT16 shows a very favorable LOD score. Though this polydactyl phenotype is intriguing, the traits of importance to the swine industry are the associated reproduction traits observed including females in this population showing various undesirable vulva characteristics as well as delayed identifiable estrus and alarming rates of pigs born dead in this limited population.

B350
Radiation hybrid maps for 12 ovine autosomal chromosomes

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An ovine whole-genome radiation hybrid (RH) 5,000-rad panel was constructed in a collaborative project between Utah State University and Texas A&M University. A framework map using this RH panel is currently under construction. In this study, microsatellite markers were selected from the latest published ovine linkage map (Australian Gene Mapping Web Site: http://rubens.its.unimelb.edu.au/%7Ejillm/jill.htm) based on their location across ovine autosomal chromosomes. Average spacing of microsatellites was about 6 cM. In total, 339 markers on chromosomes 1, 4, 5, 7, 9, 12, 14, 16, 18, 20, 22, and 24 were selected for their inclusion in the chromosome RH map. However, only 169 (51.5%) of these markers produced resolvable patterns and were analyzed across the whole panel. The average retention frequency for these markers was about 21%. For each chromosome, linkage groups were identified with $\geq$ 4 LOD support based on statistical software RHMap3.0 analysis. To date, linkage groups containing 8 and 13 markers have been constructed for chromosomes 4 and 9 respectively, and two linkage groups of 9 and 3 markers respectively were identified on ovine chromosome 1. The ovine RH framework map is progress rapidly.

B367
Porcine *OGN* and *ASPN*: mapping, polymorphism and inclusion for QTL mapping in a Meishan x Piétrain intercross

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The porcine orthologues of human chromosome HSA9q22.31 genes, *OGN* (osteoglycin) and *ASPN* (asporin), were mapped to porcine chromosome SSC3 by linkage analysis and using a somatic cell hybrid panel. This mapping was refined to SSC3q11 by using fluorescence in situ hybridization. This confirms the existence of a small conserved synteny group between SSC3 and HSA9. Polymorphism was revealed in both genes – a pentanucleotide microsatellite (*SCZ003*) in *OGN* and two SNPs (g.780G>T and g.825T>C) in *ASPN*. The two genes were included in a set of 10 markers on SSC3 for the whole-genome QTL scan in the Hohenheim Meishan x Piétrain F_2 family. Major QTLs explaining up to 6.7% of the phenotypic variance for growth and carcass traits were centered in the *ASPN – OGN - SW902* region calculated at the sex-averaged map positions 38.6 cM, 39.2 cM and 46.1 cM, respectively, by linkage analysis. The traits loin and neck meat weight, shoulder meat weight (without external fat) and daily gain (110-210 days) were significantly influenced by additive and dominance effects at $P < 0.01$ genome-wide level**;** carcass length, meat area on m.l.d. at 13th/14th rib and ham meat weight without external fat were significant at $P < 0.05$ genome-wide level; half carcass weight, ham weight including bones and external fat, weight of head, food conversion ratio, fat to meat ratio (area at 13th/14th rib) and weight of bacon meat relative to lean cuts weight were significant at $P < 0.05$ chromosome-wide level. (Supported by the Czech Science Foundation 523/06/1302)

B371
Fine mapping of the *Grey* coat colour gene in horses.

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Greying with age is a dominant coat colour common among several horse breeds. These horses are born coloured but gradually lose pigmentation in hair, resulting in a white coat colour. The phenotype has been correlated with the development of melanoma and vitiligo. We have previously used comparative linkage mapping for defining the candidate region on ECA25 as being located between genes *NANS* and *ABCA1* and corresponding to 6.9Mb region of HSA9q. Here we present an Identical-By-Descent mapping across different horse breeds as suitable strategy for narrowing down the candidate region. By using 8 different horse breeds with grey colour we have identified a region corresponding to 1.1Mb of HSA9q as harbouring the *Grey* gene and mutation. There are five known genes identified in this region, but none of them has been associated with pigmentation disorders or melanoma development. We have screened grey and non-grey individuals for coding mutations in these candidate genes, but have not identified any of the mutations as being correlated to the grey phenotype. However, a specific haplotype showing a complete association with the grey phenotype has been detected. We have initiated several experiments for evaluating the function of the genes in this interval. We are currently pursuing transfection overexpression and RNAi experiments for evaluating the role of the candidate genes in cell proliferation. Furthermore, conducting immunohistochemistry and yeast two-hybrid experiments as well as creating a knockout mouse will give us further insights into the function of this novel gene and the fascinating phenotype.

B389
The extend of linkage disequilibrium and haplotype structure in the chicken genome.

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Chicken have been, and still are, the subject of intense genetic selection with selection pressure on specific traits within defined specialized breeding populations. For the analysis of genes underlying complex multifactorial traits (quantitative trait loci or QTL) and to obtain insight in the genetic diversity of these breeds, it is essential to gain insight in the extent of linkage disequilibrium (LD) within these highly selected populations. As a first step to address these questions and related questions about the amount of inbreeding and the presence of selective sweeps in the past, we have initiated a number of studies in which a large variety of breeds are being analyzed with large numbers of SNPs. In the first large-scale public chicken SNP project, 3,072 SNPs evenly-spaced throughout the genome were genotyping on the Illumina platform, on the DNA from 2,576 birds. These included material from 1,440 elite chickens (36 lines) representing a major part of the commercial breeding stock in the world as well as numerous experimental lines. In order to obtain a more detailed description of the haplotype block structure of the chicken genome an additional 1536 SNPs were typed on 700 birds from a subset of the breeds in the first experiment. Furthermore, 600 SNPs were chosen to improve the chromosomal assignment of sequencing contigs from the whole genome shotgun assembly of the chicken genome. Preliminary results will be presented that address: (1) the validation and allele frequency of the SNPs in these farm animals, (2) the generation of a high-density SNP genetic map and its integration with the chicken genome sequence, (3) the feasibility of genome-wide marker-assisted selection (GMAS) in commercial chicken lines and (4) the haplotype block structure in the genome of chicken.

B402
A procedure to finemap mouse QTLs using dense SNP data

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Quantitative Trait Loci (QTLs) as determined in crossbred studies are a valuable resource to identify genes responsible for the corresponding phenotypic variances. Due to their broad chromosomal extension of some dozens of Mega base pairs, further steps are necessary to bring the number of candidate genes, which would underlie the detected effects, to a reasonable order of magnitude. Here, we demonstrate how murine QTLs for fatness can be dissected into smaller fragments using data of over 13,000 SNPs between inbred mouse strains. In a first approach we show that identifying informative haplotype blocks between the base lines of a cross-bred study permits the rejection of on average half of the size of a typical QTL and, thus, half of the potential candidate genes. Further reduction is achieved by focussing on chromosomal regions which are covered by informative haplotype blocks in QTL regions from several independent crosses. In a second approach we compared the genotypes of a pool of nine fat lines with a pool of six lean lines and identified 14 informative haplotype blocks. Most of these informative haplotype blocks are located within already known QTLs. Due to their short length of at most three Mb these haplotype blocks provide hints on which parts of the QTLs could harbour the gene or the genes underlying a QTL effect. Using additional information about fatness related genes in *C. elegans* and the expression of the genes in mouse fat, liver, muscle and hypothalamus tissues a further reduction of the number of candidate genes was achieved. Among the most prominent candidate genes are genes which were associated with fat deposition in previous studies.

B404
Sequencing the Pig Genome using a BAC by BAC approach

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We have generated a highly contiguous physical map covering >98% of the pig genome in just 176 contigs. The map is localised to the genome through integration with the UIUC RH map as well BAC end sequence alignments to the human genome. Over 265k HindIII restriction digest fingerprints totalling 16.2 genome equivalents form the basis of the map assembly. Most of the map coverage is from the CH-242 library generated from a single Duroc sow. The mapped clones have also been used to generate over 620k BAC end sequences with an average read length of 635bp. We are using the map as a basis for sequencing the pig genome by detecting the most economic set of minimally overlapping CH-242 BACs. Sequence clones are identified in a series of iterative sets using first mapped data and BES alignment to human with bridging clones subsequently selected on the basis of the map and BES alignments to pig sequence clones, at this stage we will also have access to end sequenced fosmids generated from the same Duroc as the CH-242 library which will improve efficiency of gap closure. The map is accessible through WebFPC (http://www.sanger.ac.uk/Projects/S_scrofa/WebFPC/porcine/large.shtml) and represents an entry point for rapid electronic positional cloning of genes and fine mapping of QTLs.

B410
A BAC contig map of the equine pseudoutosomal region

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The pseudoautosomal region (PAR) on mammalian sex chromosomes is essential for the obligatory X-Y crossover and correct segregation during male meiosis. The region is characterized by extraordinarily high recombination and silent substitution rate. Comparative data – presently available only in human/chimp and mouse - show considerable differences in size and gene content in the region. Consequently, representative information on the comparative structure and organization of PAR in mammals is limited. We herein add comprehensive data from a species representing a distinct mammalian order – the Perissodactyls - by reporting the characterization of the horse PAR and the adjacent region on the X and the Y chromosome. Primers for putative PAR genes were used to screen the equine CHORI-241 BAC library to isolate BACs containing the genes. All clones thus obtained were FISH mapped to male metaphase spreads to verify their presence on the sex chromosomes. BACs hybridizing to both X and Y chromosomes were identified and end sequenced for the development of STS markers. Chromosome walking and STS content mapping, complemented with metaphase-, interphase-, and fiber-FISH experiments permitted physical ordering of the clones and verifying overlaps between them. Collectively, the approaches enabled us to construct a BAC contig map over the entire equine PAR (~ 70 clones; 4X coverage). The contig spans the pseudoautosomal boundary (PAB) and includes the Y-specific region immediately distal to the PAR. Similarly X-specific BACs distal to the PAR were also isolated. Characterization of the contig has led to the identification of 14 PAR genes in the horse. The overall size of the equine PAR is ~ 3.5 Mb, implying that it is larger than the human or mouse counterparts. Ongoing sequencing of BAC clones spanning the PAB on ECAY and ECAX will provide details on the organization of PAB in the horse, and contribute to the comparative organization of PAR in mammals. Demarcation of PAB and identification of adjacent genes on the Y-chromosome is of particular importance for understanding the genetic consequences of male meiotic errors in horses.

B415
Molecular characterization of the canine RAGE gene

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The receptor for advanced glycation end products (RAGE) is a multiligand member of the immunoglobulin superfamily of cell surface molecules. The receptor is known to be causally involved in a variety of pathophysiological processes, e.g. immune/inflammatory disorders, Alzheimer disease, and tumorigenesis. Recently, RAGE was described to bind amphoterin, synonymously called HMGB1. The RAGE – HMGB1 complex has been shown to have significant influence on inflammation and metastasis by taking significant effect at invasiveness, growth and motility of tumour cells. *In vitro* and *in vivo* approaches showed that blocking of this complex resulted in drastic suppression of tumour cell growth. Recently we characterised the entire canine *HMGB1* gene and protein. Here we present the characterisation of the canine *RAGE* gene completing the characterization of the RAGE–HMGB1 protein complex. The canine *RAGE* gene was characterized by standard molecular methods, e.g., PCR, cDNA synthesis, cloning, Northern Blot, and *in silico* analysis. The canine cDNA sequence consists of 1384 bp spanning eleven exons. On genomic level the gene consists of the eleven exons and ten introns spanning in total 2835 bp. The deduced protein is a 404 amino acid (AA) molecule with a weight of 43113.19 Daltons. The chromosomal locus was mapped by FISH to CFA 12. Northern blot analyses in healthy tissues revealed expression in lung. The complete characterisation of the canine RAGE-HMGB1 protein complex could serve as base for future clinical studies aimed at the development of blocking strategies to inhibit metastatic behaviour of canine and human tumours.

B430
Identification of SNPs in cattle using large-scale EST sequencing

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Large scale EST sequencing efforts in a number of agriculturally important species has provided extensive information on gene sequences and genetic variation (single nucleotide polymorphisms, SNPs) within genes. In this project, the aim of sequencing 300,000 cattle ESTs using clones from 20 different cDNA libraries will provide extensive information on SNPs within coding regions of the cattle genome. The 20 cDNA libraries were constructed using mRNA from 10 different animals chosen at random for each library among a set of 24 animals from the slaughterhouse to ensure presence of genetic variants among the obtained EST sequences. Currently, after trimming of vector sequences and removal of low quality sequences, approximately 115,000 EST sequences have been obtained. Assembly of these sequences using a Phred/Phrap pipeline resulted in a total of 6,730 contigs. Analysis using PolyBayes on a subset (approx. 500) of these contigs revealed the presence of around 1,700 SNPs predicted in-silico with a p-value larger than 0.9. The A/G transition is the most abundant (approx. 29% of the identified SNPs) while the A/T transversion is almost as abundant with approx. 25% of the identified in-silico SNPs. The preliminary analysis of a subset of the contigs revealed the presence of a large number of SNPs showing that the method of combining mRNA from different animals in the construction of the cDNA libraries ensures the presence of large amounts of genetic variation in the obtained EST sequences. The identified SNPs will enable fine mapping of specific chromosomal regions of interest for QTL identification. The large number of in-house sequenced cDNA clones, which will be annotated according to the latest bovine genome assembly (www.ensembl.org/Bos_taurus), will also enable construction of a comprehensive cDNA based microarray for gene expression studies. The extensive information on gene sequences and possibilities for identification of splice variants of mRNAs will likewise provide a solid basis for the design of an oligo based cattle microarray enabling specific gene expression studies as well as studies on the differences between splice variants.

B438
A bovine whole genome linkage disequilibrium map

THE ALBERTA BOVINE SNP CONSORTIUM

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We have created a bovine whole genome linkage disequilibrium (WGLD) map. The Illumina® BeadStation was used for high throughput genotyping and allowed the development of two SNP assays which included a total of 3072 SNPs. These multiplexed SNP assays were used for high throughput radiation hybrid mapping, using the Roslin-Cambridge 3000 Rad bovine-hamster whole genome radiation hybrid panel to determine the map location of each SNP. Linkage disequilbrium was assessed using r^2 among all pairs of syntenic markers within each breed of cattle from *Bos taurus* and *Bos indicus* subspecies, including Angus, Charolais, Holstein, Brahman, Japanese Black, Limousin, Nelore and Dutch Black and White dairy cattle to create the LD maps. This enabled the analysis of variation in allele frequency and also the identification of common allelic variants between breeds. Mean minor allele frequencies among these SNPs ranged from 0.117 in Nelore to 0.269 in Charolais with 1431 loci being polymorphic in all breeds. Mean r^2 values ranged from 0.122 in Dutch Black and White dairy cattle to 0.286 in Brahman. This resource can be used in studies to associate genetic variation with economically important traits while simultaneously allowing further analysis of long range linkage disequilibrium in cattle.

Obs.: All members of the group will be listed on the poster.

B441
Evidence for a separate lineage of the insulin gene in tetrapods and fish

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While insulin plays a role in maintaining glucose homeostasis in fish and mammals, there are several differences in insulin physiology between both groups. For example, the metabolic regulatory roles of insulin and insulin like growth factor-I (IGF1) are not as differentiated in fish as in mammals, and insulin has a strong growth promoting effect in fish. Bony fish contain two insulin genes (*INS1*, *INS2*) that contain sequence similarity to mammalian, avian, and amphibian insulin. The channel catfish *INS1* and *INS2* genes were mapped to linkage group U26 and separated by 41 cM, but were unlinked to *IGF2* or *IGF1*. Catfish, zebrafish, and pufferfish contain two paralogous groups: *IGF2-TH-MyoD* and *IGF1-TH2-Myf5*, and the fish *INS* genes are not associated with either of these groups. The same two paralogues are present in human, cattle, and chicken, except that *INS* is located between *IGF2* and *TH* (a 30-50kb region). This data provides evidence to hypothesize an ancestral genomic region containing genes for an insulin-like growth factor (*IGF*), an aromatic amino acid hydoxylase (*AAH*), and a myogenic regulatory factor (*MRF*) which was duplicated before the divergence of fish and tetrapods. One hypothesis of insulin gene origin is that *INS* originally existed between the *IGF* and *AAH* genes in an ancestral vertebrate, then after duplication of the *IGF-AAH-MRF* region the *INS* gene was deleted from the *IGF1-TH2-Myf5* paralogue before the fish-tetrapod divergence. This scenario would then require translocation of fish *INS* from the *IGF2-TH-MyoD* region to a new genomic location after the fish-tetrapod divergence. A more parsimonius hypothesis is that an ancestral vertebrate *INS* existed separately from the *IGF-TH-AAH* region (perhaps originally derived from *IGF*). After the differentiation between fish and tetrapods, the tetrapod-specific *INS* gene arose as a duplication and conversion of *IGF2*, and the original *INS* in tetrapods experienced degenerative mutation due to lack of selection pressure. Localization of *INS* in separate chromosomal regions between fish and tetrapods might have led to differential gene regulation that contributed to physiological differences in glucose metabolism between fish and tetrapods.

B455
CIGENE – a core facility for detection, typing and interpretation of SNPs

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Hosted by the Norwegian University of Life Sciences the Centre for Integrative Genetics (CIGENE) is a national core facility established for the detection, typing and interpretation of single nucleotide polymorphisms (SNPs). SNPs are point mutations within the genome that are stable, frequent, well distributed and highly suitable for high-throughput genotyping. Based upon an integrative genetics approach CIGENE seeks to contribute to a deep, causal understanding of an organism's complex genetic characters for scientific and commercial exploitation. We perform SNP detection by resequencing followed by multiple alignments, and offer SNP validation and genotyping using both MassARRAY (Sequenom) and SNPlex (Applied Biosystems) technologies. In the near future we will also implement Affymetrix's molecular inversion probe (MIP) technology. In addition to supporting internal projects the CIGENE core facility is used extensively by external institutions. Consequently we are actively involved in projects investigating issues related to various plants, fish and animals, as well as humans. With respect to domesticated animals, our projects typically focus upon economically important traits in cattle (meat quality, milk production traits, mastitis, polledness), goats (casein studies), sheep (meat quality), pigs (meat quality, hernia, boar taint) and salmon (meat quality, meat colour, parasite resistance), but the projects can also be more investigative (e.g. studies of duplicated regions in the genomes in salmon, aging studies in honeybees and the production of chimeric honeybees). In addition CIGENE is one of the major participants in the Consortium for Genomic Research on All Salmonids Project (cGRASP).

B466

A radiation hybrid map of GGA25, a microchromosome absent from the first draft chicken genome assembly.

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In an effort to develop maps for microchromosomes missing in the chicken genome assembly, we develop RH maps with markers from the "unknown" fraction (chrun) of the sequence, using several criteria including the size of the supercontigs, information on conservation of synteny with human and (G+C) %. As a test case, the region between 145-160 Mb of HSA1 was selected on the basis of a sequence similarity to a high number of chicken chrun fragments, suggesting a region of conserved synteny missing in the chicken assembly. RH markers were developed from these chrun supercontigs and a RH linkage group could be built. BAC screening with the RH markers and FISH mapping attributed this linkage group to GGA25, previously unidentified in the sequence, apart for 231 kb corresponding to the genetic map linkage group E26C13. The length of the GGA25 RH framework map is 265 cR, suggesting a physical length of about 13 Mb for the chromosome. At the time of writing, the map is composed of 67 markers, including 15 genes or EST, whose location was previously unknown. By screening the Wageningen BAC library, positive clones could not be found for 5 out of 12 markers (42 %), suggesting a cloning bias for this chromosome. The GGA25 RH map was used to develop SNP markers for mapping on the reference populations. The genetic map thus obtained covers about 100 cM, demonstrating that more than half of this chromosome was overlooked when performing genome scans with markers from the E26C13 linkage group. The RH map of GGA25 can be viewed alongside all the other chicken RH maps at the ChickRH web site: http://chickrh.toulouse.inra.fr/.

B471

High-resolution comprehensive radiation hybrid maps of the porcine chromosome 2p and 9p compared with the human chromosome 11

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We are constructing high-resolution, chromosomal "test" maps for the entire pig genome using a 12,000-rad WG-RH panel ($IMNpRH2_{12,000\text{-}rad}$) to provide a scaffold for the rapid assembly of the porcine genome sequence. Here we present an initial, comparative map of human chromosome (HSA) 11 with pig chromosomes (SSC) 2p and 9p. A total of 590 markers, including 131 microsatellites (MS), 364 genes/ESTs, and 95 BESs were typed on the $IMNpRH2_{12,000\text{-}rad}$ RH panel, 273 of which were previously typed on the $IMpRH_{7000rad}$ panel. By taking advantage of the data set merging function of the CarthaGene software, we were able to rapidly and accurately construct a high-resolution framework map using the order of the common markers on both maps. This provided a platform to integrate the $IMNpRH_{12,000\text{-}rad}$ map with the $IMpRH_{7000\text{-}rad}$ map as well as the BAC fingerprint contig (FPC) maps (http://www.sanger.ac.uk/Projects/S_scrofa/), and rapidly close potential gaps between contigs prior to sequencing and assembly. At a LOD score of 10, two large linkage groups of 72 and 194 markers map to SSC2p, and two linkage groups of 84 and 168 markers map to SSC9p. Except for MS loci, approximately 91% of the typed markers have a homologous sequence (at a cutoff E-value of $1.0e^{-25}$) on HSA11 (Build 35). The comparative map indicated that although HSA11 is entirely conserved with SSC2p and 9p, significant macro-rearrangements and micro-rearrangements are present within individual synteny blocks. This work was supported by USDA, CSREES (No. 2004-35205-14244).

B487

Mapping, integration and characterization of some coat color genes in the American mink (*Mustela vison*).

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The American mink represents an economically important species especially in North Europe and North America. Within the last 2 years, newly developed microsatellite markers have facilitated the development of a genetic map, partially integrated to the physical one by means of a Chinese hamster/American mink somatic hybrid panel as well as few cosmids developed for the mink genome. This map can now be used for mapping and identification of genes that modulate monogenic traits as well as for the identification of chromosomal regions which contain genes having a major effect on economically important traits (QTL). The identification of the genetic bases for the fur color genes is one of the top aims in the first step of using this genetic map. Thus, along with the newly developed linkage map for the American mink a number of color genes have been mapped within the genome. In some families in which color genes are segregating we were able to assign and map some of the color genes of interest for this specie. The 21 offspring's from litters of 4 wild type dames back-crossed to a Pearl sire constituted the main family for genetic linkage analysis of 2 color genes: palomino and silver. The "silver" color gene has been genetically mapped on chromosome 3 and presumably associated with de "dilution" gene in dog. This is subject to further molecular investigation and genetic characterization. The "palomino" gene is expected to give linkage association and to be mapped and physically assigned to a chromosome. These are the first genetically mapped color genes for the American mink and constitute the beginning of an ample project involving resource families for many coat colors and quality features of the fur.

B490

Polymorphic Loci within the Ovine MHC Central Region

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The sheep MHC has been shown by several groups to be associated with immunity to gastrointestinal parasites. We identified BAC clones from the CHORI-243 ovine BAC library, which contain genes from within the central or class III region of the sheep MHC. In order to do this, 19 overgo primers from 12 genes were identified from DNA sequence alignment of human and mouse MHC class III gene sequences and used to screen the high-density filters from the BAC library. Positive clones were subcloned into pUC18 and random clones sequenced. End sequences were also obtained and assisted in the ordering of the BAC clones. Ten BAC clones were assembled into a contig, thereby providing the first ordered map of the central region of the ovine MHC. Analysis of the randomly cloned sequences has permitted the identification of 25 of 59 genes expected to be present in this region by comparison with other mammalian MHCs. These genes included *CYP21A2*, *APOM*, *ATP6V1G2*, *BAT2*, *BAT3*, *BAT5*, *CFB*, *C2*, *C4*, *G6b*, *G6d*, *G6f*, *HSPA1A*, *LTA*, *LTB*, *MSH5*, *NG22*, *NG36*, *NOTCH4*, *SK12W*, *TNFA*, *TNX* and *SnRNP*. Two gene sequences, complement Factor B and *TNFA*, were fully sequenced. Seventeen (17) single nucleotide polymorphisms (SNPs) were observed within eight genes covering this region. Very few polymorphic microsatellite sequences were identified in the analysis. From about 70 kb of sequence (10% of the entire region), only one polymorphic microsatellite locus (a dinucleotide) was found, which is considerably less than occurs in the human MHC central region. This study provides further evidence that the MHC central region III is evolutionally conserved in mammals in terms of genetic structure and content. Analysis of the SNP and microsatellite loci will assist in the identification of ovine MHC central region haplotypes, which will be of value in disease and productivity trait association studies.

B491

Characterization and validation of in silico developed SNP mapping to three different bovine genomic regions on 15 different cattle breeds.

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An SNP in silico detection was carried out on publicly available bovine genomic sequence data. Based on comparative mapping results three bovine regions were targeted : the whole bovine chromosome 3 (BTA03) and two regions of ~10 Mb mapping to BTA01 and BTA15. 1536 of the resulting candidate SNP were then genotyped using Illumina technology on ~2000 individuals belonging to fifteen different cattle breeds and also on 40 goats to evaluate across species conservation of polymorphisms. 900 (60%) of these 1536 in silico detected SNP were experimentally validated on the fifteen breeds. 300 could be genotyped on goat samples and 22 were polymorphic allowing the identification of the ancestral state of ~20% of the SNP. A meiotic map is currently constructed for the three targeted genomic regions based on the family structure of the Holstein population genotyped in this study. This will confirm the SNP predicted locations and permit the computation of haplotypes carried by each individual to compare the extent of LD on the different cattle breeds considered. This study will help to evaluate the power of SNP for fine mapping of genetic polymorphisms underlying traits of interest in cattle and the relationships among different cattle breeds.

B503

Identification of single nucleotide polymorphisms in pig sequence tagged-sites

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The objective of this study was to identify single-nucleotide polymorphisms (SNPs) in pig sequence tagged-sites (STS) generated from Type I (gene) amplicons. For SNP identification, we use a pool-and-sequence strategy in which a pool of DNA samples from different breeds is PCR-amplified and sequenced. SNPs are identified as ambiguous bands occurring at identical positions in a sequencing gel. For this study, we have screened 327 oligonucleotide primer pairs, developed 127 pig STS, placed 87 STS on a pig radiation hybrid (RH) map using the INRA-University of Minnesota porcine 7,000 rad RH panel, and identified 210 SNPs in 78 STS (1-6 SNPs per STS). PCR primers were designed using heterologous sequences and the identity of each STS was confirmed by DNA sequencing. Two-point analysis of RH data showed significant linkage of STS with markers on 12 different pig chromosomes. Genetic linkage analysis was performed using SNP genotypes in the USDA-ARS MARC reference families placing 19 STS onto eight different pig chromosomes. Genetic linkage analysis was also performed using SNP genotypes for nine additional STS in the PiGMaP reference families placing markers on five different pig chromosomes. Three of these STS (IGFBP2, PAX3 and PSMD1) are significantly linked to microsatellite markers at the distal end of SSC 15 in the order PAX3-(10 cM)-SW936-(11.2 cM)-IGFBP2-(14.2 cM)-SW119-(17.5 cM)-PSMD1. SNPs identified in this study contribute to the growing SNP collection for pigs and will aid in identifying genes responsible for genetic variation in economically important traits.

B512

Refined linkage mapping of the *Escherichia coli* F4ac receptor gene on pig chromosome 13.

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Enterotoxigenic *Escherichia coli* (ETEC) expressing F4 fimbriae are a common cause of diarrhea in newborn and weaned pigs. Of the three known fimbriae variants (F4ab, F4ac and F4ad), the F4ac is the most frequent. Susceptibility to ETEC F4ac adhesion is determined by a specific receptor (F4acR) on the brush borders in the small intestine and is dominantly inherited in the pig. The gene encoding the F4acR has previously been assigned to a region on pig chromosome 13q41. To refine the position of *F4acR*, a *Xba*I polymorphism in intron 7 of the Mucin4 gene (*MUC4*) and two newly developed microsatellite markers (*HSA125gt* and *MUC4gt*) were genotyped in Swiss and Nordic F4ac phenotyped resource families. The families were made-up of a combination of previously described material and recently analysed material. The refined linkage mapping of the F4acR gene was carried out using more than 650 pigs. Linkage analysis was performed using crimap. The most likely order of the loci was *SW207 – [F4acR – MUC4 – MUC4gt] – S0283 – HSA125gt – S0075 – SW1876 – SW225 – SW1030 – SW698 – SW398*. The order of the genotyped markers was in agreement with the order determined by their physical position on the BAC contig from the Porcine Genome Physical Mapping Project on the Sanger Institute web page. The most probable candidate region for *F4acR* is now narrowed down to the region between *SW207* and *S0283*, with the polymorphism in *MUC4* showing the closest linkage.

B520

An integrated RH map of the porcine genome with more than 5000 anchoring points on the human genome provides a framework for the sequencing of the pig.

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RH map and BAC physical map are the two frameworks that will guide the establishment of the porcine sequence. IMpRH panel has been widely used in the past 8 years and more than 10.000 markers have been mapped on this panel. Two reference maps were independently established. The first one was established using markers submitted by all contributors and publicly available in IMpRH database (http://imprh.toulouse.inra.fr), and one based on BAC ends mapped on RH panel and anchored on the human genome (Meyers et al, 2005). We present here the integration of both maps with the establishment of a 1000:1 framework map integrating on the map from Meyers et al as much as additional markers as possible. All additional markers associated with a sequence have also been mapped at their most likely location providing a set of dense ordered sequences that will be available to check the assembly of the porcine genome.

B521
Brindle coat color pattern in horses associated with embryo fusion and chimerism.

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Brindle is a rare horse coat color pattern. Typically, it is characterized by vertical dark striping on any color background. Owing to the striking appearance, owners have sought to reproduce the pattern through breeding but without success. Because of its rarity in horses, the genetic basis of brindle is unknown and difficult to study. It is also possible that the pattern may be heterogeneous in origin. Similar patterning is found in other mammalian species. Brindle is inherited as a dominant trait in dogs and cattle. An unusual case of an apparent parentage exclusion of a solid foal with two brindle parents led to investigations that provided evidence of embryo fusion and chimerism as a mechanism that produces this color pattern in horses. DNA extracted from blood and single hair roots from the mare and stallion was amplified for 12 microsatellites, a sexing marker and four coat color genes. For each animal, two distinct genotype profiles compatible with parental types were identified. The results showed the stallion to be an XX/XY chimera. Both cell populations were XX in the mare. Comparison of the stallion's genotypes with those of eight of his solid foals showed contribution of the male cell line only to sperm production, which is consistent with his development as a normal, fertile male. On the other hand, comparison of the mare's genotypes with two of her solid foals provided evidence of germ cell chimerism. These findings suggest that one form of brindle pattern in horses is not determined by a causative gene mutation but rather by a rare event of embryo fusion, chimerism and migration patterns of melanocytes that express contrasting coat color genes.

B522
General framework of the rabbit major histocompatibility complex.

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The major histocompatibility complex (MHC) region is one of the highest gene-dense region in mammalian genomes and comparative studies of MHC among Mammals is of high interest for speciation and evolution studies. The mapping and sequencing of the MHC locus has been completed for several species other than man, including primates, rodents, the cat or the pig. Our aim was to build a physical map of the rabbit MHC locus referred to as RLA (Rabbit Leukocyte Antigen) complex. We have already shown that the RLA complex maps to rabbit chromosome 12 at position q1.1 and we report here that the organization of the complex fits the general features of MHC in Mammals. A BAC library was screened by PCR with primers derived from rabbit sequences specific for DR, DQ, DM, DP class II genes as well as R19 and R27 sequences corresponding to RLA class I expressed genes. For the framework genes, the primers were mostly designed from human sequences. About 550 Kb of the class II region were spanned by two contigs from COL11A2 in the extended class II to DRA, with a gap close to TAP2. Another contig of about 900 Kb spanned the whole class III region and a first cluster of class I genes from BTNL to TCF19. Finally, an additional contig of about 200 Kb containing at least one class I gene was anchored close to TRIM 19. Interestingly, clones containing the R27 sequence were found only in the cluster adjacent to class III whereas clones containing the R19 sequence were split into the two clusters. We have not already identified a third class I cluster containing R19 or R27. Although the BAC library may be too small to contain the whole set of class I genes, our results suggest that the functional class I genes are distributed into only two clusters.

B533
An overview of the Bovine HapMap Project

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The International Bovine Hapmap Project was initiated in 2004 as a component of the whole genome sequencing effort. The objectives of the project are to: (1) discover single nucleotide polymorphisms (SNP); (2) validate at least 20,000 SNP by genotyping a panel representing diverse Bos taurus and Bos indicus breeds; (3) to use the genotypic data to infer common haplotypes; (4) to estimate linkage disequilibrium; and (5) examine diversity among breeds. As part of the first objective, nearly 350,000 shotgun sequences were generated from six cows representing Angus, Brahman, Holstein, Jersey, Limousin, and Norwegian Red breeds. These sequences were then aligned to the draft genome sequence from the Hereford cow L1 Dominette 01449, resulting in a total of 118,000 putative SNP. Additional putative SNP were identified from EST data and from comparison of BAC end sequences derived from Holstein animals. The second objective is in progress and will genotype breed panels with 20-50 individuals per breed. Each breed panel includes sets of trios to assist SNP validation and haplotype determination. Due to reduced costs, a total of 28,000 genome-wide SNP will be characterized. Initial results from approximately 10,000 SNP indicated that the existing coverage was insufficient to accurately examine local linkage disequilibrium. Consequently, an additional effort to discover and genotype high-density SNP was initiated. SNP discovery for this effort will use low-coverage BAC sequencing from the RPCI-42 library (derived from a Holstein bull) for selected regions on three chromosomes and a total of 4,500 SNP will be genotyped. The remaining objectives will proceed once genotypic data generation and the third whole genome sequence assembly are completed. Strategies for accomplishing these objectives will be discussed. Combined, the project will genotype approximately 33,000 SNP on 474 animals of 18 breeds, providing a resource for selection of SNP to support immediate application in high-density marker-assisted selection projects, QTL fine mapping efforts, and gene discovery research.

B551
Horse Genome Workshop: Update and Whole Genome Sequencing

ERNEST BAILEY[1] and Horse Genome Workshop

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Horse breeders have deliberately used genetic variation among horses for centuries to select for strength, endurance, speed, size, athletic ability, color, behavior and intelligence. Occasionally, genetic defects have appeared, notably those associated with developmental bone disease, muscle disease, allergic disease, reproductive dysfunction and resistance or susceptibility to infectious disease. The goal of the horse genome workshop has been to develop genomic tools that will make the horse breeder more effective in selecting for desirable traits in horses. Since 1995 the workshop participants have effectively collaborated to develop a comprehensive gene map for the horse. During 2006 a whole genome sequence for the horse will be completed for the horse under the auspices of the National Human Genome Research Institute (NHGRI) and through the work of the Broad Institute. At the time of writing of this abstract, the work is still underway and the extent of the sequencing is still being determined. The report associated with this abstract will describe the new resource and its immediate applications.

B553
An integrated map of the horse genome created using linear interpolation

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Several types of mapping data are available for the horse genome, including linkage maps, radiation hybrid maps, and markers mapped to chromosomal regions using *in-situ* hybridization technologies. Integrating the various mapping data types into a single map has allowed prediction of marker order and approximate kilobase distances between markers. Data for this integrated map of the horse genome was obtained from scientific publications between the years 1984 and 2006. It includes more than 730 radiation hybrid markers, more than 740 markers located using linkage, and more than 150 markers located using *in-situ* hybridization. This integrated map is available as both a visual representation of the horse genome and a public database that allows users to retrieve the predicted kilobase and band location of any marker and its position relative to other markers on the same chromosome. A map of the horse genome integrating all known genetic markers will be an invaluable resource in creating an accurate assembly of the horse genome. Integrated maps (such as the one described here) provide a guide to the location and orientation of sequence contigs. This integrated map was created using linear interpolation as implemented in the program LDB. Linear interpolation is not statistically rigorous but nevertheless shows very strong agreement with other more complex and rigorous integration techniques, such as the method implemented in Carthagene. The advantages of using LDB over Carthagene are the speed and ease of the calculation, the prediction of a kilobase and cytogenetic band location for each marker, and the ability to include *in-situ* hybridization data in the integration process.

B559
Development of a BAC based physical map of the channel catfish genome

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The commercial production of channel catfish, Ictalurus punctatus, is the largest enterprise in U.S. aquaculture, and the efficiency of genetic improvement in catfish populations will be improved by the identification of the chromosomal regions controlling economically important traits. Toward this goal we are striving to improve the resolution of the catfish genetic and physical maps. We have developed an initial physical map based on fluorescent fingerprinting (Luo et al., 2003) of a 7X genomic coverage of BAC clones from a gynogenetic fish. In parallel, we are producing end sequences from these clones. To date, approximately 37,000 BAC end sequencing reads have revealed over 9000 short tandem repeat (microsatellite) loci: 75.2% dinucleotide (avg. 17 repeats per locus), 15.5% trinucleotide (avg. 10 repeats per locus), 8.8% tetranucleotide (avg. 9 repeats per locus), and 0.5% pentanucleotide (avg. 10 repeats per locus). Microsatellites are highly polymorphic in the catfish genome, and the microsatellite loci in BAC end sequences will serve to increase the resolution of the genetic map and enhance our ability to integrate the catfish physical and genetic maps. We will present a progress report on the status of the physical map and the results of the BAC end sequence content analysis. These tools will be useful for gene identification and cloning, EST mapping, comparative genomic analyses, marker development for QTLs, and sequencing of the catfish genome.

SECTION C
Functional Genomics

C031

The progression of *Trypanosoma congolense* infection in susceptible and resistant mouse strains correlates with plasma cholesterol levels.

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As with breeds of cattle, laboratory inbred mouse strains show a range of susceptibility to *Trypansoma congolense* infection. For instance, the C57BL/6J strain is known to be tolerant with a mean survival time of 110 days while BALB/c is intermediate and A/J is the most susceptible of the three with mean survival times of 50 and 16 days respectively. There is increasing evidence that disease progression may depend upon the plasma cholesterol levels in the host. Decreased concentrations of total cholesterol, lipoproteins, and lipoprotein cholesterols occur in the course of trypanosomiasis in laboratory mice and may be due to decreased synthesis or increased catabolism of cholesterol. Secondly, high plasma cholesterol levels correlate with low parasite burden in inbred mice. Finally, gene expression analysis show that in the early stages of infection, the susceptible mice down regulate genes involved in cholesterol biosynthesis while up-regulating genes involved in cholesterol clearance through the bile acid pathway. Overall, there was a correlation between plasma cholesterol levels with progression of trypanosomiasis in inbred laboratory mice infected with *Trypanosoma congolense*.

C032

Association of the *BoLA-DRB3* alleles with breeding value for mastitis in Polish dairy cattle.

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Introducing resistance to mastitis into breeding programmes for dairy cattle seems to be one of the possible methods for limiting the increasing number of clinical and sub-clinical cases of udder inflammation and thus a method of improving the economic results of cattle husbandry and breeding. Looking for candidate genes is one of the strategies introducing resistance to mastitis into breeding programmes. Associations of the bovine major histocompatibility complex *DRB3* (*BoLA–DRB3*) gene with resistance/susceptibility to mastitis have been documented. The *BoLA–DRB3* alleles have considerable promise as potential mastitis marker. The objective of the study was to evaluate relationships between two *BoLA–DRB3* alleles (*BoLA-DRB3.2*16* and *BoLA-DRB3.2*23*) and estimated breeding value for somatic cell count (indication of the inflammation of the udder) in Polish dairy cattle. A total of 525 Polish Holstein cows in two experimental farms were evaluated for test-day somatic cell count. The polymorphism of *BoLA-DRB3* gene was identified using the MPT-PCR method. The variance components for test-day somatic cell count were estimated by the REML method using animal model. The breeding value of somatic cell count was calculated according to the BLUP method. The breeding value of the animals was estimated using model analogous to those used for the estimation of variance components. Statistical analysis accounted for fixed effects of herd, month and year of examination, lactation number, linear regression on milk yield and for the random effects of permanent environment and additive genetic effect of an animal. Linear contrast between model-adjusted breeding values of somatic cell count for all animals was used to test for differences between genotypes. In Polish Holstein cows population *BoLA* allele *DRB3.2*23* was associated with increase of estimated breeding value for somatic cell count in milk. It was observed increase of estimated breeding value for somatic cell count from cows carrying this allele comparing to cows carrying *BoLA-DRB3.2*16* (Pr>0.07) or other alleles (Pr<0.01).

C050

Gene expression profiling in relation to myo-genesis at different stages of muscle cell growth and development in the selected cattle breeds using cDNA-AFLP® technology

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The main objective of this study was to find out anonymous transcript derived fragments (TDFs) sequences and verification of these identified transcripts within the candidate functional genes for muscle growth and development trait in a cattle genome using an AFLP® (amplified fragment length polymorphism)-based cDNA technique (RNA fingerprints). The complete experimental procedure involved, RNA isolation from tissue samples, classification and categorization of the tissue samples from four breeds of cattle, screening and identifying the differentially display RNA fingerprints (TDFs sequences) in context to muscle growth and development within the classified group of four cattle breeds. The tissues samples were collected from 15 Polish black and White (HF), 15 Polish red cattle, 15 Hereford and 15 Limousine cattle, at the age of 6, 9 and 12 months (5 animals of each stages). Total RNA was isolated from animals slaughtered at 6, 9 and 12 months of age and used as templates for cDNA-AFLP® analysis. This technique allowed the amplification of anonymous target gene sequences by using *Pst*I^{+1}/*Mse*I^{+1} and *Eco*RI$^{+1\text{ or }+2}$/*Mse*I$^{+1\text{ or }+2}$ combinations to generate selective PCR amplification products. These selective PCR products were then separated by poly-acrylamide gel electrophoresis (PAGE) and detected using Global Edition IR^2 DNA Analyzer (LiCOR 4200). The detected fragments, so called TDFs, were then analyzed based on their differential display bands within and between breeds for the eventual excising, purification and sequencing to identify the putative expression sequence tags (ESTs) genes for muscle growth and development.

C066

Gene expression analysis in cattle resistant and susceptible to gastrointestinal nematode infections by real-time RT-PCR.

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Cytokines are proteins that play a central hole in immune mechanisms involved in defense against gastrointestinal nematodes infections. Several methods exist that allow quantification of cytokine expression in cattle, such as real-time RT-PCR (reverse transcriptase polymerase chain reaction). The present study used this methodology to quantify *Bos indicus* cytokines (IL-2, IL-4, IL-8, IL-12p35, IL-13, TNF-α, IFN-γ, MCP-1-monocyte chemoatractant protein, MCP-2) and the glycoprotein mucin-1 (MUC-1) in two groups of Nelore cattle: one resistant (R) and other susceptible (S) to this nematode. From a Nelore cattle (*Bos indicus*) herd, 100 young bulls were selected and kept together on pasture without anthelmintic treatment for at least 6 months. Seven resistant and eight susceptible animals were chosen based on fecal egg counts. At slaughter, number of *Haemonchus* was determined to confirm if the animals were resistant or susceptible to this nematode and abomasum tissue samples were collected. RT-PCR was performed using the LightCycler PCR and SYBR Green I dye. RPL-19 was used for normalization and the relative quantification of genes was calculated. Resistant animals had 9 fold less *Haemonchus* than susceptible ($P<0.01$). The expression of IL-4 ($P<0.05$) and IL-13 ($P<0.01$) was higher in resistant animals, while TNF-α was higher in susceptible animals ($P<0.01$). The higher IL-4 and IL-13 expression indicates a T_H2 response in resistant animals. This response permits the host to mount a protective reaction, facilitating the expulsion of parasites, while T_H1 response promotes susceptibility of the host, developing chronic infections. Although TNF-α is associated with both T_H1 and T_H2 response and increases the effects of IL-4 and IL-13, its expression was lower in the resistant than susceptible group without alter IL-4 and IL-13 expression. Others genes analyzed did not show statistical significant difference in expression between groups. So, it can be inferred that in resistant animals a T_H2-type response was activated. Study funded by FAPESP.

C071

Expression of transcription factors and adipogenic genes over the differentiation process of sheep omental and subcutaneous preadipocytes.

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Our previous studies shown that there are differences in the fat accretion process between depots during the lambs fattening period in a way that hyperplasia contributes more to the development of the subcutaneous than the omental depot. In order to investigate if the reported *in vivo* differences between those two depots referring hyperplasia are related to an intrinsic capacity of the depots to recruit new adipogenic cells, we have studied the level of gene expression of the main adipogenic markers involved in preadipocyte differentiation, using for that purpose primary omental and subcutaneous sheep preadipocyte cultures. The number of cells that followed the adipogenic program after induction was also assessed. In brief, stromal-vascular cells were obtained by collagenase digestion and after a period of proliferation were allowed to differentiate by adding serum-free differentiation induction media containing 1.6 μg/ml insulin, 2nM tri-iodothyronine, 10 nM dexamethasone and 10 μM rosiglitazone through the differentiation period (10 days). mRNA expression levels of C/EBPβ, PPARγ, C/EBPα, ADD1, lipoprotein lipase (LPL) and acetyl CoA carboxylase (ACC) were estimated by quantitative real time PCR. Relative gene expression was estimated by normalizing samples (three per treatment) against GAPDH or cyclophilin housekeeping genes, following the delta-delta Ct method. Number of undifferentiated and differentiated cells was assessed by flow cytometry. Expression of the main adipogenic transcription factors over the differentiation period followed this sequence: C/EBPβ, C/EBPα, PPARγ and ADD1. This was observed in in both depots, although the level of gene expression was higher in subcutaneous preadipocytes than in omental for the C/EBPα ($p<0.10$) and PPARγ ($p< 0.05$). Also the number of differentiated cells in the subcutaneous depot cultures was higher that in the omental ones ($p<0.05$), mirroring the *in vivo* observations.This results confirm that the preadipocytes response to differentiation factors is influenced by the anatomical depot and show that the lower differentiation reached by omental depot preadipocytes would be related to a lower activation of the C/EBP α-PPARγ factors.

C112

PPARGC1 mRNA expression in backfat and longissimus dorsi muscle in the pig

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Fat metabolism, especially the amount of intramuscular fat (IMF), is an important factor in determining meat quality. But it has proven difficult to select towards a lean carcass and at the same time maintain a sufficient amount of IMF to guarantee a good taste of the meat. One of the key regulators in fat metabolism is the peroxisome proliferative activated receptor γ coactivator 1α (PPARGC1). In the pig however, little is known about the expression of this possibly economical important gene, and it's very likely the expression will differ not only per animal, but also between tissues as well as within the same tissue. Therefore the mRNA expression of PPARGC1 was compared between backfat and 3 different locations of longissimus dorsi muscle of 50 slaughterhouse pigs using real-time PCR. To be able to compare the results between the different tissues and animals, the data have to be normalized with multiple reference genes, as has been extensively shown in literature. The mRNA expression of 10 reference genes (ACTB, B2M, GAPDH, HMBS, HPRT1, RPL13A, SDHA, TBP, TOP2B, YWHAZ) was analyzed using the geNorm program allowing for the selection of the most stably expressed genes. After normalization to the geometric mean of the selected reference genes, preliminary test results show a higher expression of PPARGC1 mRNA in longissimus dorsi muscle than in backfat.

C163

Isolation of differentially expressed genes in double-muscling Large White pig by screening of SSH library

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A subtracted longissimus muscle cDNA library was built to identify genes up-regulated in double-muscling Large White Pig (DM) compared to the non-double-muscling Large White Pig (NDM). All 686 single insertion clones in this library were sequenced. Using BLAST, the sequences were compared with the Genbank and Genbank EST databases and also the TIGR Porcine EST database. Eleven of the genes were not matched in any of the databases and thus might represent new genes related to porcine double-muscling trait. After differential screening was performed, 49 differentially expressed colonies were screened out from this library including insertions of RYR1, CAMK2, IGFBP-7, MHC2b, MHC2x, MyLC, α-TPM and LDH-A genes all of which are closely related to composition and development of skeletal muscle. Three of the functional genes, RYR1, CAMK2 and IGFBP-7, were chosen for quantitative PCR. The expression of these genes in the double-muscling pig longissimus muscle were 1.87, 1.90, 1.85 times higher, respectively, than in the corresponding non-double-muscling tissue. Three clones of the unidentified ESTs were also detected with quantitative PCR and they were found to 1.48, 1.44 and 1.78 times higher respectively in the double muscling pig. Northern Blot was also performed on MHC2b and MHC2x, which further demonstrated the differential expression of the genes. It has been reported that muscle fiber that expresses MHC2b and/or MHC2x is categorized as glycolytic fiber (FG), which have a larger size than either oxidative fiber (SO) or oxido-glycolytic fiber (FOG). We therefore suggest that higher expression of MHC2b and MHC2x in DM compared to NDM implies a higher percentage of FG fiber and greater conversional efficiency from SO to FG in the Double Muscling breed. These results imply that new candidate genes could be selected from the SSH library constructed in this research, and this could be a way to discover the genetic basis of the double-muscling pig.

C167

Subtracted full-length cDNA library, a powerful tool to identify transcripts associated with improved CLA production in bovine mammary gland

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Conjugated linoleic acid (CLA) is a group of geometrical and positional isomers of linoleic acid that can be found in milk fat. CLA has been reported to have potential benefits for human health due to its anti-carcinogenic, anti-atherogenic and anti-diabetogenic properties. However, the genetic factors involved in improved CLA production in the bovine mammary gland are largely unknown. In this study, a normalized/subtracted full-length cDNA library protocol has been applied to identify differently expressed functional genes from mammary gland tissues from cows which produced 6- to10-fold more CLA in the milk when fed a safflower oil/Monensin (SM) diet. Cross-subtracted libraries were constructed between the mammary gland tissues from SM diet fed cows (SM group) and control diet fed cows (CO group). Total RNA was extracted from five individuals from each group and pooled for cDNA synthesis and library construction. A standard full-length cDNA library from mammary gland was also constructed as a control. Three subtracted libraries were constructed by cross-subtracted hybridization of 1st strand cDNAs from SM with 3-fold pooled mRNAs from CO (subtracted library SM3) and 10-fold mRNA driver (subtracted library SM10), and 1st strand cDNAs from CO with pooled mRNAs from SM (subtracted library CO3). After sequencing 384, 1,200 and 384 clones from subtracted libraries SM3, SM10 and CO3 respectively, genes relevant to intra cell functions, such as genes coding for membrane signaling, and membrane transport were only identified in subtracted libraries SM3 and SM10. Genes associated with fatty acid binding protein and lactose synthesis was only identified in CO3. None of genes were identified in the control SM standard library. Our study suggests that those genes which are highly expressed in the SM tissue may affect network pathways required for CLA production in the mammary gland. To further identify the differentially expressed genes, sequence analysis of thousands of clones is in process and the global genetic factors which affect CLA production in SM-fed animals will be presented.

C168
Differential expression of genes in the bone marrow of layer and broiler chickens

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Osteoporosis is a major problem for the laying hen industry due to welfare issues and economic losses related to bone fractures. Bone integrity is known to be influenced by housing system, nutrition, production level and genetics. The objective of this research was to better understand the genetic component by comparing global gene expression profiles from bone marrow of chickens expressing phenotypic differences for a number of bone characteristics. Layer and broiler chickens were utilized for this experiment because significant differences in bone density and strength have been documented between them. Bone marrow samples were collected from the tibia of a total of 24 female birds (6 layers and 6 broilers at 15 and 60 weeks of age). Total RNA was extracted with Trizol, reverse transcribed to cDNA and labeled with one of two fluorescent dyes. The Fred Hutchinson Cancer Research Center Chicken 13K array was used to compare gene expression profiles from the layer and broiler lines. One layer and one broiler sample labeled with different dyes were hybridized to each array (6 arrays per time point). Dye swapping was used to control for dye bias, and data were analyzed separately for the two ages. Features with an intensity level greater than two times its local background for at least one hybridization were retained in the dataset. These data were normalized using lowess normalization and median centering across all hybridizations. P-values, q-values, and fold change estimates were obtained using a linear model analysis including line, dye, and slide effects. At 15 and 60 weeks, 53 and 85 features, respectively, were considered differentially expressed ($p \leq 0.05$ and $q \leq 0.25$). Genes with known importance in regulatory pathways for bone metabolism were found, including calmodulin at 15 weeks and calcineurin A beta and secreted frizzled related protein 1 at 60 weeks. Together these genes are known to have important roles in osteoblast and osteoclast differentiation, function, and survival that ultimately affect bone density. Further investigation of genes differentially expressed between layer and broiler lines will aid in understanding the genetic differences in regulation of bone integrity in chickens.

C169
Haplotypes within the bovine GHR, NPY, and UCP genes and their associations with measures of performance and carcass merit in beef cattle.

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The biological processes that influence growth, intake, and metabolism are believed to be under the control of multiple genes in animals. The regulation of these processes can affect economically important traits in beef production and therefore identification of polymorphisms within genes that show associations with production traits will have a practical implication for marker-assisted selection. In the present study, several single nucleotide polymorphisms (SNPs), in several bovine genes, have been identified. These genes include the growth hormone receptor (GHR), neuropeptide Y (NPY), and uncoupling protein 2 and 3 (UCP2 and UCP3). A range of two to six SNPs were found within each of these genes and haplotypes within each gene were constructed. These haplotypes were evaluated for associations with growth performance, feed efficiency, feeding behavior, and carcass merit in hybrid beef steers using PROC MIXED (SAS 9.1). Body weight was associated with haplotypes in NPY ($P < 0.01$), GHR and UCP ($P < 0.05$). ADG was associated with NPY and GHR haplotypes ($P < 0.05$). Marbling was associated with haplotypes in NPY and UCP ($P < 0.01$). Only UCP haplotypes were associated with backfat ($P < 0.05$) and lean meat yield ($P < 0.01$). Feed conversion ratio was associated with haplotypes in all three genes but NPY haplotypes were also associated with partial efficiency of growth ($P < 0.05$). The effects of the gene haplotypes on the traits were also estimated to be between 0.5 to 5.0%. These results have identified polymorphisms of candidate genes that affect beef cattle production traits and after confirmation in other populations these results can be applied to marker-assisted selection.

C171
Relationship between restricted fragment polymorphism (RFLP) at bovine cathepsin B and D *loci* and meat quality.

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Cathepsins D and B belong to lysosomal proteinases and are able to degrade intra- and extracellular proteins. Actin and myosin are the main miofibrillar proteins degraded by these enzymes. Cathepsin B (*CATB*) gene polymorphisms were studied in 263 bulls of Black-and-White (BW) and 114 bulls of five meat breeds (MB). In bovine *CATB* gene, new SNP was found in exon 7 (substitution C/T - no change in protein), position 540 (GenBank U16341), creates new *HpyCH4*III restriction site. *Eco*RI detected new SNP within intron 9 (transition C/T) at position 389 (GenBank AY639598). After *HpyCH4*III digestion three different *CATB* genotypes – CC, CT and TT were observed. The frequency of alleles C was 0.714 in BW and 0.707 in MB. The association between *CATB-HpyCH4*III polymorphism and the activity of lysosomal proteases (total acid autolytic activity - AAA, pepstatin-insensitive AAA - PIA and leupeptin-insensitive AAA – LIA) and meat quality traits (physical and chemical traits of meat, sensory evaluation score, pH) was studied in 76 BW bulls. All measured enzymatic activities (AAA, PIA and LIA) were higher in CC than in CT ($p \leq 0.05$) and TT genotypes ($p \leq 0.01$). Differences between CC and TT genotypes were about 13% in AAA, 17,4% in PIA and 15% in LIA. Total protein content was higher by 4,6% in TT than in CT animals ($p \leq 0.05$). The meat of TT bulls showed: the highest fat content, higher shear force and fat share in valuable cuts than meat of CC bulls ($p \leq 0.05$). *Eco*RI polymorphism within intron 9 was found only in BW bulls at position 389 with CC and CT genotypes (no TT genotype). The frequency of alleles were: 0.875 and 0.125.The bovine pro-cathepsin D gene (*CATD)* has SNP recognized with *Apa*I enzyme (G/A substitution; Gly/Ser change in pro-peptide). Only AA and GA genotypes were observed; allele A was more frequent (86,1%) than G. The *CATD*/*Apa*I SNP was associated with more tender meat (lower share force), aroma, taste and colour of the meat ($p \leq 0.05$) as well as higher cathepsin D (by 8%), pepstatin-sensitive CatD (by 6%) and LIA (by 12,5%) activities in genotype GA than that in AA animals.

C174
Analysis of novel equine defensins and psoriasin as another antimicrobial peptide.

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Defensins are evolutionary highly conserved cationic peptides with antimicrobial activities against bacteria, viruses and fungi. Defensins are components of the innate immunity, they protect organisms against infections and act as agents of the adaptive immune system. The present study deals with the investigation of a novel α-defensin, two novel β-defensins and the psoriasin of the horse, another antimicrobial peptide with a selective activity against *Escherichia coli.* Based on sequence informations primers were constructed for all four peptides. Total RNA of different horse tissues was isolated and mRNA expression was analysed by RT-PCR followed by agarose gel electrophoresis. Furthermore, the part of the cDNA coding for the α-defensin mature peptide was cloned into the pET-30 Xa/LIC, and the complete cDNA of the psoriasin into the pET-22 b vector. Both vectors were transformed into *E. coli* BL 21. The recombinant α-defensin was found to be expressed in inclusion bodies as a fusion protein with a N-terminal His-tag after induction with isopropyl-D-1-thiogalactopyranoside. The recombinant psoriasin was expressed as a soluble peptide and was purified by cation-exchange and size-exclusion chromatography. Finally, the recombinant psoriasin was tested for antimicrobial activity against different Gram-negative and Gram-positive bacterial strains by performing a radial-diffusion-assay. The activity of the psoriasin against different pathogens leads to the suggestion that it may influence infection deseases in the horse. In further investigations the antimicrobial properties of the recombinant α-defensin shall be investigated after protease digest of the fusion protein and refolding of the mature peptide.

C183
Sequencing analysis of regulatory enzyme genes in equine glycolysis

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The molecular mechanisms coordinating muscle glycogen utilization during exercise and its synthesis after exercise in horses are poorly understood. To elucidate the molecular mechanisms of carbohydrate metabolism in exercising horses, we determined the coding sequences of regulatory enzyme genes, type II hexokinase (HKII), muscle-type phosphofructokinase (PFKM), and M_1- and M_2-type pyruvate kinase (M_1- and M_2-PK) in glycolysis. Comparison of these equine enzyme genes with other mammalian HKII, PFKM, M_1- and M_2-PK homologs revealed more than 87.0%, 89.4%, 89.1% and 89.5% identity, respectively, for the nucleotide sequences in coding region, and 92.6%, 94.9%, 94.9% and 96.4% identity, respectively, for the deduced amino acid sequences. HKII had nonsynonymous nucleotide substitutions coding for potential binding sites of glucose. All deduced amino acid residues constituting various ligand binding sites in PFKM were identical to those of other mammals. Compared with corresponding amino acid residues from other mammals, M_1-PK had decreased hydrophobicity in exon-9, which codes for the intersubunit contact domain. In contrast, hydrophobicity of amino acids in M_2-PK was similar to that found in other mammals. Further investigation to clarify whether these genetic differences coding for amino acid substitutions affects catalytic properties in order to understand the distinctive carbohydrate metabolism present in horses is needed.

C186
Alleles of a bovine *DGAT1*-promoter variable number of tandem repeat associated with a milk fat QTL at chromosome 14 can stimulate gene expression.

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A quantitative trait locus (QTL) with a strong effect on milk fat percentage has been mapped to the centromeric end of bovine chromosome 14 (BTA14). This genomic region contains the *DGAT1* gene which encodes acyl-CoA:diacylglycerol acyltransferase 1, a key enzyme of triglyceride biosynthesis. Genetic and biochemical studies led to the identification of the non-conservative *DGAT1*-K232A polymorphism as causal mutation for the QTL. However, we recently found that in addition to this variant another polymorphism in the 5´-regulatory region of the gene, a variable number of tandem repeat (VNTR), also shows strong association with milk fat percentage. This promoter VNTR polymorphism affects the number of potential binding sites for the transcription factor Sp1 and might thus affect *DGAT1* expression and consequently the fat content of milk. Hence, the *DGAT1*-VNTR polymorphism could represent another allelic series of the BTA14 QTL. However, evidence for Sp1 binding to this polymorphic site and thus for the functional significance of *DGAT1*-VNTR alleles with regard to gene expression was lacking. In the current work, Sp1/VNTR interactions were analysed by electrophoretic mobility shift assays (EMSA). Additionally, effects of *DGAT1*-VNTR alleles on gene expression were measured using reporter gene analyses. Conclusions from the results are: 1) the *DGAT1*-VNTR sequence is indeed a target for Sp1 binding, 2) *DGAT1*-VNTR alleles can stimulate gene expression *in vitro* and probably *in vivo* as well, 3) although the stimulating effects of the different *DGAT1*-VNTR alleles did not show significant differences *in vitro*, their effects on transcription might be different in the chromatin context existing *in vivo*.

C189
Association of bFN1 with invasion of S. aureus in mammary epithelial cells

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Adherence to and invasion of epithelial cells in the bovine udder play a significant role in the pathogenesis of Staphylococcus aureus mastitis. Invasion of primary bovine mammary epithelial cells in vitro has been described for a limited number of S. aureus strains. Studies suggest that receptor - mediated endocytosis plays a role in the invasion of bovine mammary epithelial cells which prevent the host defense mechanisms and lead to the chronic nature of intramammary S. aureus infections. This may also explain the moderate success of antibiotic treatments for infection. In the present study, primary mammary epithelial cells of 24 cows belonging to different breeds and showing different udder health phenotypes were incubated with an S. aureus strain. The differences in invasion of the S. aureus were compared to polymorphisms in candidate genes located in QTL regions for mastitis. Fibronectins are high molecular weight glycoproteins which bind to integrins of the cell membrane and are involved in wound healing processes. Host cell fibronectin interacts with S. aureus fibronectin-binding protein (FnBP) and is a functional candidate gene for internalisation of S. aureus by epithelial cells. Bovine fibronectin 1 (bFN1) is located in the QTL region for somatic cell score in the German Holstein cattle population on chromosome BTA2 and therefore a positional candidate gene for mastitis. The SNP G/A at position 6987 in exon 46 showed association with the invasion in the 24 primary mammary epithelial cell cultures. Six cell lines from three different breeds that were homozygous for G, showed no or delayed invasion. Since both the G and the A base pair encode the same amino acid, the association is probably due to linkage.

C198
Transcript profiling of chondrocytes in a porcine impact injury model of osteoarthritis

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Osteoarthritis (OA) is a severely debilitating condition that affects articular joints resulting in cartilage degradation over time with no precise known causes. While the final stages of OA are well documented, very little is known about the early tissue changes that start cartilage down the degenerative pathway. Joint injury is a known predisposing factor making injury models useful tools in the study of early joint degeneration. We recently developed an *in vitro* injury model using impaction to the articular surface of intact porcine patellae with subsequent organ culture to examine early degenerative changes in the tissue. Understanding the chondrocyte's response is critical to understanding the progression of tissue degeneration and determining the potential for repair. Morphological changes in the development of OA have been documented in various models, but the molecular mechanisms underlying degeneration are mostly unknown. Knowing the changes that occur in chondrocyte gene expression following an impact injury will provide insights into the progression of early tissue changes that result in later degeneration. We are examining chondrocyte gene expression from impacted (2000 N load) and non-impacted control cartilage two weeks post impaction. A Serial Analysis of Gene Expression (SAGE) library from impacted tissue was constructed and approximately 11,000 tags sequenced. Four transcripts were highly expressed, representing 10% of SAGE tags. Expression of these transcripts has previously been reported in cartilage and they are thought to be involved in inflammatory response and tissue remodeling; however the significance of these findings is pending until compared with the non-impacted library. The control library is under construction and will be sequenced. SAGE tags from both libraries will be compared to identify putative differentially expressed genes. If we can gain a better understanding of the early degenerative changes that precede OA we will be better able to treat the disease and prevent the debilitating changes that occur further down the road. Controlling the early degenerative changes holds the most promise for preventing or mitigating the disease process.

C215
Differential prion protein mRNA expression in sheep blood

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Transmissible spongiform encephalopathy or prion diseases are a group of fatal, transmissible, neurodegenerative diseases occurring in human (e.g. vCJD) and animals (e.g. scrapie in sheep and BSE in cattle). These complex diseases are characterized by an accumulation in the central nervous system of PrP^{Sc}, a disease-causing isoform of the host encoded cellular prion protein, termed PrP^{C}, whose expression is necessary for the formation of PrP^{Sc}. Because PrP^{C} expressed in blood is involved in the pathogenesis, the PrP^{C} mRNA expression level was measured in triple in blood from 234 sheep using a quantitative real-time PCR assay based on SYBR Green detection, according to the standard operating protocol guidelines (e.g. DNase treatment, minus RT-control). PrP^{C} could be detected in only 10% of the samples. To correct possible experimental variation, proper normalization of the cDNA input of all samples was performed using 3 stably expressed reference (housekeeping) genes (viz. RPL13A, RPS18 and UBC), chosen from a panel of 9 tested candidate reference genes using the geNorm algorithm. Our gene expression results show true biological variation of PrP^{C} in sheep blood cells. To look for possible polymorphisms related to the observed variable expression, sequencing of the complete prion protein gene is currently carried out for 20 pairs of sheep with differential PrP^{C} expression, originating from the same flock, including 7 different races (Ardense Voskop, Bleu du Maine, Hampshire Down, Rouge de l'Ouest, Suffolk, Texel and Vlaams Kuddeschaap) and possessing the same genotype (including the 5 predominant alleles, ARR, ARH, AHQ, ARQ and VRQ).

C216
Identification of candidate genes for Brazilian chicken lines

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Years of divergent selection have produced chicken lines based on phenotypic characteristics. A considerable impact in skeletal muscle quantity and quality was reached in the broiler chicken lines using traditional methods of selection. But to date, few specific genetic factors have been identified that can account for these differences between chicken lines. To search for candidate genes that may elucidate the biological mechanisms associated with these growth rates and other differences between chicken lines, a macroarray platform containing 4,534 precursor/mature skeletal muscle genes was screened using isotopicaly labeled cDNA from *pectoralis* muscle of layer and broiler Brazilian chicken lines. A total of 99 genes were identified as differentially expressed ($P<0.05$), being 62 up-regulated in the broiler line and 37 in the layer one. QRT-PCR was used to confirm this expression data. The broiler up-regulated genes have suggested changes in the expression level of genes associated with growth and cell signaling, as a result of phenotypic selection. The search for the genetic position has indicated that some of the up-regulated genes are localized inside previously mapped QTL for the chicken genome (using QTL described for the lines used in this study and for different lines). Among the 62 broiler up-regulated genes, 23 were localized inside the chromosomes 1 to 9 (few markers and QTL information is still available for the other chicken micro-chromosomes). Of these, 17 have been positioned inside previously QTL defined regions, being 7 of them mapped for yield and five for body weight. From the 37 layer up-regulated genes, 18 have been localized inside the chromosomes 1 to 8 and, of these, 9 were inside QTL, some mapped for egg number and disease resistance. Together, these results have suggested novel candidate genes, that can be useful in the comprehension of molecular consequences of selection programs and also to improve the mechanisms of animal selection for growth rates and other economic traits.

C217
Fibronectin and cytokeratin 18 are markers for bovine blastocyst formation

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Normal preimplantation embryo development is characterized by three major transitions: (1) activation of the embryonic genome around the 8-16 cell stage, (2) compaction and (3) blastocoel formation leading to the blastocyst. At the blastocyst stage, the first important differentiation event takes place, resulting in the generation of two distinct cell lineages: the trophectoderm cells (TE) which will form the placenta and the inner cell mass (ICM) giving rise to the embryo. At this point of development, a high percentage of in vitro embryonic mortality occurs. In a first study, candidate marker genes for blastocyst formation and differentiation were selected by suppression subtractive hybridization. The RNA transcription patterns of two of the candidate genes, cytokeratin 18 (KRT18) and fibronectin (FN1) were identified by real-time qPCR throughout 4 stages of in vitro preimplantation embryo development (2-cell, 8-cell, blastocyst and hatched blastocyst). mRNA of both genes was found to be absent at the 2-cell and 8-cell stage but transcribed at relatively high levels at the blastocyst and hatched blastocyst stage. In a second study, protein expression analysis by immunostaining confirmed the differential expression of KRT18 and FN1 during in vitro bovine embryonic development. Both KRT18 and FN1 were first detected at the late morula stage and were highly expressed at the blastocyst stage. The expression of KRT18 was restricted to the trophectoderm cells, whereas the FN1 expression was predominant for the inner cell mass. These results indicate that both FN1 and KRT18 can be seen as markers for blastocyst formation.

C235
Association of IGF2 gene polymorphism with meat and milk production traits in Polish Black-and-White (HF) cattle; imprinting status of IGF2 gene in bovine liver and mammary gland

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Insulin-like growth factor 2 (IGF2) belongs to a family of structurally related peptides, which also includes IGF1, insulin and relaxin. In cattle's genome the IGF2 gene was localized to chromosome 29, the same in which QTLs for milk and meat production traits were localized. In most human and animal fetal tissues only parental allele of the IGF2 gene is expressed, but in adult tissues the expression is usually bi-allelic. It is well known that IGF2 plays a crucial role in growth and differentiation of many tissues including muscle and mammary gland, thus it may influence milk and meat production traits in cattle. The effect was studied of a polymorphism in the coding region of the IGF2 gene, a C/T transition in exon 2, on milk and beef production traits in Polish Black-and-White (BW) cattle, with 70% or more HF blood. A total of 293 young bulls were genotyped using RFLP-*Bsr*I method. The frequency of CC, CT, and TT genotypes was 0.27, 0.48, and 0.25, respectively. Bulls were slaughtered at the age of 11 or 15 months and growth, feed conversion and carcass traits were examined. The relationship was shown between IGF2 gene CC genotype and significantly higher daily gain and meat content (%) in valuable cuts. Also genotyped were 238 dairy cows from the local experimental farm. The frequency of genotypes was CC (0.23), CT (0.49), and TT (0.28). The relationships were estimated between the IGF2 gene polymorphism and milk production traits. The CC genotype appeared significantly related to higher FCM, VCM values as well as with more dry matters and fats in cow's milk. Imprinting of the IGF2 gene was studied using cDNA sequencing and RFLP analyses performed on bulls' livers and mammary somatic cells of cows' milk. The results showed that in young bulls' liver mostly the parental allele of the IGF2 gene is expressed while in the mammary gland the IGF2 mRNA is equally expressed from both alleles – maternal and paternal.

C236
Analysis of association between prion protein haplotypes and faecal egg counts in Rhoen and Merinoland sheep experimentally infected with *Haemonchus contortus*

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Since the discovery of the influence of prion protein (PrP) haplotypes on ovine scrapie susceptibility, breeding programs with the aim to increase the frequency of the PrP haplotype $A_{136}R_{154}R_{171}$ (ARR/ARR) are requested by regulation 2003/100/EC in the member states of the European Community. The aim of this study was to test different PrP haplotypes for a possible association with nematode resistance in Rhoen (RH) and Merinoland (ML) sheep and their crosses (RH x ML, ML x RH). For this purpose, faecal egg counts (FEC) at 4 weeks *p.i.* in sheep orally infected at 12 weeks of age with 5000 infective third-stage larvae (L3) of the nematode *Haemonchus contortus* have been used to select in both breeds and their crossings a total of 80 samples from sheep with high and low FEC for PrP genotyping. Mean values of FEC were 8974 and 2539, respectively. Restriction-fragment-length-polymorphism-(RFLP)-analysis was employed to analyze the samples for polymorphisms at codons 136, 154 and 171 of PrP. The PrP haplotypes ARR, ARQ, AHQ and ARH were identified, while the VRQ haplotype was missing. Neither in all sheep analyzed nor within breeds or crossings, there was a difference in PrP haplotype frequencies between the groups with low and high FEC. Therefore, no association between the four PrP haplotypes found in this study and FEC can be assumed and breeding for the PrP haplotype ARR should not have an impact on genetic resistance to *Haemonchus contortus* in the analyzed sheep breeds and crosses.

C237
Testing the *BOULE* gene as a candidate for pig XX sex-reversal.

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In pigs, the XX sex-reversal condition is detected on 0.5% of the females and it's genetically inherited. A wide genome scan of 250 microsatellites, carried out on a selected pedigree with a high incidence of intersex, pointed out four candidate regions potentially involved in this trait. One of these regions (Ssc 15q23-q25) showed high evidences of linkage disequilibrium (LOD score = 4.5). Moreover, comparative mapping to the human genome, revealed homology with a region that harbors a gene exclusively expressed in the germ cells and involved in meiosis, the *BOULE* gene. This gene is a member of the *DAZ* family (*Deleted in AZoospermia*) encompassing more two genes, *DAZ* and *DAZL* (*DAZ-Like*). They encode for closely related RNA-binding proteins, which are required for germ cell development in several organisms and therefore for fertility. Deletions in the *DAZ* gene are among the most common causes of infertility in men, and in mice, disruption of the *DAZ* homolog, *Dazl*, interferes with germ cell development in both males and females. In the same way, disruption of *BOULE* orthologous in *Drosophila* and *C. elegans* leads to meiotic defects during the first meiotic division. Previous results showed that numerous phenotypes can be found in the same litter, ranging from pig females presenting ovarian failure but no sex-reversal, to XX males without ambiguities, and this can suggest that primary germ cells depletion might be on the origin of this pathology. This fact, together with the strong evidences of linkage disequilibrium found in the Ssc 15q23-q25 porcine region support the hypothesis of *BOULE* as being one of the genes responsible for XX sex-reversal in pigs. Therefore, sequencing of its complete ORF in normal and intersex pigs is presently under development.

C244
A bovine whole genome long oligonucleotide expression array

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The design and synthesis of 24,000 long oligonucleotide probes comprising the first generation bovine whole genome expression array has been completed. The array includes 16,846 probes designed from ESTs that were aligned to homologous vertebrate proteins and to the 6X bovine genome assembly (BGA). The protein alignment step allowed removal of chimeric ESTs and selection of ESTs that match protein coding genes. After grouping ESTs with homologous proteins, each group was assembled using the TIGR pipeline, TGICL, which includes pre-clustering (MEGABLAST) and assembly steps (CAP3). Alignment of the resulting contigs and singletons to the BGA indicated that repetitive elements were abundant among the protein-coding EST clusters, despite repeat removal using RepBase. To remove protein-coding transposable elements without inadvertently removing transcription factors, the contigs and singletons were searched for PFAM domains found in transposable elements, excluding zinc-finger domains. Following transposable element filtering, 36,547 clusters (22,740 contigs; 13,807 singletons) could be aligned to the BGA which grouped the clusters to form 16,849 unique gene loci. Probes were designed at Illumina from 16,846 ESTs derived from clusters aligned to unique genes. The following criteria were used in EST selection and probe design:1) predicted constitutive exon, 2) polymorphism avoidance, 3) minimal distance to 3' end of protein coding region, and 4) optimal Tm and specificity. Constitutive exons were predicted by identifying the regions of each gene's sequence with the highest EST coverage. To sample as many different genes as possible our goal was to represent one constitutive exon rather than multiple alternative exons per gene. The probe set was supplemented with oligos designed from 703 predicted RefSeq genes, 5943 reproductive tissue ESTs with a BGA but no protein alignment, and 504 +/- controls. Probes were annotated using descriptions of homologous proteins, including Gene Ontology. Probe sequences and annotations are available at http://www.bovineoligo.org.

C249
Generation and analysis of expressed sequence tags (ESTs) from a full-length enriched cDNA library of equine superficial digital flexor tendon (SDFT) tissue.

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Injury of locomotor system is a certain part of issues in health of performance horses. Especially, the main issue is that higher the performance means higher the possibility causing tendinitis, and tendinitis doesn't recover quickly. Furthermore, the development and the physiology of the tendon tissue is still uncertain though a new treatment method and preventive that uses the stem cell are examined now. We constructed a cDNA library for ESTs data collection using equine superficial digital flexor tendon (SDFT), the tendon likely breaks down during racing, as a material. After construction of the full-length enriched cDNA library, total 14400 clones were picked and performed 5'- single pass sequence. The data will be able to use for constructing microarrays or other expression analyses in tendon tissues, as well as sequence annotations in equine whole genome sequencing.

C251
Expression pattern of transcription factors in neonatal porcine hind limb muscle

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Congenital splay leg in piglets is thought to be a developmental retardation of skeletal muscles mostly affecting the hindlimbs. The genetic basis of the syndrome however, is still poorly understood. We have selected the genes coding for 8 transcription factors with functions in skeletal muscle development and/or with relationships to human diseases. Total RNA was prepared from M. biceps femoris of 22 newborn piglets (each 6 males and 5 females of the groups "healthy" and "splayleg"). After reverse transcription of the mRNA – population with random hexamer primers gene specific primers for each gene were used for Real – time – PCR. The individual expression of the 18S rRNA was used for standardization of the values. The expression of all investigated factors could be detected in neonatal hind limb muscle. Whereas all of members of the T-box family were expressed at similar levels in all categories other homeobox factors revealed significant sex-dependent expression differences. For two genes there was a significantly decreased expression observed in male splayleg piglets compared to their healthy counterparts. Correlation analyses of the expression values indicate different relationships between sexes as well as between splayleg condition and healthy piglets. Gene networks will be constructed to identify further candidates within regulatory cascades, especially factors that act as hubs for pathways in development and growth of skeletal muscle.

C262
Detection of Sense and Anti-sense Transcripts from *Ncam1* and *Prdx2* genes in mouse tissues by *in situ* hybridization

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Recently, non-coding RNAs including anti-sense transcripts from genes have been revealed in mouse and human, using bioinformatics analysis and microarray analysis. The non-coding RNAs are inferred to be involved in control of traits expression in animals including livestock. In order to obtain a clue to understand the functions of the non-coding RNAs, the expression sites of those RNAs in tissues would be important information. In the present study, therefore, using mouse as a model animal, we investigated expression site of sense and anti-sense transcripts from *Ncam1* and *Prdx2*, which had been demonstrated by bioinformatics analysis and microarray analysis. *Ncam1* is indicated to be involved GH secretion; and *Prdx2*, in NK cell activity as well as coping with oxidative insults. Sense/anti-sense expressions of *Ncam1* and *Prdx2* have been examined in tissues of day14 mouse embryos, and 8-week mice. Mouse embryos and tissues were fixed in 4% (w/v) paraformaldehyde. Then the fixed samples were embedded in paraffin to section 4 m thick. Sections were collected on glass-slides and subjected to *in situ* hybridization using DIG-labeled anti-sense or sense RNA as a probe. Hybridization signals were detected with the NBT/BCIP system. In day14 embryos, *Ncam1* sense transcripts were detected at low levels in brain, spinal cord, and lung, whereas *Ncam1* anti-sense transcripts were detected in heart and liver as well as in the regions for *Ncam1* sense transcripts. In 8-week mice, the sense transcripts were detected in brain and esophagus at low levels, whereas the anti-sense transcripts were detected in brain, esophagus, stomach and eyeball. It appears that a part of cells expressed the sense and anti-sense simultaneously, and that the other parts expressed sense or anti-sense. For *Prdx2*, sense transcripts were detected in brain of day14 embryo and 8-week mice, whereas anti-sense transcripts (equivalent to sense transcripts of *JunB*) were detected in brain and thymus of day14 embryo; and in brain, esophagus, stomach, eyeball, and thymus of 8-week mice.

C263
Comparative Functional Genomics: Endometrial gene expression profiling during the estrous cycle and early pregnancy of Yorkshire gilts using the Affymetrix porcine GeneChip™

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Comparative functional genomics provides a powerful way for discovering the molecular mechanisms involved in endometrial gene expression changes during pre-implantation development, a stage when high rates of embryonic mortality occur in several species. In this study, RNA from quadruplicate endometrium samples from two stages and two pregnancy statuses were profiled. Endometrium from Yorkshire gilts was collected on Days 12 and 15 of the estrous cycle and early pregnancy. (Uteri of all the gilts collected on Day 12 of pregnancy contain filamentous conceptuses). The Affymetrix porcine GeneChip™ was used to identify differentially expressed genes in endometrium between pregnant and cycling Yorkshire pigs. Using an ANOVA method, we found 1,147 genes showed differential expression between d12 pregnant and cycling endometrium ($P < 0.0032$; 5% false discovery rate). We compared these genes to existing human microarray data to identify differentially expressed genes in endometrium during the window of implantation (WOI) across species. Several genes, including SERPINB7, FOXO1A, GADD45A, P8, CSPG2, NID2, MHC class IA, and COL1A1, which are reported to increase expression during the putative WOI in human endometrium, also had significantly higher expression in d12 pregnant Yorkshire endometrium compared to d12 cycling samples. Genes such as SORD, SLC25A11, KRTHA8, MSX1, and HSP40, which are reported to decrease expression in the human endometrium, also showed down regulation in the porcine d12 pregnant endometrium compared to that of cycling pigs. The above genes may be interesting for further investigation of the mechanisms controlling implantation in porcine reproduction, as well as in comparative studies of this process.

C264
Lack of a strong NFkB-dependent transcriptional response during infection with *Salmonella enterica* serovar Typhimurium as compared to that observed in *S.* Choleraesuis infection.

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Salmonellosis is prevalent worldwide and is a food safety issue and production problem. Classic salmonellae of pigs are *S. enterica* serovar Choleraesuis (SC) and Typhimurium (ST), the latter also being a significant human pathogen. To understand the host transcriptional response to infection, total RNA was collected from mesenteric lymph nodes of uninfected pigs and pigs inoculated with ST or SC at 8, 24 or 48 hours post-inoculation (HPI) (n=24 pigs). Affymetrix porcine GeneChip® were used to identify genes responding to infection. An ANOVA analysis showed that, compared with uninfected pigs, 173 genes, 224 genes and 179 genes changed their expression level ($P<0.01$, Fold change (fc)>2, $q<0.24$) at 8HPI, 24HPI and 48HPI, respectively, during ST infection. During the SC infection, 150 genes, 251 genes and 1175 genes were differentially expressed at 8HPI, 24HPI and 48HPI respectively ($P<0.01$, fc>2, $q<0.26$). The transcriptionally-induced genes were further examined using PathwayStudio software to determine which immune pathways were up-regulated. In the SC infection, the expression of known NFkB-regulated genes, such as proinflammatory cytokines (IL1B, IL6, IFN-γ), chemokines (IL-8, MCP-1), adhesion molecules (ICAM1) and many other NFkB-dependent genes (e.g., IkBa, JUNB, COX-2, PTGES, IL1A, BCL2, ALOX12) increased significantly at either 24HPI or 48HPI. Verification by QPCR is underway for these genes. Interestingly, in the ST infection, only a few NFkB-dependent genes were transiently induced at 24HPI and most decreased their RNA levels from 24HPI to 48HPI. This indicated that NFkB-dependent pathways were evaded, or even suppressed, in pigs with ST infection relative to those with SC infection. These results suggest a mechanism by which infection with ST eludes the strong inflammatory response shown with SC infection, and progresses into a carrier state in the pig.

C272

Myosin heavy chain isoform transcript abundance and muscle fiber type distribution of *Longissimus Dorsi* in high and low performing pigs and in different pig breeds

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Muscle fibers types, characterized by the content of different myosin heavy chain (MYH) isoforms, are responsible for variation of growth performance and meat quality traits in farm animals. The objective of this study was to compare the muscle fiber type composition based on relative abundance of transcripts of MYH isoforms of animals showing high and low muscularity within breeds and among pig breeds of discordant muscle growth and meat quality. The animals used were from three purebreds of Pietrain, Duroc and Mongcai, and two crossbreds of Duroc x Pietrain (DUPI) and Duroc x Berlin Miniature Pigs (DUMI). Real-time PCR quantification of MYH isoform I, IIa, IIb and IIx showed that the relative expression of MYH IIb was higher in pigs with large muscle areas in both DUPI (69.6 vs 53.0%) and DUMI (60.5 vs 47.5%). In DUPI similar transcript levels of MYH I were found in both large and small muscle (14.7 and 15.2%) whereas in DUMI animals these figures were 18.4 and 33.5% ($p<0.05$). The extreme groups in the DUPI tend to differ in MYH IIa and IIx transcripts too. The comparison among breeds confirmed the trend of high MYH IIb transcript abundance going together with high muscularity. In Pietrain, Duroc, DUPI and DUMI abundance of MYH IIb accounted for more than half of the MYH transcripts (65.4%, 59.7%, 61.3%, 54.0%). Mongcai showed very low MYH IIb (11.4%) but high type I, IIa and IIx RNA levels (24.1, 28.5, 35.9%). High consistency was seen between fiber typing by ATPase staining and quantitative RT-PCR assays of MYH isoforms with significant correlations between corresponding pairs of type I, IIa and IIx/IIb ($r=0.71$, 0.67 and 0.52, respectively). All together, both methods were in accordance and indicated that IIb fibers are the most prominent in pigs having large eye muscle area. (This project was supported by the Federal Ministry of Education and Research, BMBF grant VNB02/B06, Germany).

C278

Expression profiles of micro-RNAs in swine muscle development.

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MicroRNAs (miRs) are small ~18-22 nucleotide-long non-coding RNAs that have been shown to control gene expression by inhibiting translation or targeting messenger RNA for degradation. MiRs have been implicated in control of development, tissue homeostasis, and immune response. We examined the expression profile of porcine miRs present in neonatal biceps femoris using a digital approach of direct sequencing cloned miRs and counting the number of clones for each unique sequence observed. A total of 3,456 clones were sequenced, yielding 3,700 tag sequences representing 600 unique putative miRs. Comparison of unique tags to miRbase identified 241 matches to known miRs, providing evidence that we had successfully cloned porcine miRs. Experiments to determine potential function of these miRs were initiated by comparing the expression profile of neonatal muscle to that derived from cultured porcine satellite cells (adult myoblasts). We sequenced 4,608 clones from a satellite cell miR library, yielding 5,374 tag sequences representing 1,557 unique putative miR sequences that included 339 tags matching annotated miRs in miRbase. Comparison of neonatal muscle and satellite cell miR expression revealed a substantially different miR profile, providing the first data to indicate potential roles of specific miRs in porcine muscle growth and differentiation. The differential expression data identifies targets for siRNA-directed interference with porcine myoblast proliferation and differentiation to probe the role of miRs in muscle development in swine.

C289

An intronic insertion in *KPL2* results in abnormal gene expression and causes the immotile short tail sperm defect in Finnish Large White

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The immotile short tail sperm (ISTS) defect is an autosomal recessive disease within the Finnish Yorkshire population. The defect is expressed in males as a shorter sperm tail length and immotile spermatozoa. Histological examination of spermatozoa from ISTS affected boars indicates that the axonemal complex is severely compromised. No adverse effects on reproductive performance of female relatives have been observed suggesting that other ciliated cell types, such as oviduct cilia, are not affected. Furthermore, cilia in respiratory specimens from affected boars are physiologically normal. Recently, the disease locus was mapped on porcine chromosome 16 within a 3 cM region. In order to identify candidate genes for the defect, we used porcine BAC clone sequences from the region for comparative mapping with the human genome. The disease-associated area was located to a 2 cM region on human chromosome 5p13.2. Polymorphisms from orthologous porcine genes (ESTs) within this region defined the disease-associated haplotype to include 8 genes in the human. Sequence analysis of the most probable candidate, *KPL2*, revealed the presence of an inserted retrotransposon within an intron. The insertion affects splicing of the *KPL2* transcript by causing skipping of the upstream exon, or by causing the inclusion of an intronic sequence as well as part of the insertion in the transcript. Both changes alter the reading frame leading to premature termination of translation. The aberrantly spliced exon is expressed predominantly in testicular tissue, which explains the tissue-specificity of the ISTS defect. These findings show that the *KPL2* gene is important for correct axoneme development and provide a novel insight into abnormal sperm development and infertility disorders. However, different splice variants of *KPL2* appear to be expressed in several tissues, suggesting a wider role for at least one of the putative *KPL2* isoforms.

C292

Breeding values of Holstein sires depending on the *DGAT1* polymorphism

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DGAT1 is a microsomal enzyme catalysing the final step of the triglycerid synthese, it plays the important role in metabolism of cellular glycerolipid. Few polymorphisms were described in the locus, in the coding part of the gene the important one is in the amino acid position 232 Lysin – Alanin. Analysing breeding values of 200 Holstein sires bred by VIT Verden for milk yield, we have found significant higher values of the genotype *AA* (811 kg) and *LA* (498 kg) than of the genotype *LL* (256 kg), the allele *A* had double breeding value, than the allele *L*. Vice versa, the breeding value for fat percentage was higher at the *LL* genotype than at the *AA*, also the fat yield of *LL* sires was higher. Similarly, the breeding value for protein percentage was higher at the *LL* genotype, but the breeding value for protein yield in kg was higher at *AA* sires due to their high breeding value for milk yield. The differences among genotypes in breeding values of protein and fat percentage and yield were significant. Finally, the relative breeding value milk (RZM) was evaluated, it is the selection index, defined as comprehensive breeding value of milk performance indicators involving the breeding values for fat and protein yield, it takes the breeding value for protein percentage also into account. The relative breeding value milk was 110 at *AA* sires, it decreased at sires with *LA* (107) and *LL* (104) genotypes, but the factor genotype was not significant, while the difference between alleles (*L* 106, *A* 109) was significant at $P<0.05$. Because the breeding values of *LA* genotype sires lied within the values of homozygous sires in all evaluated indicators, the intermediary heredity on the locus could be presupposed. Based on our analyses and repeated results of other authors, the locus of *DGAT1* is of great significance in breeding. *Supported by MSMT project MSM6007665806.*

C293
LEPR gene association analysis with backfat and growth traits in F_2 gilts from an Iberian x Meishan intercross

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LEPR is a candidate gene for traits related with fatness, growth and body composition, and it is located in a region of SSC6 where several QTL have been identified in different F_2 experimental crosses. The aims of this study were the analysis of QTL on SSC6 and the association study of *LEPR* gene variants with growth and fatness traits in an Iberian x Meishan intercross. All animals (21 F_0, 114 F_1 and 280 F_2 gilts) were genotyped for 8 microsatellites and 2 neutral SNPs on *MC1R* and *LHB* genes. One SNP located on exon 14 of the *LEPR* gene (Leu663Phe) was also genotyped by pyrosequencing. A linkage map was generated, in which the *LEPR* gene is located on position 119.8 cM. Traits analyzed were: live weight (LW) and backfat thickness (BFT). Records were measured at two different stages: (1) at the end of the fattening period (around 200 days of age); and (2) one week before farrowing. QTL scanning was performed with the Qxpak software. Statistical models included one or several QTL, the batch as fixed effect, and a covariate that depends on the trait analysed: the age (for the analysis of LW) or the live weight (for BFT). QTL detection results revealed at least 3 significant QTL on SSC6, with additive and dominant effects. The most significant QTL affects LW1, LW2 and BFT1 ($p<0.001$, $p<0.001$ and $p<0.05$, respectively), and is located on 119-127 cM (between markers *LEPR* and *Sw1328*). The two other QTL affect BFT2 and are located at 34 cM ($p<0.001$) and 156 cM ($p<0.01$). *LEPR* gene was analysed as positional candidate for the QTL detected on 119-127 cM with a marker-assisted association test, applying models that include one or two traits simultaneously. The joint results indicate a significant association of the *LEPR* exon 14 SNP with all the traits analysed ($p<0.05$), with the Iberian alleles increasing both growth and fatness. Results support previous evidences related with the functional implication of *LEPR* gene variants on productive traits.

C303
Chromosome location, molecular characterization, polymorphisms and expression of *G protein-coupled receptor 54* (*GPR54*) as a candidate gene for puberty in pigs

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G-protein coupled receptor (GPR54) is a member of the rhodopsin family and plays a key role in activating the pulsatile release of hypothalamic gonadotropin-releasing hormone, which is crucial for puberty onset. The aim of this study was to characterize *GPR54* and provide a starting point for evaluating the association of the variants in this gene with puberty in pigs. *GPR54* was assigned to SSC2q21-24 by radiation hybrid mapping, and the most significantly linked marker is *SW395* at a distance of 48 cR (LOD=5.95). An 1,438-bp full-length cDNA sequence was determined with 5' and 3' RACE assay, which has a 1200-bp open reading frame encoding a protein of 399 amino acids flanked by a 141-bp-long 5'UTR and a 3'UTR of 97 bp (DQ459346). We also obtained the full-length genomic DNA sequence of this gene (DQ459345). It spans a transcription unit of 3,349 bp, consisting of 5 exons and 4 introns. Seven single nucleotide polymorphisms were identified between Chinese and Western breeds by a comparative sequencing approach. Of them, one missense mutation (T245C) was identified in exon 1 with an exchange of leucine to proline. A total of 425 animals representing 7 Chinese breeds and 3 Western breeds were genotyped for the polymorphism. The results show that Chinese breeds have obviously higher frequency of the *C* allele than Western breeds while the *T* allele is fixed in Duroc and Landrace. Semi-quantitative RT-PCR assays were performed using 18 tissues, indicating that *GPR54* is expressed ubiquitously in pigs. However, transcripts are particularly abundant in adrenal, white adipose, prostate, testis, thymus and hypothalamus and little in lung and heart. The temporal expression profile and the expression difference between full-sib F_2 animals in a White Duroc × Erhuanlian intercross with extreme puberty phenotypes are being analyzed by Q-PCR. We have constructed the White Duroc × Erhuanlian resource population and the genome scanning in 680 F_2 gilts and 240 F_3 gilts for QTL mapping is under progress. This study developed the *GPR54* polymorphic markers, which will be integrated into the genome scanning for identifying QTL affecting the puberty.

C304
Pre- and postnatal hepatic expression profiles of two pig breeds differing in body composition: insight into functional networks of metabolic regulation

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The liver plays a central role in the regulation of the metabolic status and expenditure of energy and nutrients. The aim of this study was to get insight into functional networks of hepatic metabolic regulation by comparing liver expression profiles from prenatal stages to adult in pigs representing extreme body condition types. German Landrace (DL) and Pietrain (Pi) pigs differ considerably in their metabolic status, growth rate, body composition, as well as utilization and partitioning of nutrient and thus represent a valuable model. We displayed the expression profile of liver at three prenatal stages 35 dpc (days post conceptionem) (i.e. period of differentiation and haematopoietic activity), 63 dpc (i.e. period of metabolic activity), and 91dpc (i.e. period of glycogen accumulation), plus adult stage in these two breeds. Competitive hybridization of a 13,297 porcine 70-mer oligo microarray (Qiagen-Operon) and subsequent image analysis (ImageGene), normalization, statistical evaluation (TIGR) and annotation/pathway analyses (EASE) revealed functional networks that reflect the stage-specific major hepatic activities. Moreover, the microarray analysis indicated, that Pi tend to exhibit higher expression of genes involved in changes and development of tissues and cells while DL tend to show higher transcript abundance of gene involved in intracellular energy and metabolite conversion, especially of lipids. These trends are even visible at early stages of development. RT-qPCR performed on individual samples confirmed these observations exemplarily for a number of genes of lipid- and protein-metabolism.

C305
Analysis of *Vitamin D receptor* gene as candidate gene for hairless phenotype in Iberian pigs

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Although hairless pigs are rare, there are some known examples as Labco hairless, Kapia hairless and Iberian black hairless pigs, from Mexico/Yucatan, Tanna Island and Iberian Peninsula, respectively. These strains show different coat colours and are hairless or totally naked from birth. Hair growth consists of two phases: follicular morphogenesis and postnatal hair cycle and both are controlled by the interaction between the epidermis and the underlying mesenchyme that involves multiple gene families. Vitamin D receptor (VDR) and Hairless (HR) have been proposed to integrate the necessary molecular pathway for normal hair follicle cycling. VDR protein is a member of the nuclear receptor superfamily that mediates the action of 1,25-dihydroxyvitamin D3, and its presence is required for the initiation and postnatal hair follicular cycling. In the present study, *VDR* gene has been analyzed as candidate gene responsible of the hairless phenotype of an Iberian black strain (Guadyerbas). cDNA characterization of the porcine *VDR* gene, not previously reported, has been performed from RNA samples of Guadyerbas, Wild Boar, Duroc and Meishan skin samples. Primers design from human, mouse and rat conserved cDNA sequences has been carried out for the partial amplification and sequencing of the porcine cDNA. Physical mapping of this gene has been performed using a RH pig-hamster panel (INRA-Toulouse) and linkage mapping using a polymorphism detected on exon 9, genotyped by PCR-RFLP (*Nsp*I) in a F2 cross Iberian x Landrace, by using CRIMAP software. Both maps agree in the localization of *VDR* gene on porcine chromosome 5 (SSC5q25) as expected by human- pig comparative maps. Seven synonymous single nucleotide polymorphisms have been detected on exons 4 (1 SNP), 5 (2 SNPs), 6 (2 SNPs), 9 (1 SNP) and 10 (1 SNP), none of the alleles was exclusive and fixed in the hairless Guadyerbas strain. Complementary analysis of the regulatory and promoter regions of gene sequence, as well as expression analysis would help to discover the potential implication of the gene in the hairless phenotype.

C310

Allelic variation in porcine resistin (*RETN*) gene is associated with fatness traits in a Wild Boar x Meishan reference family

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Cloning and comparative sequencing of the porcine resistin (*RETN*) gene and 5' flanking region, located at 64 cM on SSC2, revealed 9 SNPs and 2 indels. A European Wild Boar x Meishan family encompassing 335 F_2 animals measured for 46 traits including growth, fat deposition and muscle accretion was scored for a *T2094C* polymorphism by means of *Mva*I PCR-RFLP. Two haplotypes (AM157180 and AM157181) segregated in the family while the imprinted QTL in *IGF2* was fixed. The founder boar was homozygote *Mva*I *BB* while Meishan F_0 sows segregated alleles *Mva*I *A* and *Mva*I *B*. Previous QTL mapping by Lee *et al.* (2003) did not reveal any QTL for fat accretion in the *MYOD - SW395* interval where *RETN* is located. Association analysis showed that the *Mva*I *A* allele of Meishan origin accompanied by the *1472A* allele encoding for 36*Ala* is associated with higher values for fat deposition traits (fat depth at 10^{th} rib, back fat depth at $13^{th}/14^{th}$ rib, loin fat depth, average back fat depth, back fat weight, ham subcutaneous fat weight, fat cuts, fat area at $13^{th}/14^{th}$ rib, shoulder subcutaneous fat weight, shoulder fat depth), growth rate (live weight at slaughter, half carcass weight), fat to meat ratio at $13^{th}/14^{th}$ rib, food consumption from 110 to 210 day and number of teats, as compared to allele *Mva*I *B* coupled with *1472G* allele encoding for 36*Thr*. Allelic variation within the *RETN* gene accounts for six percent of phenotypic variation of average back fat depth and fat depth at 10^{th} rib. The associations measured between *RETN* variants and fat deposition traits indicate mainly additive gene effects. Further experiments are needed to elucidate whether the association between alleles and trait values is caused by the nonsynonymous *G1473A* mutation causing the Ala36Thr amino acid substitution, allelic variation in the promoter region or by DNA variants in closely linked genes.

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C311

Preliminary results on the genetic background of meat quality differences between two muscles in Avileña Negra-Ibérica calves, using cDNA microarrays

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Differences in meat quality traits and muscle characteristics between two commercial cuts have been quantified in Avileña Negra-Ibérica beef calves. The most representative muscles from each cut are *Psoas major* and *Flexor digitorum*, respectively. Muscle tissues from four hundred male calves were collected at slaughter, frozen in liquid Nitrogen and stored at -80ºC. Samples from both muscles from ten of those animals, sired by five different bulls, were used to carry out the microarray experiment. Animals were chosen in an attempt to capture the existing genetic variability. In order to assess differentially expressed (DE) genes between muscles, an experimental loop design was developed with a total of 20 hybridizations, using a bovine muscle and fat cDNA microarray of 9.600 clones printed in duplicate. Raw data were captured and processed using the GenePix Pro 5.0 software. A series of FORTRAN 90 computer programs were developed to asses the quality of the raw data, to apply a systematic data acquisition, and to perform data normalization. Background-corrected intensity signals were log-transformed and analyzed using mixed-model equations in a Bayesian context. Systematic effects of array-block combination, dye channel, muscle, interaction between array and dye, and muscle by dye were included. Random effects of genes, array by gene, dye by gene, muscle by gene and animal were considered. For each gene, a measure of possible differential expression was obtained from a linear combination of the solutions for the gene by muscle interaction effect. Over two hundred clones were identified as DE. Genes behind these clones were mainly involved in glycolytic and oxidative metabolisms, bone and muscle development and contraction, gene expression and translation or signaling pathways. These genes are expected to have a major role in the determination of meat quality.

C312

Expression profiling of bovine macrophages exposed to a *Staphylococcus aureus* strain causing clinical mastitis.

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Mastitis is the most frequent and costly disease in dairy production. Control of mastitis is therefore of great importance both for obtaining cost effective dairy production, for reducing the use of antibiotics and for improving animal welfare. Mastitis is caused by bacterial infection of the udder, and a multitude of factors influence the progress of infection, including the load and pathogenisity of bacteria present in the environment, the milking hygiene and the immune status and genetics of the exposed animal. We have chosen to use a "simple" *in vitro* system as a model, and monitor changes in gene expression of bovine macrophages as a consequence of exposure to *Staphylococcus aureus* bacteria. Bovine macrophages are important players in the first line defence of the innate immune system and *Staphylococcus aureus* is the most frequent mastitis-causing agent in Norwegian dairy production. Monocytes were harvested from fresh blood samples of heifers, and left in culture to differentiate into macrophages before exposure of the cultures with live bacteria at a ratio of 1 bacterium per macrophage cell. Cells were harvested 2 and 6 hours after infection. RNA from exposed and control cells were isolated and will be hybridized to the 20k bovine cDNA array available at ARK Genomics in a dye balance design. Initial results from these experiments will be presented.

C317

Combining mouse mammary gene expression and comparative mapping for the identification of candidate genes for QTL of milk production traits in dairy cattle

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Many studies have found segregating quantitative trait loci (QTL) for milk production traits in different dairy cattle populations (reviewed by Khatkar et al., 2004) (http://bovineqtl.tamu.edu/index.html). We hybridized Affymetrix microarray (MGU74v2), containing 12,488 murine probes with RNA derived from mammary gland of virgin, pregnant, lactating and involuting C57BL/6J mice with a total of nine biological replicates. We combined microarray data from two additional studies that used the same design in mice with a total of 75 biological replicates (Clarkson et al., 2003; Stein et al., 2003). The same normalization and filtering was applied to each experiment using GeneSpring software. Analysis of variance with false discovery rate of 5% identified 216 differentially expressed genes along the four developmental stages, common to all three experiments. The genes were anchored to their bovine map positions through comparative mapping, and thus form a list of candidate genes for previously identified QTLs for milk production traits. We present a web tool that allows navigation between the map of QTL, the overlaid candidate genes and the visual presentation of their expression in the mammary array and in the gene atlas. Of the two identified genes that have been proven to affect milk production traits in dairy cattle, *DGAT1* and *ABCG2*, only the latter was upregulated at the onset of lactation. Thus, differentially expressed genes that map to the QTL critical regions might be prioritized for quantitative trait nucleotide (QTN) identification.

C329

Profiling of Bovine MicroRNAs from Immune related and Embryonic Tissues

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MicroRNAs (miR) are small non-coding RNA transcripts (~22 nucleotides long) that have been shown to modulate diverse cell functions by interfering with translation and/or targeting mRNA for degradation. Since there is no information regarding microRNAs in bovine, we characterized this new class of RNA using tissues important for immune response and embryonic development. Alignment of human microRNA stem loop sequences (mir) against the bovine genome resulted in identification of 221 predicted bovine mirs. We validated some of these predictions by constructing and sequencing 3,209 cDNA clones made from small RNA fractions of bovine embryo, thymus, small intestine, abomasum, and mesenteric lymph nodes. This strategy resulted in identification of 129 putative mature microRNAs (miRs). Of these, 100 aligned to known human miRs, supporting their designation as bona fide miRs. An additional seven aligned to the complementary arm of a known human miR, and thus are novel miRs. Interestingly, 22 that do not have matches to human mirs displayed characteristic mir secondary structures, and 11 of these show phylogenetic conservation among other vertebrate species. Clustering of different tissues based on miR expression showed that similar tissues (abomasal and mesenteric lymph nodes) grouped together, whereas the most distinct tissue (embryonic) formed an out-group, with others (small intestine and thymus) placed in-between in the hierarchical tree. Clustering formed four major groups of miR expression, with each cluster having a characteristic signature of being selectively expressed in one or more tissue types. The miR26a was the most abundant miR, expressed in all the five tissues; whereas in human studies for the same tissues, it has been shown to be present in varying levels from high to low. This study revealed interesting insights into bovine miRNA expression such as extent of mir sequence conservation, prevalence of miR26a in all libraries and discovery of novel miRs.

C336

Trait-dependent expression and association of ferritin heavy-chain (FTH1) with muscularity and meat quality in pigs

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The iron storage protein, ferritin, has a key role in iron metabolism, which is involved in early growth and development of pigs. The ferritin heavy-chain, FTH1, is one of two subunits of that are regulated at the transcriptional or translational levels. The objective of this study was to analyze association of this gene with body composition and meat quality traits in pigs. In the present study, four discordant sib-pairs (DUPI - Duroc x Pietrain F2 resource population) were selected for microarray hybridization basing on their extreme differences in loin eye muscle area. The FTH1 gene was found up-regulated in pigs having small eye muscle area. Results were further confirmed by real-time PCR with a significantly higher number of transcripts in low performing pigs ($P = 0.023$). In order to identify single nucleotide polymorphism (SNP), comparative sequencing was used and two SNPs were found within 3' UTR at position 698 (T/C) and 714 (G/A) of the sequence (GenBank accession number D15071). For genotyping, PCR-RFLP protocols were established using restriction enzymes MspI and BbuI for the first and the second SNP, respectively. In total, 334 animals were genotyped. Analysis of variance was done by proc mixed of SAS employing a model with genotype, sex, parity and family as fixed effects and carcass weight as covariate. It was observed that the second SNP (G/A) was significantly associated with shear force ($P = 0.027$). There was a tendency for the effect of SNP1 (T/C) on pH24 ($P = 0.055$). The mapping position of FTH1 is close to SWR783 on the p-arm of SSC2. This region contains several QTL for body composition and meat quality traits, e.g. loin eye area, drip loss and pH24. In conclusion, the FTH1 gene may be an important gene for meat tenderness. (This project was supported by the German Research Foundation, DFG grant FOR753 DRIP, Germany).

C339

Studies of *MyoD* family genes and *GH* and *IGF1* receptor genes regarding their polymorphisms and expression profile in muscle and liver in growing pigs*.

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The myogenic family of transcription factors (MyoD) plays an important regulatory role in development of skeletal muscle during embrio- and ontogenesis. There are four members of this family in vertebrates, myf-3, myf-4, myf-5 and myf-6 involved in proliferation and differentiation of cells into muscle. Our study aimed to analyze the expression profile of myogenic factors genes (MYF5, MYF6), gene encoding growth hormone receptor (GHR) and insuline-like growth factor 1 receptor (IGF1R) during ontogenesis. The level of mRNA of mentioned genes determined at different age of pigs could answer the question, if there are any age dependent relationship. At the first stage of experiment, the collected data were used to compare the levels of mRNA of *MYF5*, *MYF6*, *GHR* and *IGF1R* in 60 days and 180 days old pigs.

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C343

Differential gene expression analysis in the mammary gland of Spanish Churra ewes with distinct production status

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The mammary gland can be used as a model to analyse the control of gene expression in tissues undergoing different developmental, physiological or productive stages. We have studied the differential gene expression between sheep mammary glands of animals which are in three productive stages: dry period, lactating with low milk yield and lactating with high milk yield. A total of nine ewes belonging to the three productive or physiological groups were sampled. Total RNA from the mammary gland was extracted and its quality was verified. Differentially expressed genes were evidenced using differential display technique (*RNAimage kit*, GenHunter Corporation). For each RNA sample three different anchor primers were used in the reverse transcription step and eight arbitrary primers were employed during the amplification step. After polyacrylamide gel electrophoresis in denaturating conditions, 21 cDNA bands of interest were detected. These cDNAs were cloned and their nucleotide sequences were determined. Through basic local alignment search tool (BLAST) analysis, ten potentially interesting candidates were chosen and further characterized by Northern blot. The differential expression was confirmed in six candidates, with four transcripts showing no marked differences between groups and being considered as false positive. There were five up-regulated mRNAs which exhibited significant homology with milk proteins genes and genes related to energy metabolism in mammary cells. The down-regulated mRNA could be related with solute carrier family genes although the BLAST analysis was not conclusive enough. Therefore, additional studies need to be done to prove its significance.

C345

Comparative transcriptomic analysis of the immune response in Large White pigs differing by 20 years of selection.

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Selection has been intense for the last 20 years in the French Large White pig breed with good results in the improvement of production traits, but the impact on immune capacity of pigs is unknown. Our aim is to compare the immune status of a population corresponding to pigs obtained after *in vitro* insemination of present-day sows with frozen semen of sires born in 1976 to highly selected present-day pigs. We are developing a transcriptomic approach using both *in vivo* and *in vitro* models. Five animals from each population were selected according to their SLA haplotype and blood was sampled twice, before and three weeks after vaccination against the Pseudorabies virus (PrV). Peripheral blood mononuclear cells (PBMCs) were isolated for further RNA extraction and analysis of their transcriptome profile. We are developing a complementary *in vitro* model to mimic the innate immune response. Monocytes were extracted from PBMCs collected from non vaccinated animals by immunomagnetic sorting with anti-CD172 antibody and incubated with IL4 and GM-CSF for 72h for differentiation into immature dendritic cells (iDCs). The iDCs were further infected or mock-infected with the PrV for 18h before RNA extraction and microarrays hybridization. The phenotype of iDCs was checked for CD1, MHCII and CD80-86 by flow cytometry analysis. We used a chip with 1856 pig cDNA probes comprising genes of the SLA region and PrV genes. Although preliminary results obtained with the infected iDCs of three pigs from each population allowed us to characterize the transcriptome of PrV-infected iDCs, no differences were observed between the two populations in this *in vitro* model with these samples. New hybridization experiments with additional animals presenting a different set of SLA haplotype are planned. Comparative analysis of transcriptome profiles of PBMCs and mock-infected versus PrV-infected iDCs in the two populations will be presented.

C346

Characterization of genetic changes during adipose tissue development in beef cattle

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Fat related traits are of special significance as they are associated not only with quantity (marbling, total yield), but also with the quality (flavor, tenderness) of the meat. However, it is still unclear as to which factors affect the differentiation and development of fat in cattle. Adipose tissue consists of cells that have differentiated and become specialized in the synthesis and storage of fat. Adipose tissue plays important roles in energy metabolism and its function in cattle is known to differ from adipose tissue of human and rodent. Investigation of the expressed genes during adipocyte differentiation will supply fundamental information on fat formation in cattle. In this study, we investigated gene expression profiles during adipocyte development with a long oligo microarray representing approximately 8,700 genes. Preadipocytes isolated from cattle perimuscular fat deposit (kindly provided by Dr. Michael Dodson, Washington State University) were grown with or without addition of inducing factors, such as insulin, dexamethasone and acetic acid, until the treated cells reached maturity. After hybridization of Cy3 and Cy5 labeled cDNAs generated from “treated” and “untreated” cells with the microarray chip, 21, 27, 63 and 37 elements were identified as significantly differentially expressed genes from the “treated” cells on day 0, 2, 6 and 14, respectively. Energy metabolism or protein synthesis related genes were particularly up-regulated in “treated” cells on day 6. Furthermore, fat development related genes such as IGFBP-5 and C/EBP-β were only detected from day 14 treated cells. Our understanding of above genetic network will provide evidence for biochemical pathways of induction/reduction of fat development in multiple types of tissues in beef cattle.

C351

A mutation in the 3` untranslated region of the *OLR1* gene is associated with a major effect on milk composition

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Oxidized low-density lipoprotein receptor (OLR1) is the major protein that binds, internalizes, and degrades oxidized low-density lipoprotein. The role of OLR1 in lipid metabolism and the results of previous whole genome scan studies prompted us to investigate *OLR1* as a candidate gene affecting milk composition traits. Direct cDNA and genomic sequencing of *OLR1* revealed 2 single nucleotide polymorphisms (SNP) in exon 4, five SNP in intron 4, and one in the 3`untranslated region (UTR). Four intragenic haplotypes comprising these eight SNP were inferred. Haplotype analysis showed that one of the haplotypes was associated with a significant increase in fat yield (P = 0.0022) and fat percentage (P = 0.0066). Single SNP analysis showed that allele C of the 3` UTR SNP had significant effects on fat yield (P = 0.0005) and fat percentage (P = 0.0033) whereas other SNP had no significant effects. Both single SNP and haplotype analysis strongly indicate that the 3’ UTR SNP might be the causative mutation affecting milk fat yield and percentage. To provide support for our hypothesis that this SNP is the quantitative trait nucleotide (QTN) responsible for *OLR1* effects on fat yield and percentage, we assessed *OLR1* expression levels in individuals bearing different genotypes. We found that *OLR1* expression was reduced in genotype AA individuals compared to CC and AC individuals, suggesting that C allele may be the nucleotide causing increased *OLR1* expression. The 3`UTR polymorphism found in this study to affect milk fat yield and milk fat percentage might control translation or stability of *OLR1* mRNA.

C354

Comprehensive Search of Bovine MicroRNAs Expressed in Immune and Gut Tissues by Deep Sequencing

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MicroRNAs (miRs) are small RNA transcripts (~22 nt long) that modulate gene expression by interfering with protein synthesis and/or targeting mRNA for degradation. Only 332 miRs have been described for humans, while estimates based on prediction algorithms identify approximately 800 microRNA stem loop loci from human genome sequence. Because of the paucity of information on bovine miRs, we initiated characterization of the miR portion of the transcriptome. Our intial effort was focused on gut and immune-related tissues considering the potential role of miRs in regulating cellular proliferation, differentiation and immune response. Small RNA derived from bovine thymus, abomasal and mesenteric lymph nodes, spleen, bone marrow, Peyer’s patches, fundic abomasum, and small intestine was isolated by gel-based size fractionation. Samples were pooled to increase the potential diversity of miRs prior to ligation of 5’ and 3’ RNA adaptors and cDNA synthesis. This cDNA was cloned and sequenced by Massively Parallel Signature Sequencing (MPSS) to generate approximately 1 million sequence tags ranging from 17 to 20 nt in length. A total of 3,001 distinct tags were identified and these tags could be clustered into 1,692 sequences. Subsequent filtering against rRNA, tRNA and snoRNA (Small Nucleolar RNA) sequences and alignment to the bovine genome assembly yielded 1,252 tags potentially corresponding to miRs. Comparison of these 1,252 tags against miRBase resulted in the identification of 178 putative bovine miRs with 130 of these having very significant matches to known vertebrate miRs. The tags that matched miRBase were 83% and 68% of the total tag counts, respectively. The remainder of the sequence tags are being analyzed as potential novel miRs. These results indicate a moderate diversity of miR expression in adult cattle tissues and suggest that less intensive sequencing of different tissues at multiple developmental stages will be optimal for miR discovery and expression profiling.

C357
Linkage mapping of the silver coat color locus in the horse

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Silver dapple is a highly desirable coat color in some horse breeds. The phenotype shows an autosomal dominant inheritance in this species. The responsible mutation/s for this coat color only affect the eumelanin, which is diluted. A black silver horse has a slightly diluted body, often with dapples, and a shiny white mane and tail. In contrast, the body color of a bay silver horse stay more or less unchanged apart from the legs that are dark greyish, and the mane and tail that are diluted to a mixture of white and grey hairs. The aim of this study was to identify the gene and thereafter the mutation/s for the silver dapple coat color locus in the horse genome. Linkage mapping was performed within a half-sib family comprising one stallion with 34 offspring and 29 different non-silver colored mothers. Within the pedigree, we typed for 11 blood group and protein systems, and genotyped 40 microsatellite markers, as well as one single microsatellite marker (TKY284) close to a candidate gene called SILV on ECA6q23. In total, 52 markers were analyzed for pairwise linkage using the TWOPOINT option of the program CRI-MAP. Significant linkage was found between three pairs of markers within this pedigree; the silver phenotype and the marker TKY284 ($\theta = 0$, $z = 9.0$) at ECA6; ASB2 and Albumin ($\theta = 0.05$, $z = 4.6$) at ECA3; as well as COR075 and HMS1 ($\theta = 0.09$, $z = 3.0$) at ECA15. These data strongly support SILV as the gene responsible for the silver dapple phenotype in horses. This gene encodes a transmembrane protein called Pmel17. We are currently sequencing the SILV gene in silver and non-silver colored horses. So far, one SNP has been discovered with full association to the silver phenotype. Furthermore, studies are conducted to evaluate the consequences of the mutation for Pmel17 localization and function in cell culture models.

C362
An 8579-bp cDNA isolation, polymorphism identification and expression profile of the porcine *nuclear receptor corepressor 2 (NCOR2)* gene

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Nuclear receptor corepressor 2 (NCOR2) is a member of the N-CoR family and acts as a key corepressor protein in many pathways. NCOR2 and other transcription factors assemble a large protein complex to mediate transcription of targeted genes, such as those involved in morphogenesis, development, cell differentiation and gonada differentiation. Also, it can represses the activity of peroxisome proliferators-activated receptor-γ by docking Sirtuin and NCOR1, resulting in reduced fat storage in mice. We previously assigned *NCOR2* to SSC14q21 by radiation hybrid mapping. Here we report the full-length cDNA isolation, polymorphism identification and expression profile in different tissues of the porcine *NCOR2* gene. A 451-bp and 1144-bp fragments were amplified with 5' RACE and 3' RACE assay, respectively. A total of 23 primer pairs were designed according to the porcine ESTs or the homologous sequences between mammals, and then used to amplify a set of overlapping products covering the gap between the 5' and 3' RACE fragments. These amplicons were bidirectionly sequenced and aligned, providing an 8,579-bp full-length cDNA sequence. The porcine *NCOR2* gene is predicted to have a 7416-bp open reading frame encoding a protein of 2471 amino acids flanked by an 163-bp-long 5' UTR and an 1000-bp 3' UTR. Its genomic structure is clarified by comparison of the obtained mRNA sequence with human *NCOR2* DNA sequence. It consists of 47 exons and 46 introns with exon sizes ranging from 1249 to 53 bp. A 3-bp-deletion was identified in exon 43, leading to loss of a serine in the encoded protein. Approximate 400 unrelated animals from Chinese and Western breeds are being genotyped for the deletion. Semi-quantitative RT-PCR assays were performed using RNA from 18 porcine tissues. The results show that *NCOR2* is expressed ubiquitously in pigs. However, transcripts are particularly abundant in ovary, thymus and bladder and little in white adipose.

C365
Relative quantification of selected differentially expressed genes in porcine fetal skeletal muscles

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The aim of this study was to verify and quantify differential expression of selected genes in the porcine fetal skeletal muscles using the real-time quantitative PCR. On the basis of the sequences of clones obtained from the subtractive hybridization and the results of linkage and comparative mapping the specific primers were designed to analyse expression of genes *ACTC*, *CDK4*, *CNN3*, *GNAS*, *MYH3*, *POSTN*, *TP53BP1* and *YWHAQ*. The skeletal muscle-specific RNA was isolated from the adult Large White pig (m. biceps femoris) and the 50-day fetus (hind limbs) and the total RNA was reverse transcribed into cDNA. The samples were analysed using SYBR Green PCR Master Mix and ABI 7500 real-time PCR System. Relative quantities of samples were normalized against the relative quantities of the housekeeping genes *GAPDH*, *HPRT* and *PPIA*. It was found that all these genes were expressed in both the adult and the fetal samples. The expression levels of the investigated genes were significantly higher in the fetus than in the adult muscle and the relative ratios were approximately 13 000, 9 500, 4 300, 1 500, 800, 500, 72 and 16 for *POSTN*, *MYH3*, *ACTC*, *CDK4*, *CNN3*, *YWHAQ*, *TP53PB1* and *GNAS*, respectively. The study provides new knowledge on genes playing role in muscle development and growth in pigs, with the prospect of their application in selection.
(This work was supported by the Czech Science Foundation 523/06/1302)

C368
Isolation, location and expression of two *IL1* family members as possible candidate genes to *scrapie* in sheep.

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c
Scrapie (SC) is a transmissible spongiform encephalopathy (TSE) of sheep and goats. Susceptibility to this neurodegenerative disease is mainly controlled by point mutations at the *Prn-p* locus. Other genes, apart from *Prn-p*, have been reported to contribute to modulate susceptibility to SC. On the basis of several expression studies in human Alzheimer disease (AD) and in different TSE models, and taking into account that the correct homeostasis of cytokines is crucial in the brain, *Il-1B* and *Il1rn* were chosen as positional (at least in mice) and functional candidate genes that might be involved in the polygenic variance mentioned above. The first objective of this work was to determine the exact position of these two genes in the ovine genome in order to verify if their locations were in concordance with previous reports describing QTLs associated with susceptibility and incubation period for different TSEs in ovine and murine models. This was carried out by FISH, and confirmed by linkage analysis in nine families belonging to the Ag International Mapping Flock. Both genes were located in OAR3p22. OAR3 was previously described as a chromosome containing a QTL for SC incubation period. Furthermore, this region matches exactly within the corresponding interval where a QTL peak for the incubation period of bovine spongiform encephalopathy (BSE) was found in murine chromosome 2. The next step was to verify if there was any variance in the expression of these genes between and within SC infected ($n = 8$) and uninfected ($n = 5$) sheeps with the same genotype for *Prn-p* (ARQ/ARQ). This comparison was performed using Real Time RT-PCR in spleen and cerebellum, which play an important role in the dissemination process of various TSEs. Preliminary results have shown differences in the expression of both cytokines in cerebellum but not in spleen.

C373

Characterisation and analysis of five cholesterol metabolism-related genes in a Duroc pig population.

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The knowledge concerning the genetical bases of cholesterol metabolism is scarce in pigs. We have used a high intramuscular fat commercial Duroc line, used in the production of fine quality cured ham, to investigate the effect of several candidate genes on cholesterol parameters. An experimental population of half-sib families was generated by mating five parental boars with 370 sows, and taking only one castrated male per litter. Two blood samples were taken at 40 and 180 days of age for measuring total plasma cholesterol (CHOL), LDL, HDL and triglyceride (TG) concentrations. In this scenario, we have characterised the coding region of four cholesterol metabolism-related genes, some of them not previously described in pigs. Two of the characterised genes were related to the uptake of cholesterol from bloodstream: the LDL-receptor (LDLR; 1850bp) and the LDL-receptor adaptor protein (ARH: 550 bp). The other two characterised genes participated in cholesterol/lipid synthesis: the 3-hidroxi-3-methylglutaryl-CoA reductase (HMGCR; 2650 bp) and the carnitine palmoyl transferase 2 (CPT2; 1970 bp). By sequencing these genes in animals of different breeds (Duroc, Meishan, Landrace, Large White and Iberian) we identified a total of 13 polymorphic sites, 10 synonymous and 3 non-synonynous mutations. Those polymorphisms segregating in our Duroc population were genotyped by RFLP-PCR or real time quantitative PCR. In addition, the hypercholesterolaemic ApoB5 allele from the porcine Apolipoprotein B-100 gene was also genotyped. Preliminary association analyses indicate a relationship between ApoB5 allele and higher levels of TG in our pig population, while SNPs of the LDLR coding region are particularly associated to LDL and CHOL levels at 180 days of age. Analyses of ARH, HMGCR and CPT2 genes are underway.

C395

Expression of *PRKAG3* gene and possible association with glycogen content in cattle.

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The protein kinase, AMP-activated, γ3 non-catalytic subunit (*PRKAG3*) gene is a member of the 5'-AMP-activated protein kinase (AMPK) gene family. The AMPK is a heterotrimer consisting of a α catalytic subunit, and non-catalytic β and γ subunits. AMPK is an important energy-sensing enzyme that monitors cellular energy status. Mutations affecting the genes encoding the regulatory γ subunits have been shown to influence AMPK activity. In pig, mutations on the *PRKAG3* gene affect meat quality by influencing muscle glycogen content. The *PRKAG3* gene encodes the γ3 isoform of the regulatory γ subunit. This isoform is mainly expressed in white skeletal muscle, where it is the predominant γ isoform suggesting that it has a key role in this tissue. The bovine *PRKAG3* gene, located on chromosome 2 (BTA2), harbours 14 exons spanning 6.8 kb on genomic DNA. We identified 36 SNPs, in both introns and exons of the gene, as well as alternative splicing sites at two positions of the gene. Multiple *PRKAG3* transcripts have been identified that might encode a set of sub-isoforms of the γ3 subunit. A preliminary work has been done in order to find possible associations between specific polymorphisms and phenotypic traits. In this study, 100 Holstein animals were genotyped for 11 markers and 3 major haplotypes were identified (74%, 12% and 11.5%). Different phenotypic traits including meat characteristics (pH value, glycogen content, drip loss) were measured for these animals. We found a significant association ($p < 0.05$) between glycogen content and one polymorphism present on the *PRKAG3* gene. We also identified, in another set of animals, a polymorphism influencing the expression level of the gene in a glycolytic skeletal muscle. These preliminary results may represent important clues for assessing the impact of the *PRKAG3* gene on meat quality in cattle.

C396

Time course microarray studies of gene expression in pig cells infected with the Pseudorabies virus (PrV).

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The Pseudorabies virus (PrV) is a well-studied pathogen of the pig species but molecular mechanisms involved in physiopathology of infection during viral infection are not well characterized. In order to better understand the PrV-cell interactions and to identify cellular genes which are induced or repressed during viral infection, our aim was to simultaneously analyse modifications of cellular and viral transcriptomes during time-course infection. On one hand PrV shows a strong tropism for epithelial cells and one the other hand immature dendritic cells (iDC), which play a central role in innate and adaptative immune response against pathogens, are the first immune cells interacting with the virus. We chose to study the *in vitro* kinetics of transcriptome modifications in these two cells. Epithelial renal PK-15 cell line and primary iDC that were *in vitro*-differentiated from pig monocytes by incubation with GM-CSF and IL-4 were mock-infected or PrV-infected. Cells were collected for RNA extraction just after infection and 1, 2, 3, 4, 8 and 12 h post-infection in case of PK15 cells and 8, 12, 18 and 24 h post-infection in case of iDC. Four kinetics replicates for the PK15 cells and kinetics from iDC of 4 animals were analysed. Two complementary DNA chips were hybridized. One array corresponded to a laboratory-designed cDNA chip comprising 1856 probes including 530 probes targeting the swine leucocyte antigen (SLA) complex region that contains many genes with immune functions, 80 immune genes located outside the SLA complex, 80 PrV genes and 1056 randomly chosen probes for data normalization. The second array referred as Qiagen-NRSP8 is an oligo chip comprising 13297 probes. Genes that were found differentially expressed and transcriptomic profiles will be presented.

C401

Rapid and low cost real time qPCR assays to validate pig genes using pre-designed human LNA (Locked Nucleic Acid) probe libraries.

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In previous work, the molecular pathogenesis of PRRSV (Porcine Respiratory Reproductive Syndrome Virus) had been investigated to identify host factors of resistance/susceptibility to European PRRSV challenge by complementing Suppression Subtractive Hybridization libraries and microarray analysis. Genes activated and down regulated in response to infection were identified and assigned to specific pathways of the immune response. The following step of validation of many differentially expressed genes required rapid and cheap real time RT-PCR methods. The Universal Probe Library concept seems to successfully combine specificity and flexibility for real time qPCR assays, in that up to 99% mRNA transcripts within one organism can be quantified using a set of 90 probes (Roche Diagnostics). Each probe is a short (8 or 9 nt), dual labeled probe that contains Locked Nucleic Acid (LNA) to ensure compatibility with standard real time qPCR methods, where annealing and extension are performed at 60°C. Due to the similarities of human and pig genome, we used the Human Universal probe library to screen several pig genes modulated by PRRSV.
Preliminary data were obtained for HPRT1, NFKBIA, Mx1, Mx2, AMCF-I, CD163 pig genes and for the PRRSV (ORF7). First results showed that the new assay has comparable sensitivity with classical SYBR green methods.
Additional data on the performance of the new assay vs. classical qPCR will be presented. This work is partially supported by Lombardia Region ("D.G. Agricoltura, Programma regionale di ricerca in campo agricolo 2004-2006").

C405
Decreased abundance of Asb15 mRNA by siRNA increases C2C12 myoblast differentiation.

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Ankyrin repeat and SOCS box protein 15 (Asb15) is a novel Asb gene family member that is predominantly expressed in skeletal muscle. We have previously shown that Asb15 mRNA abundance increases upon differentiation and that overexpression of ASB15 delays differentiation of C2C12 myoblasts. The objective of this study was to evaluate the knockdown of Asb15 mRNA by small interfering RNAs (siRNA) in differentiating myoblasts. Preliminary studies evaluated three siRNAs for Asb15 on day 1, 2, and 3 of differentiation. Two of the three siRNAs decreased Asb15 mRNA abundance by approximately 72% compared to a control siRNA. The siRNA with the greatest effect on endogenous Asb15 mRNA abundance was evaluated in a creatine kinase activity assay for differentiation. Creatine kinase activity was measured on day 1, 2, and 3 of differentiation in cells for the Asb15 siRNA, a control siRNA, and a stable cell line overexpressing ASB15. Decreased mRNA abundance of Asb15 increased differentiation as indicated by increased creatine kinase activity on day 1, 2, and 3 of differentiation by 11%, 24%, and 51%, respectively ($P < 0.01$). Additionally, overexpression of ASB15 decreased creatine kinase activity on day 1, 2, and 3 of differentiation by 20%, 36%, and 28%, respectively ($P < 0.01$). Together, these results suggest that Asb15 functions in signaling pathways to regulate differentiation, such that increased Asb15 delays and decreased Asb15 stimulates differentiation of C2C12 myoblasts. The identification of signal transduction pathways in which Asb15 participates to regulate differentiation will provide important insight to the biological function of this novel gene.

C424
Molecular characterization of a new porcine skin and lung phenotype – a potential model of human lung emphysema

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A new pig phenotype initially characterized by juvenile hairlessness and thin skin has been discovered in a Danish pig herd. The trait shows autosomal co-dominant inheritance with all three genotypes easily distinguishable. The phenotype is comparable to the integrin subunit β6 knockout (*ITGB6-/-*) phenotype seen in mice, and *ITGB6* is therefore considered an obvious candidate gene for this trait. Integrins are a family of cell surface transmembrane proteins each consisting of an α- and a β-subunit. Integrin $\alpha_v\beta_6$ is known to play a role in epithelial proliferation and repair. Furthermore, this integrin has been identified as a virus receptor. Comparative mapping predicts that the porcine *ITGB6* ortholog maps to SSC15. An experimental family (n=113) showing segregation of the trait has been established. The candidate region has been confirmed by linkage analysis with four microsatellite markers giving LOD scores from 8.4 to12.1. Sequencing of the *ITGB6* coding sequence from affected and normal pigs has not revealed evidence of a causative mutation. A preliminary immunological analysis of nine pigs (three of each genotype) has shown that the leukocyte profile of the pigs homozygous or heterozygous for the causative mutation differs from that of normal pigs. Histologically, affected pigs have significantly thinner skin and fewer hair follicles. Histological examination of lung tissue from an adult boar, homozygous for the mutation, has revealed emphysema (enlargement of alveolar airspaces) and focal accumulation of alveolar macrophages comparable to observations seen in adult *ITGB6-/-* mice. The present porcine phenotype may constitute a general model of pulmonary emphysema, which is a world wide health problem in humans. Ongoing work focusing on expression studies of splice variants and ITGB6 protein expression is in progress.

C425
Cloning and expression analysis of the PLUNC family genes in the pig.

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The PLUNC gene family is named after their expression pattern, as they are expressed in the palate, lung and nose epithelium. They are related to the LBP/BPI proteins which play an important role in innate immunity during gram negative bacterial infections. This relation together with their expression pattern at sites of infection, and the fact that PLUNC proteins have been shown to bind to LPS of the bacterial cell wall, indicate that the PLUNC family is of potential functional importance in innate immunity. The PLUNC family can be divided into long (*PLUNC*) and short (*PLUNCS*) genes. Thus, the long PLUNC genes have two domains similar to LBP/BPI while the short genes only have one. Five porcine PLUNC genes and two PLUNCS genes were identified in the EST sequences generated in the Sino-Danish pig genome project. Full length sequences were obtained by 5'and 3' RACE for these sequences and their expression pattern in the upper respiratory tract was determined. Since the genomic organization of the PLUNC genes differs between species a porcine BAC clone containing the PLUNC region has been subcloned and sequenced in order to find all porcine PLUNC genes and to determine the genomic organisation of this region in the pig. This has resulted in the identification of three additional putative SPLUNC genes. Cloning of cDNA from these genes and expression studies are underway.

C447
Microarray Analysis of lipopolysaccharide-treated porcine alveolar macrophages

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The innate immune responses comprise the initial defense against microbial pathogens. The phagocytic cells involved in the first line defense are macrophages and neutrophils. The activation of macrophages consists in a complex strict controlled process with functional, morphological, and biochemical modifications. LPS (lipopolysaccharide), also called endotoxin, is the main structural component of outer membrane in Gram-negative bacteria and a potent activation agent of monocytes and macrophages. LPS induced a wide variety of cellular responses that are responsible for the pathophysiologic reactions. Activated macrophages orchestrate innate immunity by phagocytosing pathogens and coordinating inflammatory responses. The main objective for understanding the host-pathogen relationships is the identification of genes, gene products and molecular pathways of animal host involved in host pathogen. In order to understand the molecular mechanisms of pathogen specific immune defense, we used a pig specific cDNA microarray to study differential expression of porcine alveolar macrophages with LPS activation versus LPS non activation cells. This array is a global porcine cDNA-microarray consisting of 27.742 elements spotted in duplicate developed by DIAS.Samples of porcine alveolar macrophages from 4 adult Landrace-White pigs were hybridized to the arrays using a reference design. A linear model was fitted to the data and our analysis identified several candidate genes for the genetics underlying immune defense. Among these candidate genes several important groups of genes are significantly upregulated, including cytokines, chemokines, transcription factors, and receptors and genes that code for signal transduction and cell structure.In summary, we have evidenced differential gene expression patterns between porcine alveolar macrophage in the basal state and in the LPS-activated state.

C448
Systematic identification of regulatory elements in cattle

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Comparative genomics provides a powerful approach for the systematic discovery of functional elements, by identifying highly conserved sequences due to evolutionary constraints. Besides protein-coding, these functional elements are likely to function as regulatory elements, RNA genes and structural motifs. However, it has been difficult to recognize these short degenerated functional elements in a systematic fashion. Most of these elements remain unidentified, such as *cis* DNA elements in promoters acting as transcription factor binding sites (TFBS) and microRNA target sites in 3' UTR. We designed a systematic approach combining position-weight matrixes and phylogenetic footprinting to systematically identify functional elements in bovine promoters and 3' UTRs by cross-species comparison of several mammals. To verify our approach, we analyzed some well-studied promoters and the initial analysis yielded most previously known TFBS and several new candidate sites. We began to test these new sites by experimental validation and the preliminary results are promising. We are in the process of evaluating and customizing existing matrixes and algorithms in order to apply them to individual pathways and the whole genome. This study will provide a comprehensive catalog of functional elements in bovine promoters and 3' UTRs and may provide the first insights into the fundamental understanding of genome-wide regulation and variation in the bovine gene expression.

C451
Construction and pilot analysis of full-length enriched cDNA libraries from Korean native pigs.

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Full-length enriched cDNA libraries from spleen, neocortex, brainstem, kidney, liver, lung and testis tissues of Korean native pigs were constructed. A total of 3390 chromatograms from 5' end sequencing of spleen, neocortex, brainstem and liver were analyzed for SNP identification and characterization of transcriptional start sites (TSS). Our sequencing results were combined with 50000 sequencing trace files of pig ESTs retrieved from the Genbank. Sequences with Phred quality value>30 were selected and assembled using CAP3. The process yielded 442 contigs and 3277 singlets (2593 singlets from our sequences and 684 singlets from Genbank sequences). A large number of singlets reflect low redundancy among our sequences. Twelve putative SNPs from 8 contigs consisted of more than two Genbank as well as two korean native pig sequences were selected and analyzed for SNP conformation using genomic DNA. Especially, we found four SNPs from the *Sus scrofa myostatin* gene. To design primers for SNP confirmation, information of genomic sequences and exon-intron structures were used. Four different breeds of pigs (*Korean native pig, Yorkshire, Duroc, Landrace*) were used for SNP analysis. Allele frequencies were determined for each of the breeds. TSS of *lipophilin, citrate synthase, stathmin, cyclophilin* and *ubiquitin* were compared among *Homo sapiens, Bos Taurus, Mus musculus, Sus scrofa* for understanding the differences in the promoter structure as a pilot analysis. In conclusion, large scale analysis of our libraries will provide useful information for pig genome analysis and annotation.

C456
High resolution genotyping of chicken MHC class-I locus BF1 by Pyrosequencing™.

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The MHC (Major Histocompatibility Complex) is a genomic region that plays a central role in immune response, notably through a set of genes coding for protein involved in antigen presentation to T-cells. In chicken, the MHC B locus is involved in immune responses to pathogens (disease resistance or response to vaccination), as in the case of Marek's virus or coccidiosis. This region presents one of the highest level of polymorphism in the genome, and its characterization is critical to understand the role of MHC in host-pathogen interactions, and to derive, if possible genetic criteria to improve disease resistance. Current typing methods of this region (serology, microsatellite markers) are not precise enough to fully characterize the local variability. Moreover, functional diversity, as the one of highly variable areas of class I and class II genes, is difficult to analyze with classical SNP assays, because of the high density of such SNPs. The polymorphisms of class I and class II genes can be explored by classical Sanger sequencing or SSCP. In Human, pyrosequencing technology, based on light detection during sequential addition of nucleotides to a DNA template, allows high resolution MHC genotyping, with the aim to check for histocompatibility between donor and recipient prior to a graft. The same approach has been used here to determine the genotype of BF1 (classical class-I gene, encoding a class Iα chain) in several inbred chicken lines with different MHC haplotypes. For pyrosequencing, dispensing order of nucleotides has been determined after alignment of previously known sequences of the BF1 gene. The results indicate that, regarding the specific animals tested, four polymorphic sites on 78 bp are present in BF1 highly variable region, and that the genotypes obtained, mostly heterozygous, are not in agreement with the expected status of homozygosity of these chicken lines, as determined by classical serology. Genotyping new target genes in MHC will allow us to define precisely the functional polymorphism of this region, in order to better understand the role of this region in disease resistance.

C460
Bovine Fatty Acid Synthase (*FASN*) gene promoter analysis: functional role of a new polymorphism in the 5' flanking region.

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Fatty Acid Synthase (FASN) plays a central role in de novo lipogenesis in mammals by catalizing all the reactions involved in the synthesis of palmitate from acetyl-CoA and malonyl-CoA. The bovine *FASN* gene is organized in 42 exons, constituting the first and last ones the 5' and 3' mRNA untranslated regions respectively. We have identified a G/C substitution in the bovine *FASN* exon 1 which alters a putative Sp1 transcription factor binding site. As exon 1 can be involved in controlling *FASN* gene expression by regulating either transcription or translation, we have started the functional characterization of this SNP. Association studies of this polymorphism with fat related traits in different cattle breeds have been carried out and significant associations have been observed in each case. Quantification of the promoter activity determined by each allele and transcription factor binding to the DNA sequence have been tested by luciferase reporter assays and electrophoretic mobility shift assays (EMSA) respectively. Transient transfections with the bovine *FASN* 5' flanking region (promoter and exon 1) cloned in the pGL3 control luciferase reporter vector have been carried out in 3T3L1 and HepG2 cells. On the other hand, EMSA and supershift assays with anti-Sp1 and anti-Sp3 antibodies have been also done using HepG2 nuclear extracts. Differences in luciferase reporter activity have been detected in both cellular types showing that this SNP could alter the *FASN* promoter activity. Both Sp1 and Sp3, transcription factors have been detected to bind to the putative Sp1 binding site of exon 1. In addition, binding differences between alleles have been observed. These results suggest that the G/C SNP in exon 1 could control *FASN* promoter activity by Sp1 or Sp3 binding what supports, at least in part, *FASN* involvement in determining fat related traits in cattle.

C461

Analysis of apoptosis related genes in Central Nervous System of scrapie infected sheep.

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Neuronal loss is a salient feature of prion disease; however, its causes and mechanisms are unclear. Some experimental studies show that neuronal death could occur through an apoptotic process. In the present study this process was analysed in the Central Nervous System (CNS) of seven naturally scrapie infected sheep and five controls. All animals had the same sex, age and PRNP genotype. In situ end labelling (TUNEL) and immunodetection of the activated form of caspase-3 were used to identify apoptotic cells in different brain regions from scrapie and control sheep. Both methods revealed a reduced number of stained glial cells and a very low amount of positive neurons with apoptotic appearance. These results could be expected as only a few neurons are supposed to die at a given time point in a chronic disease like natural scrapie. What is observable using these techniques may represent a minute proportion of all apoptotic events going on in TSE affected brains. The analysis of early apoptosis related markers could facilitate both, the identification of this process and the determination of the molecular pathways involved. We have investigated the existence of differences in transcript levels of genes involved in the mitochondria pathway (*BAX, BCL-2, BCL-X_L, BCL-X_S, BCL-W, BAD, BAK, MCL-1, CYT-C, HSP-27 and APAF-1*), as well as other genes with functions in other apoptosis cascades (*FAS, FASL, TGFb1, TNF-R1, AIF, p53, HSP70*). When sheep sequences were known, specific primers were designed to amplify cDNA fragments. In most cases, heterologous primers were used to identify sheep genes and, after sequencing, to design specific primers. Gene expression was quantified using Real Time RT-PCR. PCR reactions were performed using the TaqMan and SYBR-Green assays. *GAPDH, ACTB* and *18S rRNA*, three of the most common housekeeping genes, were used as internal controls against which all samples were normalized. Expression results indicate that genes belonging to the mitochondrial pathway are involved in the neuropathology observed in scrapie.
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C464

Molecular decipherment of porcine hernia inguinalis/scrotalis

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Scrotal and inguinal hernias (also called rupture) are very common congenital disorders in pigs. It is generally accepted that both genetic and environmental factors contribute to the phenotype. However, not only is the mode of inheritance still unknown. Also the estimated heritability differs remarkably according to the pertinent studies (h^2 = 0.21 to 0.86). Under the assumption of 2,5 million dams and an average number of 23.6 born piglets per dam and year, and conservative frequency estimates of 0.5% resp. 1%, 295,000 resp. 590,000 affected newborn piglets would be a realistic estimate for Germany alone. Based on a genome-wide DNA-marker scan we recently identified five chromosomal regions (SSC3, SSC6, SSC7, SSC12, and SSC15) that are associated with the phenotype in our DNA repository of affected full- and half-sib families (specimens were provided by German pig breeders joined in the FBF). Some of the regions could be confirmed by means of genomic mismatch scanning (GMS). Currently, different approaches to shed light on the pathophysiology of porcine hernia scrotalis and inguinalis and to further fine-map the selected chromosomal regions are under way. At the time of abstract submission, PAC clones containing nine potential positional-functional candidate genes were isolated from an appropriate library. So far, five of the nine genes have been assigned (by hybrid panel mapping and/or FISH) to chromosomal regions associated with the defect. A sixth gene, *TAC1*, maps to SSC9q12-14 (FISH) linked to *SWR915* at a distance of 67 cR (LOD score of 5.79). Because of its chromosomal assignment, *TAC1* can no longer be regarded as candidate gene for the defect. Region-specific microsatellites were isolated from the PAC clones by targeted-oligonucleotide-mediated microsatellite identification (TOMMI). Up to now, STS-markers *S0701* and *S0702* (isolated from the *GUSB*-containing PAC clone) were genotyped on the DNA repository. After characterization, two SNPs (SNP1 located in exon 5, SNP2 located in exon 6) within porcine *GUSB* (assigned to 3p16-p14) were analyzed (Transmission Disequilibrium Test) and rejected as causative for the transmission of congenital hernia inguinalis and scrotalis ($p_{TDTSNP1}$=0.229; $p_{TDTSNP2}$=0.195).

C476

Functional Analysis of a PRKG2 nonsense mutation in American Angus dwarf cattle.

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Dwarfism in American Angus cattle is an undesirable phenotype that reduces the profitability of beef cattle. Dwarf cattle have greatly reduced growth rates and frame sizes compared to normal cattle, making them difficult to finish and undesirable to packers. Carriers spread the disease, making them a future liability. Therefore, a genetic test is needed to control the dwarf allele so that favorable genetics in carriers can be preserved in the Angus breed without producing dwarves. Our lab has recently discovered a nonsense mutation in exon 15 of cGMP-dependant, type II, protein kinase (PRKG2). This C→T transition is predicted to truncate 85 amino acids from the mature protein, including a large portion of a kinase domain. Previous studies have shown that the PRKG2 kinase domain is critical in signaling SOX9 to translocate to the nucleus for expression of collagen 10 (COL10) and repression of collagen 2 (COL2). These processes are required for proper chondrocyte maturation and organization of the growth plate. Mutations of PRKG2 in the mouse (knockout) and rat (natural) both cause dwarfism. Dwarf rats show COL2 levels similar to wild-type individuals. These observations suggest that the bovine PRKG2 mutation may show a similar collagen expression phenotype. To determine if the Angus PRKG2 mutation alters collagen expression, we created expression vectors of bovine wild-type and PRKG2 exon 15 nonsense mutation for transfection experiments in cell culture. We present data in HUH7 cells describing the effect of the PRKG2 exon15 nonsense mutation on collagen markers vital to growth plate development.

C477

Mining of polymorphisms in the *leptin receptor* gene in two chicken lines and their association with performance and carcass traits[1]

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Leptin is a polypeptide hormone secreted mainly by adipose tissues and plays an important role in regulating feed intake and energy metabolism. The effects of leptin are mediated through its receptors at the cytoplasmic membrane. Polymorphisms of the chicken *leptin receptor* gene (G*ga* 8) have been associated with abdominal fat deposition making it a candidate gene for association studies with carcass fat deposition traits. The function of this gene has been intensively studied in mammals, but in chickens only a few studies have been reported. Considering its importance on regulating body growth, fat deposition and endocrine function, the objective of this study was to investigate the occurrence of polymorphisms in the *leptin receptor* gene in a broiler (TT) and in a layer (CC) line, both developed by Embrapa Swine and Poultry Research Center. Polymorphism analysis was performed using DNA of 6 males TT and 6 females CC by PCR amplification and sequencing. Primers were designed according to the sequence of the chicken *leptin receptor* gi33867936 from the *GenBank*. The sequences were analyzed using the *Phred, Phrap, Consed and Polyphred* programs. In intron 19, five single nucleotide polymorphisms (SNPs) were identified only in the CC line (G323A, G440A, T646C, G920A, G935T), while the TT line was found to be fixed for the major allele, and a 5 bp deletion (GGAAG in position 628) was detected in both lines, with higher frequency in TT. The potential association of these polymorfisms with economically important traits is under investigation in an F_2 resource population generated by crossing TT and CC chickens. At present, 136 F_2 chickens were genotyped for G920A and no significant association was found with this polymorphism and performance and carcass traits. Other regions of the gene and a larger number of animals are being genotyped and will be included in future analyses.

C478
The genetic characterization of the A and B blood type in the domestic cats.

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The domestic cat has one major blood group system with 3 different types (A, B and the rare AB). Knowledge of the cats' blood type is important in veterinary medicine to avoid transfusion reactions and neonatal isoerythrolysis (NI). The B blood type is recessive to type A and has been found with different frequencies worldwide and among domestic cat breeds. The presence in the type B cat of strong naturally occurring alloantibodies against the A antigens leads to NI for A and AB kittens born from a B queen and can cause a severe immune response if A blood is transfused in a B type cat. Milder reactions occur if the type B blood is transfused into an A type cat, while the AB blood type cat can be considered the universal recipient. Currently cat blood typing is performed with a haemoagglutination (HA) test. The aim of this study was to find the gene and the mutation that controls the A and B blood type in order to develop a genetic test. Using a candidate gene approach we have identified the gene that is responsible for determining the different antigens that cause the feline A and B blood group variant. The gene has been characterized and the full length cDNA sequenced. The segregation of a potentially causative SNP was observed in three different pedigrees of cats representing these breeds: British Short hair, Birman and Devon Rex. Furthermore, several non-related cats were genetically tested and results compared to the HA test results. The SNP and HA results are 100% concordant for 64 cats representing 4 breeds and 3 random bred cats, confirming the accuracy of our findings. Developing a genetic test, hence the chance to identify the B-type allele carrier, would also be helpful for cat breeders to select matings to avoid NI problems. Besides, the genetic test would allow a non invasive and early test from a buccal swab sample, which does not require the veterinarian to draw blood.

C484
***PPARD* – a candidate for a backfat QTL on porcine chromosome 7**

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A quantitative trait locus (QTL) for backfat has been identified repeatedly on porcine chromosome 7 by several groups using different resource families. The gene encoding peroxisome proliferative activated receptor delta (*PPARD*) represents both a positional and functional candidate gene for this QTL given that it has been mapped to 7q1.1-1.2 and that its product is a key regulator of lipid metabolism. We determined the genetic structure of *PPARD*, and screened eight exons , their flanking intronic sequences, and approximately 2 kb of the promoter region for variation in the parental animals of a Mangalitsa x Piétran cross. A total of 20 variants, comprising 18 single nucleotide polymorphisms (SNPs) and two insertion / deletion polymorphisms, were identified, none of which affect the amino-acid sequence. Five haplotypes were derived, which could be distinguished using three representative "tag SNPs"; the latter were used in combination with restriction enzyme assays to genotype the F2 generation of the cross. Statistical analyses revealed that heterozygous carriers of haplotype 4 had significantly more backfat than non-carriers. Interestingly, this haplotype stems from the Piétran breed, which exhibits considerably less backfat than does Mangalitsa. This finding agrees with those from other QTL studies involving the high-fat breed Meishan, in which the Meishan-derived QTL allele also reduces backfat. Genotyping of 24 Meishan animals failed to detect haplotype 4, indicating that it is probably rare, or possibly even absent, in this breed. Altogether, these results indicate that variation within *PPARD* could indeed be responsible for the variation associated with the backfat QTL, although it cannot be ruled out that the actual cause is due to variation within another gene linked to haplotype 4.

C492
Mutational analysis of the porcine UDP-N-acetylglucosamine transporter SLC35A3.

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The solute carrier family SLC35 comprises enzymes that transport nucleotide-sugars from the cytosol into the lumen of the endoplasmic reticulum and the Golgi apparatus. In these organelles, nucleotide-sugars are used by glycosyltranferases to synthesize the sugar chains of glycoproteins, glycolipids and carbohydrate polymers, thereby linking nucleotide-sugar transporters closely with diverse cellular functions. For example, the *SLC35A3* gene which encodes a UDP-N-acetylglucosamine transporter has recently attracted much interest because of its role as causative gene for the disease complex vertebral malformation (CVM) in cattle. To better understand the functions of nucleotide-sugar transporters in development and disease, we have cloned and characterized the porcine *SLC35A3* gene and begun to define functionally important amino acids using site-directed mutagenesis.

C498
Studying mitochondrial proteomic expression patterns in beef cattle that differ in for net feed efficiency

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Net feed intake is the feed intake of an animal after adjustment for its average weight and weight gain while on the feed test. High efficiency animals have a low net feed intake, so the aim is to select animals that have a high net feed efficiency (NFE). The goal of present study is to identify genes controlling feed efficiency in beef cattle. Four NFE quantitative trait loci (QTL) were mapped in a Jersey-Limousin backcross. As a number of candidate genes within these QTL are involved in oxidative phosphorylation, mitochondrial function was examined in liver and muscle samples from high and low NFE Angus beef cattle differing in NFE by 4.85 kg/day. The samples were used to study the activity of enzymes involved in the mitochondrial oxidative phosphorylation and ATP turnover pathways. The expression pattern of mitochondrial proteins was also studied by two-dimensional differences in multi-fluorescent gel electrophoresis (DIGE). The DIGE technique has the capacity to separate and visualise up to 3 protein samples on a single 2-D gel. Mitochondrial proteins were extracted by differential centrifugation. Samples from high and low NFE animals were compared by labelling their mitochondrial proteins with different fluorescent dyes (Cy3 and Cy5) and separating the proteins on a single 2-D PAGE. Cy2 dye was used to label an internal standard, which prepared by mixing high and low NFE pooled mitochondrial protein samples in equal amounts. The fluorescent signals were captured by a laser scanner based on the specific wavelengths of the dyes. Differences between individual spot intensity and quantification were determined by using Decyder-2D version 6.5 (Amersham) image analysis software. Individual protein spots were quantified by comparison to the internal standard to generate a ratio of relative expression. There were 960 protein spots, of which 60 spots had a two-fold difference between high and low NFE cattle. Identification of the 60 differential expressed proteins by mass spectrometry is in progress and should assist in the selection of candidate genes for sequencing.

C501

Qua*LIPID* - a FUGATO project aiming at the comprehensive analysis of genes involved in lipid metabolism in cattle and swine

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The quality of animal products - namely their processing ability, palatability and dietary value - is largely determined by the quantity and quality of their lipid constituents. The Qua*LIPID* project (*Functional analysis of genes involved in lipid metabolism in cattle and swine for the identification of product quality relevant DNA variation*) aims at the comprehensive identification and functional analysis of genes involved in lipid metabolism. Through the collaboration of groups with expertise in bioinformatics, structural and functional genomics, lipid biochemistry and high-throughput lipid analytics, and animal nutrition the project is structured as follows. Genes involved in lipid metabolism are systematically pinpointed through bioinformatics. Porcine BAC clones containing key functional and positional genes are identified through *in silico* BAC library screening and sequenced to a low coverage. The resulting porcine sequences as well as sequences derived from the bovine draft sequence are re-sequenced to examine for DNA variation. Lipid parameters are scored for large numbers of animals by applying high-throughput methods. Association of DNA variation with lipid parameters is studied both in resource families and in animals with extreme phenotypes or breeding values. Microarray analysis in animals with known QTL and candidate gene genotypes will be employed to identify new candidate genes and to evaluate the causality of candidate gene polymorphisms. Genotype by nutritional environment (GxE) interaction will also be investigated for selected polymorphisms. Qua*LIPID* was launched in July 2005. It is conceived for three years and is supported by the German Ministry for Education and Research, BASF, FBF and the Bavarian Milk Producers.

C514

Gene expression profiling of two hypertrophied muscles in callipyge lambs.

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The goal of this study was to identify changes in gene expression during the onset of muscle hypertrophy in order to identify the pathways that are involved in the callipyge phenotype of sheep. Gene expression patterns in the longissimus dorsi (LD) and semimembranosus (SM) were measured using the Affymetrix Bovine Array in callipyge (+/C) and normal (+/+) lambs at 10, 20, and 30 days of age. Muscle weight data indicated hypertrophy had begun by 30d in the LD and at 20d in the SM. A two-way ANOVA for age and genotype effects was performed with the Benjamini and Hochberg False Discovery Rate method used for an overall p-value of 0.10. Twenty probes were identified to have a significant age effect over the two muscle tissues. Fourteen probes exhibited a significant genotype effect ($P < 0.10$) in the LD and eight were significant ($P < 0.10$) for genotype in the SM. Only two of these significant probes overlapped between muscles: *DLK1* (from the callipyge locus) and a transcription factor DNTTIP1 for a total of 20 differentially expressed transcripts. Seventeen were analyzed by quantitative RT-PCR in a larger group of animals (six ages from 10 to 200d, +/C and +/+, N = 42 lambs). Twelve genes (70.6%) had a significant genotype effect in the subsequent analysis. Differentially expressed genes included a transcription factor, a ribosomal protein methyltransferase, two cAMP phosphodiesterases (PDE), a calcium-activated potassium channel, and two muscle-specific metabolism enzymes. Using K-means clustering, the transcription of *PDE7A* most closely mirrors the expression pattern of *DLK1* (0.95 correlation), indicating it may play an important role in the early development of hypertrophy in callipyge muscle. Gene expression profiling in callipyge lambs during development of hypertrophy has provided potential candidate genes for a signal transduction pathway or regulatory network initiated by the up-regulation of *DLK1*.

C518

Real-time quantitative RT-PCR analysis of myogenic factors expression (MyoD, myogenin and MRF-4) in two chicken lines (broiler and layer).

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In poultry, skeletal muscle formation is controlled by a complex network of factors controlling the activity of muscle protein genes, as well as genes negatively regulating the myogenic program. The MyoD superfamily is composed by the transcription factors: MyoD, Myf-5, MRF-4 and myogenin which are related to the activation of the myogenic program. MyoD and Myf-5 are needed for cell determination, whereas myogenin and MRF-4 are involved in the cellular differentiation. Despite all progress in the study of gene regulation of muscle formation during the early stages, only a few studies describing the action of these genes at late development stages and at the post-hatch period have been reported. This study compares MyoD, myogenin and MRF-4 expression between two chicken lines with different growing potential: a broiler line (TT), characterized by high growing rates and great muscle deposition, and a layer line (CC), which demonstrate low growing rates and poor muscle mass. Real-time quantitative RT-PCR analysis of five animals, in duplicate, of each line in two different development stages was performed: embryonic (9 and 17 days of incubation) and post-hatch stage (1 and 21 days old). Relative quantification method was applied using the β-actin gene as control. Comparison of means was performed using *t*-test. MyoD expression was higher ($p<0.05$) in the broiler than in the layer line at the embryonic stage and in the first day post-hatch. On the other hand, at 21 days, MyoD expression decreased in the broiler line and became higher in the layer line. Myogenin and MRF-4 expression was greater ($p<0.05$) in the broiler line at all development stages analysed. However, the difference in MRF-4 expression between the lines was greater than the differences observed for the other two factors, especially at embryonic stages. Gathered together, these data suggest higher muscular cell determination and differentiation in the broiler line, compared to the layer line.
Financial support: EMBRAPA/PRODETAB, FAPESP.

C523

A further look at candidate genes for glycolytic potential: association with meat quality and production traits in Italian Large White pigs.

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Glycolytic potential (GP) is a measure related to the energy metabolism of the skeletal muscle. This parameter is correlated to several meat quality traits, like pH, meat colour, drip loss and processing yield. Previously, we investigated in pigs tens of candidate genes for GP chosen among those involved in the glycogen metabolism and glycolysis pathways as well as their regulation in skeletal muscle. Polymorphisms identified or analysed in these genes have been screened in a first association study with meat quality traits in commercial pig hybrids. Significant results were obtained for muscle pyruvate kinase (*PKM2*), muscle phosphoglycerate mutase (*PGAM2*) and gamma 3 non-catalytic subunit AMP-activated protein kinase (*PRKAG3*) genes. Here we further investigated these three genes to evaluate their putative effects on meat quality and production traits in pure bred Italian Large White (ILW) pigs. Glycogen and lactate content, GP, pH_1 and pH_2 were determined on muscle *longissimus dorsi* of 270 ILW sib tested animals. Estimated breeding values were calculated by ANAS for average daily gain (ADG), back fat thickness and lean content (LC). These animals were genotyped for single nucleotide polymorphisms of the *PKM2*, *PGAM2* and *PRKAG3* (T30N, G52S and I199V mutations) genes. Association analysis between genotypes at these loci and the indicated traits was performed using the GLM procedure of SAS. Significant results ($P<0.05$) were obtained for *PKM2* (pH_1, GP, ADG, LC) and *PRKAG3* I199V (pH_1).

C529

The callipyge mutation does not alter *DLK1* expression in visceral or subcutaneous adipose tissue in sheep.

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The callipyge phenotype is characterized by muscle hypertrophy in the loin and pelvis limbs as well as a significant reduction in overall body fat. The *DLK1* gene lies adjacent to the callipyge mutation and is significantly up-regulated in the hypertrophied muscles of paternal heterozygous (callipyge) sheep. *DLK1* is also known to be involved in the differentiation of adipocytes; therefore, we hypothesized that decreased carcass fat may be due to altered *DLK1* expression in the adipose tissue of callipyge animals. Subcutaneous (SC) and visceral adipose samples were collected from lambs of all four possible callipyge genotypes at 10, 20, 30, 80, 150, and 200 days of age (N=65). The expression of genes from the callipyge region including *DLK1* and *GTL2* as well as two genes involved in adipocyte function, *PPARG* and *LEP*, were measured by quantitative PCR. There was no effect of genotype (P>0.05) on *DLK1* or *GTL2* transcript abundance in either SC or visceral adipose tissue. There was a significant effect of age (P<0.04) on *DLK1* and *GTL2* expression in SC and visceral adipose tissue with transcript abundance decreasing over time. Similarly, there was no effect of genotype on expression of *PPARG* or *LEP* but there was a significant effect of age. At both 150d and 200d, SC and visceral tissues expressed significantly more *LEP* (P<0.0001) than younger time points. *LEP* expression is known to increase with age and total body fat accretion in many mammals. These results show that expression of the genes from the callipyge region are not affected by maternal or paternal inheritance of the callipyge mutation in adipose tissue, contrary to effects in skeletal muscle. The results for *PPARG* and *LEP* indicate normal genetic regulation and cytokine secretion of adipose tissue in callipyge animals. Therefore, the decrease in body fat accretion in callipyge animals is not likely to be a direct effect of the mutation on adipocyte function, but an indirect effect of muscle hypertrophy.

C531

Polymorphisms in the *calmodulin* gene in two chicken lines (*Gallus gallus*).[1]

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Brazilian broiler production yields a considerable amount of income for the nation. Brazil is currently the world's third broiler producer and the first chicken meat exporter, contributing with 41% of the whole world market. Knowledge of gene action on muscle development may provide information to be used for increasing production of high quality meat for human consumption. Calmodulin is a ubiquitous calcium binding protein modulating diverse cellular processes. Because of the importance of Ca^{+2} in the myotube formation, calmodulin seems to be related with muscular cell proliferation rate. Therefore, studies on this gene may contribute to the understanding on muscular development. Our group had formerly described polymorphisms in this gene based upon EST identification. The present study searched for polymorphisms in the *calmodulin* gene in two different chicken lines: a broiler (TT) and a layer (CC) line, both developed by Embrapa Swine and Poultry Research Center. These lines present great phenotypic differences in body growth and muscle mass deposition. TT line is fivefold heavier than CC line at 41 days of age when both lines are raised as broilers. Polymorphism analysis was carried out using DNA of 6 males TT and 6 females CC. A 735 bp fragment comprising a region started at position 283 of *GenBank*, access GI L00101, corresponding to the exon 6 of *calmodulin* gene, was amplified and sequenced. Six SNPs were identified in the two strains: C479T, G480A, C522T, T727G, C734G, C739T. The SNP C479T is in agreement with that previously described by our group, also using TT and CC lines. These polymorphisms are located at 3'UTR of the *calmodulin* mRNA and may be related to mRNA stability, which can interfere in the amount of protein produced, consequently affecting both growth and muscular development. Moreover, these polymorphisms can be linked to other traits related to muscle development. The C522T was only observed in the CC line and its association with economically important traits is being investigated in a F_2 resource population originated from the cross of TT x CC lines.

C532

Use of transcriptional profiling to understand genetic mechanisms controlling feed intake and efficiency in pigs

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Feed is the major variable cost in pork production. Determination of genetic mechanisms that control feed intake (FI) and feed efficiency (FE) remains a major challenge for the improvement of FE and FI. Although feed is associated with production traits such as growth and composition, considerable variation in FE and FI exists independent of these traits. This variability is called residual feed intake (RFI) and has heritability of 15-40% in pigs. In this study, using transcriptional profiling of key tissues, we aimed to identify genetic mechanisms differing between control pigs and pigs that have been under selection for low RFI for three generations. A further aim was to determine the pathways responding to feed restriction within these lines and any line x treatment interactions resulting in gene expression differences. Low RFI pigs (n=4) and control pigs (n=4) were allowed feed *ad libitum* or were feed restricted to 80% of maintenance for 7 days in a complete 2 x 2 factorial design. Total RNA was isolated from liver and fat tissues from all pigs, and analyzed using hybridization to the 24,123 probe set Affymetrix Porcine Genechip™. A mixed linear model was fit to each tissue and each gene using SAS Proc Mixed. Preliminary results indicate that 2,809 genes in fat ($p<0.04$, $q<0.2$; among which 1,219 genes with $q<0.15$) and 61 genes in liver ($p<0.001$, $q<0.2$) showed differential expression in response to feed restriction. Also, 1,247 genes ($p<0.02$, $q<0.2$; among which 344 genes with $q<0.15$) showed differential expression between low RFI and control pigs and 38 genes ($p<0.001$, $q<0.2$) showed a line x feed interaction in liver. Based on these results, candidate genes will be selected as a first step in hypothesis formation. Along with blood hormone assays, confirmation of expression of candidate genes will be performed to determine potential pathways that control FI and FE in pigs. Supported by USDA-NRI-2005-3560415618.

C548

Comparative profiles of gene expression of *Bubalus bubalis* and *Bos taurus*

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We investigated the expression profile of skeletal muscular tissue taken from *Bos taurus* and *Bubalus bubalis* using the SAGE method (Serial Analysis of Gene Expression) on total RNA. A total of 30,283 tags were generated for *B. taurus* and 30,553 for *B. bubalis*. These values equated to 7892 (*B. taurus*) and 8574 (*B. bubalis*) genes respectively. Analysis using a Venn diagram revealed 1879 genes active in tissue of both species, with 6000 genes specific to *B. taurus* and 6600 genes specific to *B. bubalis*. Of these, two transcripts had a higher expression in *B. taurus.* The first, presenting a ratio of 108, was recognized as a gene protein similar to that found in *Canis familiaris* (Bt. 2947, GTGACGCCAA). The second, presenting a ratio of 87 (CTAATTATAA), has no information available about its function or structure. In *B. bubalis* five transcripts were the most highly expressed, presenting the following ratios: 644 (Bt. 7434, GTGGCCTTAA), 375 (TAATTATTGG), 214 (GTGGCCTTAC), 115 (GCGACGCCAA), 102 (GATTGGAGGA). The next step will be the characterization of the structure and function of the predicted genes, and validation of their expression. This study presents for the first time an RNA gene expression profile of muscular tissue taken from *B. bubalis*. This allows the comparison of genetic differences between two species of both a biological and economic interest.
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C554

Microarray analysis of host gene expression in response to Bovine Leukemia Virus infection

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To understand the role of BLV *tax* in inducing lymphoproliferation, cell transformation and immune disorders in the host, we performed gene expression profiling of a BLV-infected bovine B-lymphoblastoid cell line (BL3*) and its uninfected parental cell line (BL3°) using a 7,872-element cattle cDNA microarray. mRNA from seven independent aliquots of cells from each cell line were labeled with Cy3 and Cy5 dyes and assayed using a dye swap design. 1,025 unique transcripts were differentially expressed in BL3* in comparison to BL3º cells (false discovery rate, $p<0.05$), of which 39 genes were upregulated two-fold or more, while another 13 genes were down-regulated at least two-fold. Among those, 14 genes involved in apoptosis, cell growth and maintenance, transcription regulation, and/or protein biosynthesis were subjected to SYBR Green-based quantitative PCR (qPCR) to validate the results obtained in the microarray analysis. Cattle-specific primers targeting *EEF1E1*, *EIF4A1*, *ETS1*, *MFHAS1*, and *RPS14* (down-regulated genes), and *BTG1*, *EAF2*, *ID3*, *MARCH6*, *MLLT4*, *NFATC3*, *SPP1*, *TFDP2*, and *USP9X* (up-regulated genes) were used in the qPCR assay. Results of this validation confirmed differential expression of all 14 genes (*t*-test, $p<0.05$), while BLV *tax* expression was detected only in the BL3* cells. A biological interaction network was built with genes exhibiting two-fold or higher differential expression in order to understand the specific pathways by which BLV drives leukomogenesis in the host. All genes except *EIF4A1* presented relevant interactions on the basis of their role in transcriptional regulation. The transcription factor *ETS1* showed the largest number of interactions, which indicated its importance in the host cellular response to BLV infection. These results clearly demonstrate that transcriptional-transactivation of host genes by BLV occur on a genomic scale. Our experiments provide further understanding of the likely importance of BLV *tax* in B-cell transformation and its effects on the host immune system.

C555

Molecular analysis of the Equine TFAM gene

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Mitochondrial transcription factor A (TFAM) is an integral part of the mammalian transcription machinery and is necessary for transcription of mammalian mtDNA from the light-strand (LSP) and heavy-strand (HSP) promoters. In addition, transcription form LSP also produces an RNA primer, which is required for mtDNA replication. This qualifies TFAM also as an important factor regulating mtDNA copy number. In mammals, *TFAM* gene has already been isolated and mapped in human, mouse and rat. In man *TFAM* gene is located on HSA 10 in the vicinity of the *CDC2* gene. In our study we applied comparative approach using *TFAM* sequence information already available for the *TFAM* gene in human, cattle, pig, chicken, mouse, rat, and frog to design primers for selective amplification of the equine *TFAM* gene regions. The sequence of the horse *TFAM* gene (3269 nt) including 348 bp of the coding region (exons 1-4), first three introns and 467 bp of the proximal promoter was obtained. The translated amino acid sequence showed 85% identity with cattle and pig, 82% with dog, 72% with human, chimpanzee and orangutan, 68% with silvered leaf monkey, 56% with rat and 54% with mouse. The comparative walking generated a 467 bp sequence of the equine *TFAM* proximal promoter region which contains one NRF1 and two Sp-1 putative binding sites which have both been reported as prevalent *cis* factors associated with respiratory genes. In addition, binding site for activating transcription factor, which belongs to the CREB/ATF family of transcription factors and is conserved in five mammalian species, was also identified. Two additional conserved sequence blocks were found in eight mammalian species: the sequence TCCCA which does not correspond to known transcription factor consensus sites, and the sequence GCGGGCATGATA which contains the putative binding site for tumor suppressor p53. Characterization of the *TFAM* gene is of interest in animal species where physical performance plays an important role. Polymorphisms in the promoter region of the *TFAM* gene might be related to the expression level of the *TFAM* gene and consequently to the mitochondrial number and physical ability of animals.

C586

A regulatory mutation in *IGF2* affecting skeletal muscle, heart and back fat thickness in pigs.

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A single point mutation (G to A transition) was identified in the *IGF2* gene in an intercross between Large White domestic pigs and Wild boar. The mutation causes a 3-4 % increase in muscle mass in domestic pigs compared to the wild type in the Wild boar pigs. It also affects heart size and back fat thickness. The mutation is located in a regulatory element in intron 3, a conserved CpG island, and affects transcription from promoters 2, 3, 4 and an *IGF2* antisense promoter. (Van Laere et al. Nature. 2003, Braunschweig et al. Genomics. 2004). We detected a three-fold increase in IGF2 mRNA expression in postnatal skeletal muscle in pigs carrying the mutated domestic allele compared to wild type. No difference in expression was observed in liver or in prenatal tissues. *In vitro* DNA-protein interaction experiments have shown that a nuclear factor binds the wild type sequence but not the mutant form. Since the wild type allele shows a reduced expression, this nuclear factor must be a repressor. (Van Laere et al. Nature. 2003). We are currently pursuing several approaches to identify this repressor. A South-Western screening of an expression library as well as biochemical approaches are under way. Recent data show that this protein should be clonable since South-Western hybridization of nuclear extracts showed reproducible higher binding efficiency to the wild type allele compared to the mutant allele.

SECTION D
Genetic Markers and Selection

D037

A genome wide linkage disequilibrium (LD) screen in Tying-up syndrome.

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A genome wide LD screen is increasingly used to identify genetic risk factors for complex diseases. In this study, a genome wide association study was performed for mapping susceptible loci of Tying-up syndrome in horses. Tying-up syndrome, the common muscle problem in Thoroughbreds, has also been called recurrent exertional rhabdomyolysis. It has been recognized for more than a century as a syndrome of muscle pain and cramping associated with exercise. Recent evidences suggest that it is a heritable disorder (heritability of 0.43), showing that it is difficult to use a traditional linkage study for mapping susceptible loci. Therefore, a genome wide association study was designed to isolate genes governing Tying-up syndrome. Each 144 affected and unaffected horse DNAs were pooled to facilitate efficient genotyping of 986 microsatellites as first screening. Allele frequencies between the two populations were compared using the ΔTAQ, and 10 markers (7 chromosome regions) were selected. The markers were then individually genotyped in the affect (n=144) and unaffected (n=144) groups. Of those markers, 7 markers (5 chromosome regions) also showed significant P-values (P<0.10 or 0.05). To confirm the detail regions, neighbored microsatellites of 5 markers (4 chromosome regions) except of 2 unknown mapped markers were genotyped, and case-control studies for individual markers and permutation tests for the two neighbored markers to evaluate haplotype frequency differentiations were performed. Using this 3-stage approach, we have been able to find two candidate regions on ECA12 and ECA20 for Tying-up syndrome in horses. Those regions showed significant values in permutation test for haplotype frequency differentiations (P=0.0108 on ECA12, P=0.0025 on ECA20) as well as each P value (P<0.10 or 0.05). Although the markers, which were used in this study, might not be enough for genome wide LD screen in Thoroughbreds, the two regions must be the susceptible regions for Tying-up syndrome in horses. In addition to this study, we also describe prospect for genome wide LD screen in Thoroughbreds, because this study would be first attempt to identify genetic risk factors for complex diseases in Thoroughbreds.

D041

Quantitative trait loci for infectious bovine keratoconjunctivitis.

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Infectious bovine keratoconjunctivitis, also known as pinkeye, is an economically important disease in cattle. The objective of this study was to detect quantitative trait loci associated with infectious bovine keratoconjunctivitis in offspring from a Brahman x Hereford sire. The sire was mated to Hereford, Angus and F1 cows to produce 288 offspring in 1994, and mated to MARC III (¼ Hereford, ¼ Angus, ¼ Red Poll, and ¼ Pinzgauer) cows in 1996 to produce 259 offspring (547 animals total). Infectious bovine keratoconjunctivitis was diagnosed by physical examination in 36 animals of the family. Records included unilateral and bilateral frequency, but not severity. Records were binary; "zero" for unaffected and "one" for affected cattle. A putative quantitative trait loci for infectious bovine keratoconjunctivitis was identified on chromosome 1, with a maximum *F*-statistic ($F = 10.15$; $P = 0.0015$) at centimorgan 79 of the linkage group. The support interval spanned centimorgans 66 to 110. There was also evidence suggesting the presence of a quantitative trait loci for infectious bovine keratoconjunctivitis on chromosome 20, with a maximum *F*-statistic = 10.35 (P = 0.0014) at centimorgan 16 of the linkage group. The support interval ranged from centimorgan 2 to centimorgan 35. No clear candidate gene can be identified as responsible for infectious bovine keratoconjunctivitis on these chromosomes.

D102

Development of a commercial multiplex marker assay

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High throughput microsatellite multiplex genotyping has become a quick and reliable method for single and multiple gene tests in commercial genomics. In the New Zealand livestock industry there is considerable demand for DNA test services. Commercial pedigree tests are available for sheep, deer and cattle, and gene tests are rapidly becoming available for a number of economically important traits such as Calpain1 and Myostatin in cattle, and LoinMAX® and MyoMAX® in sheep. In order for these tests to be cost effective in a commercial environment, the cost of the DNA test process must be kept to a minimum. This may be achieved partly, in the design of highly robust, high throughput DNA multiplexes, where multiple markers are analysed concurrently in a single experiment. Here, we describe a troubleshooter's guide for the development and validation of a high throughput ovine multiplex assay. The aim is to integrate five new markers into an existing 6 marker multiplex which can then be analysed in a single run on an Applied Biosystems ABI3730 DNA analyser. Bovine primers previously designed from intronic regions of 5 target sequences, were tested for amplification in sheep DNA samples using magnesium and temperature gradients to optimise PCR conditions. The number and size range of alleles for each marker was obtained by scoring panels of DNA samples from multiple breeds. The multiplex was then configured using the optimal temperature and magnesium concentrations, allele size range and dye label for each marker. Some changes were required to the original 6 marker multiplex to achieve a final configuration that allowed all markers to be analysed together. Where allele size ranges overlapped within the same dye range, primers were redesigned using ovine sequence data to increase/decrease the size of PCR products. The multiplex was validated using samples previously analysed by other multiplex/uniplex assays.

D166

Analysis of bovine muscle DNA polymorphisms and their association with meat quality, in particular intramuscular fat, in the Irish cattle population

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Meat quality is a complex set of properties and the factors underlying meat quality have a molecular basis. Advances in molecular genetics have led to the identification of genetic markers and variations in DNA (polymorphisms) associated with genes that affect meat quality traits. The objectives of this research were to determine known single nucleotide polymorphisms (SNPs) in bovine candidate genes, to establish the frequency at which these SNPs occur and to evaluate an association between these SNPs and intramuscular fat (IMF) levels in the Irish herd. Three candidate genes were chosen for this study: the leptin, thyroglobulin (TG) and diacylglycerol O-acyltransferase1 (DGAT1) genes. Blood samples (383) were collected from different breeds of pedigree bulls from Artificial Insemination (AI) stations in Ireland. Bovine muscle samples (270) [*M. longissimus dorsi* (LD)] were collected from slaughter-weight commercial cattle and meat quality measurements were analysed. Genomic DNA was isolated from blood and muscle samples and two known SNPs in the leptin gene, one SNP in the TG gene and two SNPs in the DGAT1 gene were detected by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) genotyping. For the DGAT1 gene, the two SNPs are positioned adjacent to each other, therefore, samples were cloned into a pCR®4-TOPO® vector and sequenced to distinguish between the different alleles. Three genotypes CC, CT and TT, were detected for the leptin and TG genes and AA, KA and KK for the DGAT1 gene. For the frequency study, the genotype frequencies were performed using the FREQ procedure of SAS allowing differences between breeds and genotype to be discerned. To date, breeds tested include Aberdeen Angus, Charolais, Hereford, Limousin and Simmental. Associations between genotypes and IMF values were assessed using the GLM (one-way ANOVA) procedure in SAS. Results to date show a non-significant (P>0.05) association between genotype and intramuscular fat values in Irish beef for these candidate genes.

D176
QTL analysis of body composition and fatness in a reciprocal cross between BFMI860 and C57BL/6 mice.

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To constitute a valuable resource for studies on the genetic basis of body composition and fatness, a novel mouse model for fundamental research, the Berlin Fat Mouse Inbred line 860 (BFMI860), was established. This mouse line has been long-term selected for low body weight and high fatness. At the age of 20 weeks, the selected inbred line showed on average only a 1.5 fold increase in body weight compared to the unselected control line C57BL/6 (B6) (33.3 g *vs.* 22.6 g), but BFMI860 mice disposed of approximately nine times the mass of reproductive fat tissue (3.4 g *vs.* 0.4 g). In this ongoing study, BFMI860 and B6 animals are used for a reciprocal cross to construct a segregating F_2 population consisting of more than 1300 individuals. Finally, 500 BFMI860xB6 and 300 B6xBFMI860 F_2 mice will be fed a high fat diet, 500 BFMI860xB6 F_2 animals a normal maintenance diet. All mice will be analysed for various traits related to fat deposition and growth. Body fat mass and body lean mass, for example, are determined in all mice by quantitative magnetic resonance analysis weekly from three to ten weeks of age. Weights of different fat depots and inner organs are recorded at ten weeks. A least squares Quantitative Trait Loci (QTL) analysis will be performed on these data to identify natural genetic variation affecting the complex traits growth, body composition, and fatness in this pedigree. Moreover, the chosen pedigree design allows for exploration whether the type of reciprocal cross had significant effects on the recorded traits. 200 additional F_2 animals derived from a BFMI860xB6 F_1 x B6xBFMI860 F_1 reciprocal cross will lead to the detection of maternal effects and genetic differences in mitochondrial DNA.

D177
Double muscling in Marchigiana breed in Brazil

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Double muscling (DM) is characterized by hypertrophy of muscles, caused by an inactivation of myostatin gene (GDF-8). Cattle showing DM, have higher meat yield, a higher proportion of more valuable cuts, and leaner and more tender meat. This trait was initially reported in Belgian Blue and afterwards in several cattle breeds. A new variant was recently found in Marchigiana breed in Italy. In the present study, 377 bovines Marchigiana and 20 Marchigiana x Nelore hybrids raised in São Paulo and Paraná states – Brazil were tested for identification of myostatin gene. The results showed 6.9% individuals mutant homozygous (DD), 55.2% heterozygous (DN) and 37.9% normal (NN). In hybrids, 14 showed the normal allele and six the mutant allele. Checking the pedigrees of the animals it was possible to detect an intensive use of some bulls. It could be an explanation for the considerable high frequency of the mutant allele in the Brazilian herds.

D197
Differences in SNP allele frequencies between chicken lines divergently selected for antibody response to sheep red blood cells

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Many studies have focused on identifying quantitative trait loci (QTL) for disease resistance in the chicken. As a precursor to developing an intercross population from lines that had undergone long-term divergent selection (32 generations) for high or low antibody response to sheep red blood cells, the founding parents were subjected to single nucleotide polymorphism (SNP) typing. Sixteen individuals from the founder High Antibody Select (HAS) and Low Antibody Select (LAS) lines were genotyped using the Illumina platform for 2733 SNPs distributed across the genome. Of the loci genotyped, 1667 appear to be fixed within the population, as indicated by a single allele present in all of the individuals genotyped. Another 129 SNP loci were observed to have segregating alleles in one, but not both, of the selected lines, while 937 SNP loci had alleles segregating in both selected lines HAS and LAS. Allele frequencies were calculated both within and across lines. Interestingly, no loci were found where both of the lines were fixed for alternate alleles. Significant differences in allele frequencies observed in the selected lines from that of population wide allele frequencies were calculated using a Chi square analysis. There were significant differences between the HAS and LAS lines for allele frequencies at 43 loci. These loci were located on 15 chromosomes with specific regions of concentration on GGA2, GGA4, and GGA10. These three regions have been previously associated with disease resistance or antibody response; however it was not within a single population. The results of this preliminary analysis suggest that multiple loci with relatively small effects contribute to the variation in the phenotype on which selection is based. The nature of selection appears to favor the contribution of a large number of loci with relatively small effects as opposed to single loci with large effects. Further characterization of these selected lines and their intercross population will provide additional information on the complexity of antibody response in the chicken as well as the genetic basis of selection in general.

D218
A SNP in the horse KIT gene as a new marker for the tobiano spotting pattern.

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Tobiano (*To*) is a dominant spotting gene in horses. Horses homozygous for the tobiano gene are highly valued because all offspring will express the tobiano pattern. Yet the actual mutation for the tobiano color pattern in horses has not yet been identified. In 2002 Brooks, et. al identified a single nucleotide polymorphism (SNP) in *KIT* that is strongly associated with the presence of the tobiano gene. This marker, designated KM1, has proven to be highly effective as a screening tool to identify homozygote versus heterozygote horses. However, we found that the KM marker was not concordant with the tobiano phenotype or genotype in a herd of British riding ponies. Fifteen samples were submitted to our laboratory for testing and of these, three tobiano individuals were negative for the KM1 marker. We have identified a new marker in Intron 5 of *KIT*, designated UK5, which not only corresponds to results obtained with KM in other breeds, but discriminates between tobiano genotypes in the spotted British ponies. UK5 identified two of the KM1-negative individuals as being heterozygous for tobiano and one individual as being homozygous for tobiano. This concurred with pedigree and production records for the herd. We therefore present the UK5 marker as a more accurate marker for the *Tobiano* gene.

D231

Bayesian multiple QTL mapping with epistasis for half-sib populations of beef cattle incorporating non-random mating under field records

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Economically important traits of livestock, such as carcass traits in beef cattle, are generally controlled by multiple quantitative trait loci (QTL). To detect the QTLs that control the carcass traits of Japanese beef cattle (so called Wagyu), numerous half-sib families are designed based on field records from carcass markets. Such records are generally impacted by various environmental effects. In addition, the random mating of the dams of the family cannot be expected. Therefore, to address such unpredictability, the Multiple QTL with Epistasis Mapping (MQEM) method for a half-sib design (denoted as the MQEM-HS) which is based on the Bayesian inference approach with MCMC (Narita and Sasaki 2004; Narita 2004) was developed and evaluated. The objective of this study was to investigate the accuracy of the detecting QTLs using the MQEM-HS under the condition of non-random mating based on computer simulation. The MQEM-HS deals with the multiple QTLs, epistatic QTL pairs, fixed effects, and the polygenic effect with pedigree information of the family. We assumed a simulated half-sib family, where the number of the maternal grandsires (MGSs) of the 300 dams in the family was set as 20 or 100. The following three analyses with the MQEM-HS were applied; I) considering the pedigree information with 1-generation, II) with 2-generations, and III) with 2-generations incorporating additional 200 steers related to the maternal grandsires with their phenotypic records but no marker genotype information. When the number of the MGSs was 20 where the bias from the random mating was large, the false-positive detections of the QTL pairs in the analysis-I were most clearly recognized. By considering more pedigree information (the analysis-II) the degree of the false-positive detections became slightly reduced. The most accurate result was obtained in the analysis-III where these results suggest the importance of the adjustment of the non-random mating of the dams. Also revealed by this method was the usefulness of the MQEM-HS under the such difficult conditions of the multiple QTL mapping in beef cattle using field records.

D239

Investigation on the genetic background of coat colour inheritance in a Charolais x German Holstein F_2 resource population

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Coat colour inheritance in cattle comprises the effects of essentially four factors: Agouti, Extension, Spotting, and Dilution. While the Extension locus has been identified and characterized comprehensively, the precise molecular background of the other factors is still under investigation. The Dilution locus is responsible for a dilution effect of the original coat colour. Two different loci affecting dilution of pigment over the entire body were assumed for the Charolais (Dc) and the Simmental population (Db), but for both loci no linkage mapping information on chromosomal location is available. To enable chromosomal mapping of the Dc mutation an F_2 fullsib resource population (F_1 and F_2 individuals) generated from the Charolais (Cha) and the German Holstein (GH) breed was scored for coat colour regarding the dilution phenotype. For an initial mapping approach phenotypes were classified as Dilution homozygous (white), non-dilution homozygous (black) and heterozygous (grey). All red or yellow individuals (homozygous e/e at the Extension locus) were excluded from the primary analysis, because different shades of the coat colour phenotype "red" are observed in the German Holstein population. Linkage analysis revealed a significant linkage of the Dilution locus Dc with microsatellite markers on BTA5. The marker interval RM500 – IGF1 harboured the three unit LOD score support interval. No recombination was observed between marker ETH10 and the Dc mutation ($\theta = 0.0$). The mapping result is in agreement with a recent report about a trait-associated mutation in the silver homolog (SILV) gene. SILV is located at 33.648 Mb on BTA5 on the same sequence contig carrying also the ETH10 locus. Further investigation of the colour inheritance within the F_2 resource population revealed that a single diallelic mutation at the SILV gene cannot explain the total variation of coat colour dilution observed in our Cha x GH resource population suggesting further variation at the Dilution locus.

D248

Associations between single nucleotide polymorphism markers on bovine chromosome 14 with carcass merit in beef cattle

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A chromosome-wide QTL scan on bovine chromosome 14 (BTA14) of 28-half-sib families comprising of 420 steers has yielded 4 significant QTL affecting carcass merit in beef cattle: ultrasound marbling, 21 to 27 cM ($P<0.05$), ultrasound backfat thickness, 64 to 65 cM ($P<0.01$), ultrasound rib-eye area, 98 102 cM ($P<0.01$) and carcass gradefat, 21 to 22 cM ($P<0.05$). Single nucleotide polymorphism (SNP) markers spanning those QTL areas were analyzed for association with carcass merit of those animals using the mixed model by SAS. In the ultrasound marbling QTL area 3 SNPs were significantly associated with this phenotype ($P=0.0394$, $P=0.0402$ and $P=0.0466$). For 2 of the those SNP markers, animals inheriting the CT genotype had the highest marbling score, while TT genotype animals showed the lowest marbling score, with no significant difference between CT and CC genotype animals. For the other SNP, AG genotype animals showed the highest marbling, followed by GG genotype animals and AA genotype animals, with no significant difference between AG and GG genotype animals. In the ultrasound backfat QTL region, 1 SNP marker was found to have a significant association with this trait ($P=0.0172$). Animals inheriting the AG genotype had the lowest backfat thickness, while the AA genotype showed the highest backfat thickness, with no significant difference between the AA and GG genotype animals. In the ultrasound-rib-eye area QTL region, 1 SNP marker was significantly associated with this phenotype ($P=0.0216$). Animals inheriting the CC genotype showed the highest rib-eye area compared to both CG and GG genotype animals. In the QTL region for carcass grade fat, no SNP was found to be significantly associated with the trait. Further studies are underway to validate the associations between the SNP markers and carcass merit using different populations.

D256

Bilateral convergent strabismus with exophthalmus in German Brown cattle is linked to BTA5 and BTA18.

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Bilateral convergent strabismus with exophthalmus (BCSE) is a widespread inherited defect in several cattle populations. Affected cattle show a permanent anterior-medial rotation of both eyes with a bilateral symmetric protrusion of the eyeballs. The disease is incurable and shows a progressive course, which may lead to complete blindness. Complex segregation analysis showed that an autosomal dominant major gene was the most likely explanation for the segregation of BCSE in German Brown cattle. The objective of the present study was to map gene loci responsible for bilateral convergent strabismus with exophthalmus (BCSE) in German Brown cattle. For this purpose, a total of 92 animals out of 10 families was used for a whole genome scan. We selected 164 highly polymorphic microsatellite markers from published bovine linkage maps to achieve a uniform coverage and a mean marker distance of less than 20 cM. We used non-parametric linkage analysis to test the proportion of identical by descent (IBD) alleles which affected individuals share across the marker loci irrespective of the mode of inheritance of the phenotype. The analysis revealed significant linkage between two genomic regions and the occurrence of BCSE. These BCSE loci are located on the bovine chromosomes 5 and 18, i.e. BTAs 5 and 18, respectively. The region of significance on BTA18 ranged from 72 cM to 85 cM. The Z Mean peaked at the marker DIK5109 (77.6 cM) with a genome-wide error probability of less than 0.00001. For BTA5, the significant region extended between 25 cM and 46 cM. The Z Mean peaked at 42 cM (BMC1009 and DIK2465) with a genome-wide error probability that was below 0.0004. These results suggest that the major gene causing BCSE is located on BTA18. The locus on BTA5, however, may contain a gene, which independently contributes to the defect by means of affecting the characteristics of BCSE such as delaying its time of onset and/or its progression.

D267
Genome scan for QTLs related to tick resistance in bovine

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In tropical countries losses caused by bovine tick infestation has a tremendous economic impact in production systems. Genetic variation between *Bos taurus* and *Bos indicus* to tick resistance together with molecular biology tools might facilitate the use of molecular markers linked to resistance traits as an auxiliary tool in selection programs. The objective of this work was to identify QTLs associated with tick resistance/ susceptibility in a bovine F2 population derived from the Gyr (*Bos indicus*) x Holstein (*Bos taurus*) crosses. Using multiple ovulation and embryo transfer (MOET), four Holstein sires were mated with 28 Gyr cows generating 120 F1 animals. Five F1 sires and 62 F1 cows were mated producing 370 F2 animals. A total of 305 animals were evaluated to tick resistance and a genome scan for 10 chromosomes (1, 2, 3, 4, 8, 9, 13, 18, 19 and 17) was performed using microsatellite markers. Log of tick count + 1 was used as dependent variable in a model including year, sex, contemporary group, hair type, coat color as fixed effects and age at tick count as covariable. Statistical analysis was performed with the QTL express web-based package using additive plus dominant (a+d) and additive (a) models. Animals with short-straight hair were more resistant to tick infestation than animals with long-curl hair and animals with whiter coat color had fewer ticks also. Chromosome 4 showed a QTL with additive plus dominant effect ($P<0.05$), located at the far end from centromere. Among resistant F2 animals, most of them received a Gyr exclusive allele in this QTL. Chromosome 18 showed a QTL with dominant effect ($P<0.01$), located at 60 cM from centromere. It is not clear the origin of resistance in this QTL, since both breeds share alleles that most impact resistance or susceptibility in the F2. Additional chromosomes are currently under investigation in order to cover the whole bovine genome.

D275
Complex segregation analysis of megaesophagus in a large pedigree of German Shepherd dogs

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Megaesophagus, an abnormality of the esophagus, renders affected dogs with an inability to properly pass food through the esophagus into the stomach. Affected dogs present with varying symptoms from clinically appearing to be unaffected all the way to severely affected and unable to ingest solid food in a "normal" pattern. Definitive diagnosis was obtained by barium swallow radiography using standard procedures. To meet the need for dogs trained as guides for blind people, The Seeing Eye breeds over 200 German Shepherd puppies per year. This breeding program produced a single pedigree of German Shepherd Dogs containing 5,230 animals, 88 of which were diagnosed with megaesophagus. Looking at the prevalence of megaesophagus by birth year, an increase began in 1991, peaked in 1999/2000 with a prevalence of 10%, and subsequently declined as a result of selection efforts. A segregation analysis using the Pedigree Analysis Package (PAP) focused on the part of the pedigree informative for the disease. A mean prevalence was estimated to be 5% based on the birth years 2000 to 2005, where offspring were systematically tested for megaesophagus by barium swallow radiography. Due to the complexity of the single pedigree its informative part had to be split into 9 families in order to run PAP. The mixed inheritance model explained the data much better than the environmental model ($P < 0.001$), but also better than the major gene model ($P = 0.018$) or the polygenic model ($P = 0.025$). The large heritability, estimated to be 0.954, and the low penetrance (0.135) for the genotype homozygous for the unfavorable allele point to a very strong polygenic component with low influence from the environment, or to the presence of one or more additional major genes. The fact that the prevalence of megaesophagus could be reduced to 2% in this population within only 5 years by selection makes an exclusively polygenic background of this disease highly unlikely. It rather supports the hypothesis that an oligogenic mode of inheritance plays a role in the expression of megaesophagus in German Shepherds.

D277
Characterization of the aldo-keto reductase 1C gene cluster on pig chromosome 10 and association with age of puberty and ovulation rate.

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The rate of pubertal development and weaning to estrus interval are positively correlated and affect reproductive efficiency of swine. Selection for growth rate and leanness in modern commercial pigs has resulted in a delay in the onset of puberty. A QTL for age of puberty has been identified in a Meishan-White composite population on pig chromosome 10q (SSC10) near the telomere, which is homologous to human chromosome 10p15. This region in the human contains an aldo-keto reductase (AKR1C) gene cluster with at least six family members. Aldo-keto reductases (AKR1C) are tissue-specific hydroxysteroid dehydrogenases involved in interconversion of preferred steroid substrates. Screening the porcine CHORI-242 BAC library with a full-length AKR1C4 cDNA identified 7 positive clones and sample sequencing of 5 BAC clones revealed 5 distinct AKR1C genes (AKR1CL2 and AKR1C1-4), which mapped to 126-128 cM on SSC10. TIGR contigs were identified for 4 of the five AKR1C genes; AKR1C1 was not represented in EST libraries. These mRNAs were confirmed by RT-PCR of overlapping fragments or by sequencing individual cDNA clones. Using the IMpRH (7000rad) and IMNpRH2 (12000rad) radiation hybrid panels, these 5 genes mapped between microsatellite markers SWR67 and SW2067 and the gene order was the same as human. Comparison of sequence data with the porcine BAC fingerprint map show that the cluster of genes resides in a 300kb region. SNPs were identified in genomic DNA from parents of the resource population and 10 SNPs were genotyped in 184 gilts observed for age at first estrus and 284 females with ovulation rate from the F8 and F10 generations of one-quarter Meishan descendants of the MARC resource population. Age at puberty and ovulation rate data were analyzed for association by the General Linear Models procedure of SAS with sire and maternal grandsire fitted as fixed effects. One SNP, a phenylalanine to isoleucine substitution in AKR1C2, was associated with age of puberty ($p < 0.02$) and ovulation rate ($p = 0.11$) and a SNP in AKR1C4 was associated with ovulation rate ($p < 0.01$). These 2 SNPs are also segregating in commercial breeds and may be predictive for reproductive performance.

D287
Analysis and selection of microsatellite markers for an individual traceability system in Korean cattle (Hanwoo)

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The current animal industry is technology-intensive based on biotechnology and information technology. It will undoubtedly become more globalized, as the Free Trade Agreements and Doha Development Agenda are expected to take effect. To test applicability to the Korean cattle (Hanwoo) traceability system, 20 microsatellite (MS) markers were analyzed. Four hundred sixty-two Korean cattle from 19 different geographical regions in Gyeongnam Province, Korea were used in order to obtain fundamental data. The samples were analyzed with the use of the ABI-3100 Genetic Analyzer (Applied Biosystems, USA). The MSA, CERVUS, FSTAT, GENEPOP, API_CALC, and PHYLIP statistical programs were employed to estimate heterozygosity, polymorphic information content, F-statistics, identity probability, exclusion probability and genetic distance. We selected 11 MS markers (*TGLA227, BMS2113, BL1009, SPS115, BMS1747, TGLA122, INRA23, ETH3, BM4305, BM1824, and TGLA53*) in which the heterozygosity (*He*) value was found highly-ranked among the 20 MS markers. A multiplex PCR set was accomplished with these 11 MS markers. Two pairs of primers were combined for the determination of sex with the multiplex PCR, accomplished with a total of 13 markers. Identity probability (3.42×10^{-23}) of using the marker set was shown to be ten times higher than when using StockMakers™(3.50×10^{-22}) of Applied Biosystems. This result indicated the selected marker set is appropriate and effective for use in the Korean cattle traceability system.

D298

***FASN* gene association analysis with litter size in a F2 Iberian x Meishan intercross**

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The *fatty acid synthase* (*FASN*) plays a key role in the *de novo* lipogenesis in mammals. Furthermore, in mice, fatty acid synthesis is essential in embryonic development. The aims of this study were to identify QTL for litter size on SSC12 in an *Iberian* x *Meishan* intercross, and to carry out an association study of *FASN* gene variants with this trait. Data consists of 881 litter size records (number of born alive, NBA) from 256 F2 sows. The whole pedigree (21 F0, 114 F1 and 256 F2 sows) was genotyped for 10 microsatellites and 1 neutral SNP on *GH* gene. Three missense SNPs were detected on exons 9 (Arg418Gln), 21 (Thr1063Ile) and 39 (His2182Asn) of the *FASN* gene. These SNPs were genotyped by pyrosequencing, and were linked in two haplotypes in this intercross. The *FASN* gene maps on position 0 cM, and it is just 1 cM far from the first mapped microsatellite. QTL scanning was performed assuming a multitrait animal model, considering as different traits litter size at parities 1, 2, 3 and 4 or more. The fitted models included additive and dominant effects of one or two QTL and the year-season effect. The results revealed one QTL located on position 12 cM, with highly significant additive effect (LR=18.20, $p<0.001$), that increased only litter size of the first parity in 0.93 (± 0.29) piglets per copy of the *Iberian* allele. The comparison between models allowing for two-QTL versus a single-QTL, did not provide significant evidence of two QTL. The effects of the *FASN* haplotypes were tested with a standard animal model, and a marker assisted association test, to account for between breed linkage disequilibrium. Significant results are obtained (LR=14.06, $p<0.007$) when the standard animal model is applied. However, the marker assisted association test results indicate a lack of statistically significant association of the *FASN* haplotypes with litter size. The joint results suggest that the effects on NBA found for the *FASN* polymorphisms may be due to linkage disequilibrium between the *FASN* alleles and the true causal mutation.

D299

Confirmation of QTL for the inverted teat defect in porcine dam lines

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The inverted teat defect is a condition characterized by the failure of teats to protrude from the udder surface. The inverted teat defect has a polygenic background with heritability estimates ranging between 0.2 and 0.5. The inverted teat defect is a quantitative trait with regard to the number of inverted teats and the degree of inversion. Similar disorders are described in mouse, cattle and man but no information on the genetic cause is available. A genome scan within an experimental three generation F2 population based on Duroc x Berlin Miniature Pig with 42 % prevalence of the defect led to the identification of several QTL regions supporting the polygenic model of inheritance. Significant QTL were detected on SSC 1, 2, and 6 (NPL scores ≥ 4.5). In order to evaluate the relevance of these QTL in commercial dam lines, German Landrace (DL) and Large White (DE), a linkage analysis was performed. Therefore affected sibling and their parents comprising 120 full sib families and 500 individuals were genotyped at 28 microsatellites. The QTL analysis confirmed the segregation of loci that modulate the occurrence of inverted teats, on chromosome 2 and 6. Moreover, the breed dependent analysis (DL, respectively) of the data showed that on chromosome 6 at least two QTL are segregating. In order to complement the map-based approach of QTL analysis and to identify causal genes we aim to display trait-associated and QTL-genotype-dependent expression profiles of mesenchymal tissue of the teat ground employing genome-wide commercial and application-specific custom-made microarrays. (This project is supported by the FBF and the German Federal Ministry of Education and Research, BMBF: 0311701 and FUGATO–HeDiPig FKZ0313392)

D309

Analysis of associations between alleles of eight microsatellites and *Mycobacterium avium* subsp. *paratuberculosis* antibody response in German Holstein cattle.

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Paratuberculosis in ruminants is characterized by chronic granulomatous enteritis, resulting in persistent diarrhoea and progressive wasting of cattle infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease occurs worldwide with increasing frequency, leading to growing economic losses in beef as well as dairy industries. The objective of this study was to investigate possible genetic influences on susceptibility to MAP in cattle. Eight microsatellite markers known to be located near or within genes involved in response mechanisms to bovine paratuberculosis were chosen for genotyping. Pedigree information existed for 4686 German Holstein cows originating from 13 German farms. All of these animals had routinely been screened for MAP status within a surveillance programme using commercially available ELISA tests. The MAP specific immunoglobulin G antibody cutoff level was used to classify all animals positive or negative for paratuberculosis. A total of 637 (13.6%) cows were tested positive for paratuberculosis. The negative control group comprised 622 (13.2%) animals, each of which correlated in age at the time of testing, descended from the same sire and originated from the same farm as each of the positive animals. The microsatellites (*BB704*, *BB705*, *BB717*, *BB719*, *BMS1617*, *BB702* and *BOBT24*) showed five to twelve alleles with equally distributed frequency, microsatellite *BMC 9006* had only three alleles, two of which displayed very low frequencies in the present data set. Chi-square test revealed no significant differences in microsatellite allele frequencies between the two groups of Holstein cows tested positive or negative for paratuberculosis. Additional diagnostic measures such as faecal culture and expanded sampling may give further insight into the contribution of the investigated microsatellites to paratuberculosis susceptibility in German Holstein cattle.

D315

The role of growth hormone receptor and prolactin receptor in milk production in dairy cattle

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Many studies with diverse breeds of dairy cattle suggest that QTL affecting milk production segregate on bovine chromsome 20. In a previous study in Holstein-Friesian dairy cattle an *F279Y* polymorphism in the transmembrane domain of growth hormone receptor (GHR) was found to be associated with an effect on milk yield and composition. However, the possibility that two or more QTL exist within the region was not excluded. Multimarker regression analysis by graddaughter design suggests segregation of two distinct QTL on the chromosomal region including growth hormone receptor (GHR) and prolactin receptor (PRLR) in Finnish Ayrshire. The result is supported by combined linkage and linkage disequilibrium analysis. Both GHR and PRLR have important roles in the regulation of growth hormone and prolactin function in the mammary gland and are thus potential candidate genes that could be resposible of the observed QTL effect. By sequencing the coding sequences of GHR and PRLR and the sequence of three GHR promoters from individuals of known QTL genotype, we identified two substitutions that were associated with milk production traits: the previously reported fenylalanine to tyrosine substitution (*F279Y*) in the transmembrane domain of GHR and a serine to asparagine substitution (*S18N*) in the signal peptide of PRLR. The effect of the GHR *F279Y* on milk content in Finnish Ayrshire is in good agreement with observations in Holstein-Friesian cattle. The effect is clearly detected on protein and fat percentage and to some extent on milk yield. Protein and fat yields were not affected by *F279Y*. The PRLR *S18N* substitution is significantly associated with all three yield traits but not with the content traits. The association was confirmed in two independent samples of the Finnish Ayrshire population. We herein provide strong evidence that the effect of PRLR *S18N* polymorphism is distinct from the GHR *F279Y* effect. We also show preliminary evidence on the effect of interaction between the two loci.

D319
Strong positional concordance of plasma lipid QTL in pigs, human and mouse

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Genome-wide searches of LDL, HDL and triglyceride (TG) QTL in human and mouse have revealed that most of them map to homologous positions. The present study aimed to extend this comparative analysis to pigs, which belong to a different taxonomic superorder (Laurasiatheria). We have performed a genome scan for total cholesterol (TC), HDL, LDL and TG levels in a Duroc population of 370 individuals distributed in five half-sib families. Our study revealed highly significant QTL for plasma lipids levels at pig chromosomes 3, 4, 5, 6, 8 and 12, most of them showing a striking correspondence with regard to the chromosomal location of plasma lipid QTL in human. Thus therefore, QTL affecting LDL, HDL, TC and TG have been found at pig 3q1.4-2.6, which is orthologous to human 2p13-p25 where a QTL for TG, HDL and LDL has been reported. Similarly we have described QTL for TG at pig 4p1.3-1.5, 5p1.1-1.3 and 6q3.1-3.5, which are orthologous, respectively, to human 8q22-24, 12q12-24 and 1p31-36, where also QTL for TG have been mapped. Our results also revealed QTL at pig 8p2.1-2.3 (TC, HDL) and 6p1.4-q1.2 (TC, LDL), which are orthologous to human 4p13-16 (QTL for HDL) and 19q13.1-13.4 (QTL for LDL). Most of these QTL shared by human and pigs have a correspondence in the murine genome. This positional concordance might indicate that diverse polymorphisms located in a common set of genes, shared by distantly related mammalian species, are involved in the genetic variation of plasma lipid levels.

D322
Analysis of quantitative trait loci on chromosome 7 affecting milk production traits, cow herd-life and female fertility in Israeli Holsteins

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Genotypes of 5391 cows, daughters of 11 Israeli Holstein sires, were determined for 27 microsatellites spanning *Bos taurus* (BTA) 7, and analyzed by the daughter design for nine quantitative traits: milk, fat, and protein production, fat and protein percent, somatic cell score (SCS), herdlife, female fertility, and persistency of milk production. Preliminary analysis was by interval mapping of all 11 families jointly, with chromosome-wise significance levels determined by permutation tests. Protein yield, fertility, and herd-life were significant at 0.01, SCS was significant at 0.001, fat percent was significant at 0.0001, and fat yield was significant at 10^{-7}. Peak F-values considering only families with significant contrasts were obtained at 38 cM for fat and protein yield, at 31 for fat %, at 65 for SCS, at 76 for herd-life, and at 11 for fertility. It is likely that a single QTL is responsible for the effects on the three milk production traits, while two QTL are apparently segregating for herd-life. Confidence intervals were determined by the nonparametric bootstrap for each trait, considering only families with significant contrasts. The 95% confidence interval for female fertility spanned positions 2 to 30, with a median at position 12, and corresponds to a QTL affecting twinning rate segregating in the US Holstein population. Four of the 11 families had significant contrasts for female fertility. The 90% confidence interval for fat yield spanned positions 15 to 40, with a median at position 34. Five families had significant contrasts for this trait. Effects for fertility and herd-life were verified by application of the modified granddaughter design to a single family with significant contrasts for these traits. Significant effects of the grandpaternal allele were found for both traits. Based on the modified granddaughter design, the alleles that increase herd-life and female fertility are apparently the less frequent alleles in this population. Thus there is significant scope for selection on both of these genes.

D328
FUGATO - Functional Genome Analysis in Animal Organisms

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In the year 2004, the German Federal Ministry of Education and Research (BMBF) implemented the German genome analysis program FUGATO (Functional Genome Analysis in Animal Organisms), which complements the National Genome Research Network. The funding initiative aims to clarify the molecular basis of features with economic importance and to develop processes and technologies that enhance classical farm animal breeding. The main research focus is on animal health, animal welfare, and product quality. FUGATO, as a public-private partnership, is supported by the business platform FUGATO (IVF), which represents the interests of the industry and guarantees the implementation of the research results into practice. Until now, six cooperative projects are established in the fields of hostpathogen interactions (cattle, pigs, and chicken), biology of reproduction (cattle) as well as hereditary diseases (pigs). The first consortium, IRAS, aims the development of genetic markers for immune defense and resistance in the porcine respiratory tract. Main focus of the *E. coli*-chick project is to study host-pathogen interactions in *E. coli*-resistance in chickens and its application in breeding programs. The identification of genes causing hereditary diseases like inverted teats, anal atresia, and splay legs and their avoidance is the objective of the HeDiPig consortium. The fourth project, *QuaLIPID*, deals with the functional analysis of genes involved in the lipid metabolism in cattle and swine to identify relevant DNA variation, especially for product quality. Fertilink investigates the molecular mechanisms of fertility problems in cattle with a view to an early diagnosis of fertility problems. Finally, the M.A.S.- Net project analyses the genetic mechanisms determining the variability of protective ability and resistance against mastitis in cattle. The involved partners of the different cooperative projects establish a network of the leading academic institutes and the industry, mainly the livestock breeding economy, in Germany.

D330
Mapping quantitative trait loci for osteochondrosis in South German Coldblood horses

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Osteochondrosis (OC) is a developmental disease in growing horses due to disturbed differentiation and maturation of cartilage particulary at predilection sites of the fetlock, hock and stifle joints. Horses with radiographically visible osteochondrotic lesions are at a higher risk of developing orthopaedic problems later in life than unaffected horses.
The aim of this study was to identify quantitative trait loci (QTL) for osteochondrosis (OC) and osteochondrosis dissecans (OCD) in a whole genome scan of South German Coldblood horses. In 117 South German Coldblood horses belonging to nine paternal half-sib families standardized radiographic examinations of fetlock and hock joints were performed. The size of the examined half-sib groups ranged from 5 to 28 and 77% of the horses were female. The average age at radiographic examination was 17 months.
The genome scan included 157 polymorphic microsatellite markers in the first step which were equally spaced over the 31 autosomes and the X chromosome with an average distance of 17.7 cM and a mean polymorphism information content of 63 %. Putative QTL regions were confirmed in a second step by extending the marker set with 79 additional microsatellite markers. Chromosome-wide QTL for OC were found on 17 equine chromosomes. Eleven QTL were linked with fetlock OC or OCD and two QTL were associated with hock OC. None of the QTL was linked with OC in fetlock and hock joints, QTL significant for palmar/plantar osteochondral fragments in fetlock joints were shown on seven equine chromosomes. This genome scan is an important step towards the identification of genes responsible for OC in horses.

D332

A genome scan in a Duroc-Pietrain resource population reveals most prominent effects on meat quality, leanness, and fatness on SSC1 and multiple expression on SSC2.

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This study presents QTL detection in a F2 resource population resulting from a reciprocal cross between Duroc and Pietrain, with a main focus on the finemapping of SSC1, SSC2, SSC5, and SSC18. A 599 F_2 progeny within 31 full-sib families were produced. Genetic maps consisting of 106 informative microsatellites were constructed by CRI-MAP resulting in a sex average map of 1990.6 cM Kosambi. Least square regression interval mapping was conducted for QTL detection between these markers and 32 phenotypic traits. These traits included body weight, growth traits, body composition, and meat quality. Significant thresholds were determined by permutation test. In total, 70 QTLs were identified on all autosomes by the one-QTL model. Among these, 21 QTLs reached the 5 % or 1 % genomewide significant level. The largest significance levels ($P < 3.7*10^{-11}$) were found for QTLs between S0312 ~ S0113 on SSC1 affecting two kinds of meat pH value. In the same confidence interval an 1% genomewide QTL for meat colour and a 5 % chromosomewide QTL for meat conductivity were identified. Also on SSC1 between SW2166 ~ SW1957, two QTLs affecting meat content exceeded the 1 % genomewide threshold. Furthermore, 1 % genomewide significant QTLs for seven fat traits were identified between S0312 ~ SW1957 on SSC1. The above results on SSC1 strongly suggested multiple QTL expression. By using two-QTL model with imprinting effect, two QTLs were found simultaneously segregating on SSC2: one indicated imprinting expression, mainly paternal expression in the proximal region; the other indicated Mendelian expression in the middle region. These multiple QTL mainly affected the fat traits and meat content. (This project was supported by the German Research Foundation, DFG grant FOR753 DRIP, Germany).

D334

Developing new microsatellite markers on cattle chromosome 9 using Ensembl database

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We have carried out a study to detect quantitative trait loci for mastitis resistance in three Nordic cattle breeds, mapping a QTL on BTA9q2. The aim of this study was to identify new microsatellite markers using the cow genome sequences available in Ensembl database in order to increase the number of markers available for the fine QTL map. As BTA9 is orthologous to HSA6q, 11 human genes and ESTs were chosen along HSA6q (*C6orf55, AIG1, Pex3, Rab32, Shprh, Hivep2, Deadc, Stx11, Utrn, Epm2a* and *Grm1)* for BLAST searches in the cow Ensembl database. Cow traces were screened for tandem repetitions using the software "equicktandem" and the sequences were confirmed by sequencing small fragments containing the microsatellites. Microsatellite polymorphisms and allele number were determined by running the microsatellites in a capillary electrophoresis equipment (MegaBace) in a set of 20 individuals that belong to different cattle breeds and populations. We found nine new microsatellites, eight of which were polymorphic, the allele number ranged between three and nine and two of them showed null alleles. Marker positions on BTA9 were confirmed by physical mapping using the 3000-rad Roslin/Cambrige bovine RH panel and six of the developed polymorphic microsatellites were successful mapped on BTA9.

D340

Comparison between the biochemical and molecular tests to identify the probable homozygous Tobiano animals in Pampa horses.

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The Pampa horses present the coat color pattern Tobiano as their main characteristic. This pattern is caused by the gene Tobiano (To) inherited as single dominant gene. In several horse breeds the Tobiano gene is linked to Albumin (Al) and vitamin D binding protein (Gc) and also shows phase conservation with alleles Al^B and Gc^S. This genetic linkage can be used in the detection of the probable dominant homozygous for the gene To. However, exceptions to this linkage phase have been demonstrated in some breeds. The equine homologue of proto oncogene (KIT) has also demonstrated to be strongly associated to the gene To, being a new marker in the identification of the probable dominant homozygous for the gene To. In this study 159 samples of Pampa horses and a control group of 32 Paint horses were used in a comparative study between the biochemical (Al and Gc) and molecular (KM1) markers, revealing that in the Pampa horses only the molecular test can be used to detect the probable homozygous Tobiano horses.

D347

QTL scan for physiological variables related to vitality in newborn piglets

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Preweaning mortality generally accounts for 12 to 20 % of total piglets born alive, thus leading to major economic losses for pig breeders. Mortality is related to the piglet's ability to adapt to the new extrauterine conditions that demands respiratory, digestive, thermoregulatory and immunological changes. We have performed a whole genome scanning focused on the search for QTL harbouring genes that control physiological and vitality variables of newborn piglets from an F_2 *Iberian* by *Meishan* intercross. A total of 449 piglets were monitored for heart rate (HR-0, HR-1), arterial oxygen saturation (OS-0, OS-1) and rectal temperature (RT-0, RT-1) at birth (-0) and 1 h later (-1), time to reach the udder (TU) and time to the first ingestion of colostrums (TS). A linkage map was constructed with 109 microsatellites and 10 SNP. A whole genome scan was carried out within the context of an animal model, which allows joint estimation of QTL and polygenic effects. We have detected 23 QTL significant at the genome-wide level, of which two were related to HR-0 (SSC8 and SSC10, with $P<0.05$), one to HR-1 (SSC12, $P<0.05$), one to TS (SSC5, $P<0.05$), four to OS-0 (SSC7, SSC10, SSC11, and SSC12, $P<0.05$), and fifteen to OS-1 (SSC2, SSC7, SSC8, SSC11, SSC12, SSC13, and SSC16, $P<0.001$; SSC6, SSC14, SSC15, and SSC18, $P<0.01$; SSC1, SSC3, SSC4, and SSC17, $P<0.05$). Only dominance genetic effects were significant, displaying positive values in all cases. The comparison between a model allowing for two-QTL versus a single-QTL model provided significant evidence of two QTL for OS-1 mapping on SSC6 at positions 36 and 110 cM. The main result of this work is the detection of a considerable number of chromosomal regions harbouring QTL with effect on physiological traits. However, the magnitude of the detected dominant effects must be cautiously considered because it can be probably overestimated. For this purpose, a joint analysis of QTL mapping on different chromosomes and their interaction will be carried out.

D348

Quantitative trait loci associated with parasitic infection in a bovine F2 population

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Gastrointestinal nematode infection causes significant losses to the cattle industry. It reduces meat and milk production, increases mortality, requires anthelmintic use and changes in herd management. In New Zealand, anthelmintic expenses are about $27.9 million/year, while in the U.S.A., these parasites cost the American livestock industry approximately $2 billion per year in losses regarding productivity and increased operating expenses. In Brazil, nematode infections are responsible for losses of 40kg liveweight/animal/year. In untreated animals, mortality can reach up to 30%. This study aimed to identify quantitative trait loci associated with endoparasitic infection in a Holstein x Gyr F2 population. Data were collected from 310 animals over a 5-year period. All animals were continually exposed to a mixed nematode infection by grazing during 20 weeks. Faecal samples were collected from each animal in two seasons (winter and summer). Strongyloidea super-family predominates over other super-families of nematodes (Fecal Egg Count – FEC was performed separately). This super-family is important because it contains the most pathogenic genus to bovine. Each animal from the F2 population was genotyped with 57 microsatellite markers from 10 chromosomes (1, 2, 3, 4, 8, 9, 13, 18, 19, and 27). Statistical analysis was performed with the QTL express web-based package using both additive plus dominant (a+d) and additive (a) models. Evidence were found for quantitative trait loci (QTL) associated (P<0.05) with Strongyloidea super-family FEC on chromosomes 2, 8 and 9. This study has shown that some aspects of host resistance to gastrointestinal parasites are under genetic control, therefore these QTL could be utilized in a marker-assisted selection scheme to increase host resistance to gastrointestinal parasites. Additional chromosomes are currently under investigation in order to cover the whole bovine genome.

D356

A high density genome wide SNP analysis in Australian dairy cattle

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A high throughput single nucleotide polymorphism (SNP) genotyping screen was performed on 1551 Australian progeny-tested dairy bulls using 15,000 SNP markers for a quantitative trait analysis as part of an Australian dairy research program (Cooperative Research Center for Innovative Dairy Products, CRC-IDP). A commercial SNP genotyping platform (Parallele-Affymetrix) was used incorporating 10,000 public domain SNP markers and 5,000 proprietary SNP markers. The proprietary markers were selected to cover regions in the genome predicted to be marker-sparse, known QTL regions, and candidate genes from the CRC-IDP candidate gene data base. The 22.5million data points resulted in the following summary performance statistics; 99.4 % conversion rate to genotype assays; 88.1 % informative SNP markers; 86.4 % placed in a predicted position based on an integrated map; 74.8 % with minor allele frequency >0.05. A reproducibility of 99.2 % was achieved for repeat informative assayable SNPs based on repeat genotyping of 24 bulls (20 bulls were genotyped 2x and 4bulls 3x). A data base of high-quality SNP scores was built after corrections for segregation distortion. A linkage and integrated map is being built for all 15,000 SNPs. LD structure across bovine genome including the X chromosome (Khatkar et al. these proceedings) is being built. A multi-pronged approach is being used to analyze genome-wide marker information for 41 traits, representing 38 traits with estimated breeding values and 3 bull semen fertility phenotypes. In addition the SNP information is being used assessment of genome wide and population diversity (Zenger et al. these proceedings), and study of inherited disorders (Tammen et al these proceedings).

D360

The Acyl-coA dehydrogenase, medium-chain (*ACADM*) gene has no effect on fat deposition traits in an Iberian x Landrace intercross

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Previous studies have indicated a strong possibility of the existence of QTL for body composition located in the position between 125 and 132cM on SSC6q (region defined by markers *DG32* and *LEPR*) in an Iberian x Landrace intercross. In this study, physical localization of Acyl-coA dehydrogenase, medium-chain (*ACADM*) gene was detected with the use of the radiation hybrid (RH) panel. The results of two-point analysis implied that the location of *ACADM* was between *DG32* and *LEPR* on SSC6q. Because of the function related to β-oxidation of fatty acids, porcine *ACADM* was considered as a candidate gene affecting fat deposition, due to its physiological function and position on the pig genome. Sequence analysis of the coding region from an Iberian pig and that from a Landrace pig by RT-PCR revealed no difference between the two coding sequences. In order to determine the nucleotide sequence of the promoter region and the transcription initiation site, we performed 5'-RACE and direct BAC sequencing. We found four SNPs (A-41G, G-77T, C-100G, and C-499T) and an insertion (AACA) mutation. Among the SNPs, the SNP G-77T was on a potential *NRRE* following two *SP1* binding sites. In the linkage map, the position of *ACADM* was located at 127.2cM, and the deduced order and distance were *SW1376*-9.9cM-*SW316*-8.1cM-*SW71*-8.2cM-*S0228*-3.3cM-*DG32*-2.5cM-*ACADM*-5.8cM-*LEPR*-3.4cM-*SW1881*. The two SNPs (A-41G and G-77T) in the promoter region were used for testing relationships with fat deposition traits using PCR-RFLP with the Iberian×Landrace population. The results allow us to refine the QTL mapping with a slight improvement of the statistical significance and location estimate, and to discard the *ACADM* polymorphisms analyzed as potential causal mutations for the observed QTL.

D361

Single nucleotide polymorphisms (SNP) at the Calpastatin gene and meat tenderness in cattle.

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The protein Calpastatin (CAST) has long been known to affect meat tenderness in cattle and other species, but only in recent time has confirmed genetic linkage been demonstrated between genomic regions including CAST and meat tenderness, and now a genetic test for meat tenderness in cattle partly based on CAST is offered for commercial testing. The coding sequence for CAST was sequenced and 12 coding SNP were discovered, 3 of which alter the amino acid sequence. One of the amino acid changing SNP, CAST-155, is close to an intron exon boundary, and in the absence of intronic sequence, an allele specific oligonucleotide assay was designed that included as the last bases the consensus sequence of the intron exon boundary. MGB-Taqman™ assays were designed for the other SNP. The minor allele frequencies (MAF) of these SNP are CAST-155 = 0.37, CASTE13-16 = 0.06 and CAST3-84 = 0.21. There were no homozygotes and only 5 heterozygotes for CASTE13-16 in 103 taurine animals so taurine animals were not genotyped further for this SNP. The CAST-155 SNP showed association to peak force (N=589, t = 2.69, P = 0.014). CASTE13-16 showed a weak association to peak force in zebu and zebu-cross animals (N=921, t=2.07, P = 0.067, MAF = 0.07), although the Belmont Red was highly significant but the Santa Gertrudis was not. The CAST3-84 SNP showed highly significant association to peak force in the combined sample as well as separately in taurine as well as in zebu cattle (N=4936, t=4.59, P < 0.00001) and in individuals breeds within those groups.

D363
What sort of genes affect milk production?

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Using DNA markers 410 positions across the bovine genome were tested for the presence of genes affecting one or more of 3 independent milk production traits, Australian Selection Index (ASI, which is (3.8*protein) + (0.9*fat) – (0.048*milk), protein percentage (P%) and fat percentage corrected for protein percentage (F%-P%). The half-sib design consisted of 6 sires each with 100 high ASI and 100 low ASI daughters. A maximum likelihood analysis was conducted to estimate the proportion of positions that affect two of these traits. Genes that affect only ASI would result from a larger or more active mammary gland affecting all milk components. Genes that affect only P% correspond to increased or decreased lactose synthesis within the mammary gland resulting in the volume of milk being changed in the same direction, but without a change in protein or fat yield. Protein synthesis would remain constant causing a change in the percentage of protein in the milk and also a small change in ASI. Genes that affect both ASI and P% in the same proportions result from an increase in protein synthesis. Genes that affect F%-P% correspond to increased or decreased fat synthesis within the mammary gland while the volume of milk remained relatively constant. Results indicate that of the 410 chromosome positions that detected a gene, most affected one trait and not the others, though a small proportion (2.8%) affected ASI and P% in the same direction. Therefore there are few genes that cause an increase in protein synthesis.

D377
Development of mutagenically-separated PCR assays for equine genetic screening

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Screening tests for Equine genetic defects are predominantly performed using PCR based assays that rely on a common primer plus two allele-specific primers that differ at the mutation site, generally only a single 3` base. We have found that these allele specific primers are able to cross-amplify the non-target allele, particularly when primers are partially degraded. To ensure the reliability of genetic screening assays our laboratory is developing mutagenically-separated PCR (MS-PCR) assays to detect specific mutations, such as those responsible for Overo-Lethal White Foal Syndrome (OLWFS). MS-PCR uses one common primer and two allele specific primers that incorporate additional deliberate mis-matches between the allele specific primers and the target DNA sequence. This results in absolute allele-specific amplification which we have demonstrated in negative control amplifications using wild type DNA template with just the mutant specific primer and common primer, and *vice versa*. The essential features of the MS-PCR allele-specific primers are: multiple, deliberate mismatches within 3-5 bases of the allele specific 3′ bases; 10-30 base size difference between allele-specific primers; allele specific primers labelled with different fluorescent dyes; and 2-3 mismatches in the longer allele-specific primer at the position corresponding to the 5′ end of the shorter allele-specific primer. OLWFS is caused by a 2 bp missense mutation in the first exon of the endothelin-B receptor gene (ENDRB, 353-354TC>AG), resulting in substitution of isoleucine with lysine at position 118 (ENDRB, Ile118Lys). We have designed and validated an MS-PCR assay for this mutation. This enables one tube diagnosis, based on PCR fragments which differ in size and fluorescent tag, by either agarose gel or high throughput capillary electrophoresis. This assay has been validated with individual non-competitive PCRs, with only one of the allele specific primers present at a time, and has consistently amplified the target allele only thus eliminating the problem of cross-allele amplification even in a non-competitive reaction. This greatly improves the accuracy and robustness of genetic screening assays.

D379
Exploring the molecular basis for morphology and physiology that regulate athletic performance traits in North Swedish trotters

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The horse constitutes an exciting model for physiological genetic research in a functional genomics context. The origin of the North Swedish trotter provides a unique opportunity to identify genes influencing body constitution and racing performance. The North Swedish trotter originates from the North Swedish horse, that is a draught horse used in farming and forestry. It is well known that there has been a degree of cross-breeding between Standardbreds and North Swedish trotters before obligatory paternity testing was introduced in Sweden. We hypothesize that we will be able to detect a gene flow that has occurred between Standardbreds and North Swedish trotters due to this cross-breeding, and a subsequent strong selection for racing performance in the North Swedish trotters. A remarkable improvement in racing performance of the North Swedish trotter has occurred during the last fifty years. This process should leave “genetic footprints” in the genome of North Swedish trotters in the form of chromosome segments originating from Standardbreds.We are using a population genomics approach to identify chromosome regions under positive selection to achieve our goals. Chromosome regions under strong positive selection are likely to harbor genes affecting morphological and physiological traits important for physical performance traits in this system. We will compare the genetic makeup of North Swedish trotters (20 individuals), North Swedish horses (10 individuals) and Standardbreds (10 individuals) by performing a high-density genome scan. To date, 102 microsatellite marker have been analyzed within the horse material. Detection of genes that regulate racing speed is of particular interest as this could give a model for further studies of the genetic basis for complex traits such as muscle capacity and oxygen uptake. In both horses and humans these traits show clear genetic variation.

D382
Allele frequency of markers of tolerance to diarrhoea (FUT1, MUC4) and virus disease (Mx1) in pigs in the Czech Republic

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The oedema disease and post-weaning diarrhoea in 4-12 weeks old pigs is associated with the colonization of the small intestine by toxigenic *Escherichia coli* strain with fimbriae F18. The FUT1 (α-1,2 fucosyltransferase) gene has been determined as a marker gene for *Escherichia coli* F18 receptor locus. The M307 polymorphism of the FUT1 gene influences susceptibility to adhesion of *E. coli* F18 to intestinal mucosa and an outbreak of illness. Enterotoxigenic *Escherichia coli* expressing fimbriae F4 frequently colonizes the intestine of neonatal and weaned pigs. Susceptibility to enterotoxigenic *Escherichia coli* with fimbriae F4 is determined with the marker gene MUC4 (sialomicin complex). Mx1 gene affects the capability of inhibition the proliferation of RNA virus. A 11-bp deletion in Mx1 gene results in the loss of the ability to suppress viral propagation. The main objective of this study was to evaluate presence of resistant alleles of FUT1, MUC4 and Mx1 genes in population of pigs in the Czech Republic. Samples of blood and/or tissue and/or hair roots of 213 pigs were analysed by PCR – RFLP methods in FUT1 and MUC4 genes. The same amount of animals (213) were used and PCR based detection of the 11-bp deletion in Mx1 gene was performed. There were analysed files of 81 breeding boars (different breeds – Large White (LW), Landrace (LA), Duroc (D)), 77 breeding sows of LW breed and 59 the Přeštice Black-Pied pigs (PBP). Frequence of „resistant“ allele of marker FUT1 in particular files - breeding boars; breeding sows of LW breed and PBP pigs was: 0,20; 0,24 and 0,08. Frequence of „resistant“ allele of marker MUC4 in particular files - breeding boars; breeding sows of LW breed and PBP pigs was: 0,52; 0,52 and 0,80. Frequence of Mx1 allele associated with the capability of inhibition the proliferation of RNA virus was 0,85 in breeding boars; 1,00 in breeding sows of LW breed and 0,85 in PBP pigs. The study was supported by grants of Ministry of Agriculture of the Czech Republic number 1G58073 and QG60045.

D383

Evaluation of probability of exclusion of 16 microsatellites from Brazilian Mangalarga Marchador equine breed.

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In these past few years the efficacy and reliability of DNA tests have made it the choice for human genetic identification and paternity tests. This approach has also being used for animal identification . In this study 1500 paternity tests cases have been analized using a set of 16 microsatellites, including the nine loci recommendedby the international standard panel – ISAG (AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10 AND VHL20). DNA was purified from total blood and hair roots. The material was amplified in two multiplex PCR reactions. Each reaction contained 1x of Buffer 10x (500mM KCl, 100mM Tris –HCl and 15mM MgCl2), 2mM of each dNTP, 0,5 U of Platinum Taq Polymerase (Invitrogen), working solutions of primers in variable concentrations (1 to 5uM/sample) and 50ng of genomic DNA. The PCR products were analised by capillay electrophoresis using a MEGABACE 1000 sequencer (GE HealthCare). Among the 1500 cases we have found 123 cases where the offspring was not confirmed, whether sire- offspring or dam-offspring. Based on these data the combined probability exclusion of our multiplex have been analised resulting an estimated CPE= 0,9999998. The estimated CPE of the international standard panel is 0,999648. Another result is that the microsatellites with the lowest PE in our multiplex is HTG6, and the microsatellite with the highest PE is ASB2. These results confirm that the multiplexes are efficient and recommended to be used in Mangalarga Marchador breed genetic identification and parentage testing.

D390

Genetic analyses of six stocks of tilapia (*Oreochromis spp*) using microsatellite markers

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The production of Tilapia in the state of Minas Gerais has grown in the last years, trying to attend the lack of the consumer's market. Thus, the genetic identification of stocks is extremely important, once the genetic variability is the base for the commercial success, as well as for the implantation of any program for commercial selective breeding. In this study, 235 individuals from six commercial stocks of tilapias (Ceará, Chitralada, Israel, Nilótica, Taiwan and Red) from the Southeast region of Brazil were genetically characterized using five microsatelittes loci. Analyzing the stocks it was possible to identify the existence of genetic differences among stocks, estimated through the fixation allele index (Fst = 0,3263), and that a considerable loss of heterozigosity is occurring in almost all the stocks, according to the inside population inbreeding coefficient (Fis=0,0486). The stocks Israel and Nilótica were the most genetically similar (Ig=0,6663), while Chitralada and Taiwan were the ones that presented less genes in common (Ig=0,2463). The stock named Red was the most distinct among all of them. Differences in the identity matrix were observed between results from the present study and the literature, regarding origin of stocks. These results indicate that without a better genetic control of the stocks, is not possible to conduct effective programs of genetic improvement of Tilapias.

D394

Mapping and polymorphism of bovine ghrelin gene

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Bovine ghrelin, a 27-amino-acid peptide has been identified in bovine oxyntic glands of the abomasum. It is an endogenous growth hormone secretagogue. Total mRNA was extracted from abomasum and complete ghrelin mRNA was sequenced by rapid amplification of cDNA ends. The gene contains five exons and four introns with a short noncoding first exon of 17 bp similar to mouse and human ghrelin gene. Using a radiation hybrid panel, the gene was mapped to chromosome 22 near microsatellite markers UWCA49, BM4102, BMS1932, BM2613 and URB035 with good LOD Score. Some studies detected different QTLs near these markers like for milk fat percent, milk protein percent and somatic cell score. So, it would be interesting to study the polymorphism on the bovine ghrelin gene. Screening for polymorphisms in the five exons and the introns II and IV on ten Belgian Blue bulls, ten Holsteins bulls and ten Limousin bulls revealed a total of three single nucleotide polymorphisms. In order to evaluate if ghrelin could be involved in genetic variation for milk fat percent, milk protein percent and somatic cell score an association study between SNPs on ghrelin gene and these traits could be performed in a major cattle population.

D397

Genetic relatedness of Caribbean hair sheep (Preliminary results and analysis)

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Highly variable loci such as microsatellites provide a large amount of genetic information based on individual genotypes, permitting alternative approaches to the traditional ways of investigating and clarifying the genetic relationships between populations or breeds (Arranz *et al*., 2001). Microsatellite DNA polymorphisms were used to study genetic relationships between hair sheep breeds and to identify genetic markers for the Barbados Blackbelly sheep. Breeds investigated were the Barbados Blackbelly (Barbados population and St. Croix, US Virgin Islands, population), West African (Barbados), 'Mixed' breeds (Barbados), "Sugarlands Black" , a black off-type of the Blackbelly (Barbados), the St. Croix White (St. Croix, US Virgin Islands) and the Dorper (US Virgin Islands) as an out group. Fifteen *ovine* and *bovine* primers recommended by the International Livestock Research Institute (ILRI), Kenya were used and all showed polymorphism. These were *ILSTS017, OarFCB20, SR-CRSP-5, MAF214, ILSTSO19, BM1818, OarAE129, OarFCB304, MAF209, MAF035, TGLA53, BM827, ILSTS049, HSC and OarJMP29.* Preliminary results indicate that thirty alleles show frequencies ranging from 0.4 to 1 in the Barbados Blackbelly sheep and are potentially markers for the identification of the Barbados Blackbelly sheep breed. Five markers amplified showed frequencies ranging from 0.54 to 0.86 in both sub-populations of the Barbados Blackbelly sheep. These were: {*SR-CRSP-5* (157bp), *ILSTS019* (183bp), *BM1818* (231bp), *BM827* (220bp) and *OarAE129* (155bp)}. Calculation of average heterozygosity, Wright's F_{ST} and dendrograms constructed, using Microsoft Office Excel® and Minitab® Version 13.1, showed that the Barbados Blackbelly was genetically more similar to the mixed sheep found in Barbados than to the West African and the Sugarlands Black. Variation within the Barbados Blackbelly population supported the expected results based on the calculated heterozygosity within the sub-populations. The degree of genetic differentiation within the sub-populations ranged from significantly high in the Barbados Blackbelly sheep to very little in the mixed sheep and the off-type black sheep found in Barbados.

D406

Detection of a highly significant QTL for tick resistance on bovine chromosome 5 (BTA5) using a F_2 experimental population

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Ticks and tick-borne diseases affect animal health worldwide, and are the cause of significant economic losses, being considered the most important vectors of diseases affecting livestock and companion animals. The tick *Boophilus microplus* has spread throughout the tropics including Australia, East and Southern Africa, and South and Central America. Resistance to tick infestation, or at least the ability of developing an effective immunological response to infestation, is genetically determined. Uncontrolled *Boophilus spp.* infestations in climatically favorable conditions severely affect European cattle, but have little effect on Zebu cattle. We decided to explore these resistance differences between *Bos taurus* and *Bos indicus* by generating a Gyr x Holstein F_2 experimental population to be used in search for QTL (Quantitative Trait Loci) controlling resistance to tick. A total of 282 F_2 animals were evaluated for the incidence of ticks, and DNA samples of these animals were used to perform PCRs for eight microsatellite markers on chromosome 5 (BTA5), where the gene encoding interferon gamma (IFN-γ) is located: this class of proteins have been associated with parasite resistance. The QTL analysis was performed by the method of multiple interval mapping for F2 families using the *QTL Express* software. Tick countings were normalized using a Box-Cox transformation, and the effects of coat type and year in which the animal was evaluated were assumed as fixed effects. For QTL analysis, we used the BTA5 linkage map assembled for this F_2 population with the *Cri-Map* software. A highly significant QTL ($P < 0.01$) for tick resistance/susceptibility was detected in BTA5. The highest F-statistics value ($F = 13.06$) was at 132cM from the most centromeric marker. The trait average was 3.13 ±0.233 ticks/animal, while the addictive effect of the QTL was of -0.344 ±0.095 ticks.

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D407

Putative QTL for Parasite Resistance in Sheep

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Gastrointestinal parasites have a profound effect on sheep production. In a collaborative study between Utah State University, Louisiana State University and Roslin Institute, a genome-wide QTL scan was implemented to identify chromosomal regions in the ovine genome that play a role in resistance to gastrointestinal parasites. A sheep population segregating for parasite burden (measured by fecal egg count or FEC for *Haemonchus contortus* and packed cell volume or PCV) was constructed at Louisiana State University and included F2 offspring of F1 parents produced form Gulf Coast Native (resistant) and Suffolk (susceptible) crosses. Selective genotyping of the upper and lower 20% of the lambs for FEC after natural challenge has been used to identify potential QTL regions. To date, 1008 cM (33% of the genome) have been investigated for possible QTL. Suggestive QTLs have been identified on ovine chromosomes 1, 6, 9 and 19. Additional markers in these regions are then genotyped across the full population, including grandparents. The most significant results were detected on chromosome 1 for FEC at weaning, with a putative QTL localized on the central region of this chromosome. Another interesting region associated with PVC after natural challenge is located in the center of chromosome 9. Additional markers on chromosomes 6 (37 markers) and 9 (25 markers) have been genotyped and will be analyzed in the near future.

D412

A chromosome-wide scan for quantitative trait loci affecting carcass merit on bovine chromosome 19 in beef cattle.

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The objective of the study was to detect quantitative trait loci (QTL) affecting carcass merit on bovine chromosome 19 in a beef cattle population. Nine microsatellite and ninety four single nucleotide polymorphism (SNP) markers, with an average spacing of 1.18 cM, were genotyped on a total of 448 hybrid cattle (28 bulls and 420 steers). Animals were produced from a cross between Angus, Charolais, or University of Alberta hybrid bulls and the University of Alberta's hybrid dam line. Traits observed on live animals included slaughter weight and ultrasound measures of backfat thickness, marbling score and ribeye area. Carcass traits included hot carcass weight, grade fat, average backfat thickness, ribeye area, marbling score, lean meat yield, yield grade and quality grade. The study detected QTL for ultrasound marbling score (109 cM, $P < 0.05$), carcass weight (8 cM, $P<0.05$), carcass average backfat thickness (109-110 cM, $P<0.10$), carcass ribeye area (77-86 cM, $P<0.05$), lean meat yield (109-113 cM, $P<0.05$), carcass marbling score (34-52 cM, $P<0.05$), yield grade (109-115 cM, $P<0.05$) and quality grade (94 cM, $P < 0.05$) based on across-family analyses. These results provide a valuable reference for association studies and positional candidate gene research.

D413

Effect of growth hormone gene copy number in milk yield in Serra da Estrela ovine breed.

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Growth hormone (GH) has been described to influence milk production. In sheep, the *GH* gene is duplicated (Valinsky *et al*., 1990). Sheep can be homozygous for one copy (*GH1*, Gh1 allele), homozygous for both copies (*GH2-N* and *GH2-Z*, Gh2 allele) or heterozygous for the copy number (Gh1 and Gh2 alleles). The objective of this work was to study the influence of progenitor GH copy number genotype on ewes' milk yield. Southern blotting analysis of the genomic DNA of 89 "Serra da Estrela" animals digested with *Eco*RI showed the three expected *GH* genotypes. The observed genotypic frequencies were 2.2%, 15.7% and 82.0% for genotypes Gh1/Gh1, Gh1/Gh2 and Gh2/Gh2, respectively. Allele frequencies were 10.1% for allele Gh1 and 89.9% for allele Gh2. The impact on milk yield adjusted to 150 lactation days of the probability of a ewe to receive the Gh2 allele from either progenitor was statistically analysed by restricted maximum likelihood (REML) through univariate analyses with repeated measures using the BLUP - Animal Model and the MTDFREML program (Boldman *et al.*, 1993). Univariate analyses were performed using 1247 lactations from 390 ewes. The probability of a ewe to receive allele Gh2 was tested as a covariate; and as a fixed effect. The first analysis showed that ewes which received allele Gh2 yielded on average more 21.9 ± 0.26 L/150 d than ewes that received allele Gh1 ($P<0.001$). The second analysis showed that the ewes which received allele Gh2 yielded on average more 26.4 ± 0.29 L/150 d than ewes which received allele Gh1 ($P<0.001$), and more 3.0 ± 0.25 L/150 d than ewes which could have received either Gh1 or Gh2 alleles ($P<0.001$). These results suggest a dominant positive effect of the Gh2 allele upon milk production in Serra da Estrela ewes. We thank Associação Nacional de Criadores de Ovinos Serra da Estrela for blood and milk records. This study was financed by the European Community - III Framework Programme for Research and Technological Development, co-financed by the European Social Fund (ESF) and by Foundation for Science and Technology (PhD grants SFRH/BD/1140/2000 and BD/18061/98).

D419

Fine mapping of the porcine Arthrogryposis Multiplex Congenita (AMC) region on SSC5 and development of a genetic marker test.

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AMC is a very common congenital defect in swine. The disease reported here is caused by an autosomal recessively inherited mutation recently mapped to pig chromosome 5 (*SSC5*). Piglets born with this syndrome have multiple defects of the legs and spinal column and do not survive. To identify genetic markers for fine mapping and association studies, 35 partial gene sequences of 24 genes, all located in the *AMC* region, were sequenced and compared between genetically healthy and diseased piglets. Three SNPs in *TUBA8*, one SNP in *CNTN1* and a newly developed microsatellite (*bE77*) co-segregated with the AMC phenotype in 250 pigs (of which 60 had AMC) of our experimental herd without recombination events (LOD scores of 38.5, 24.1 and 51.2, respectively). A BAC contig, combined with the information of recombinant pigs from commercial herds, enabled the precise delineation of a new order of loci on the q-arm of SSC5: centromere-*SW1987-UMNp1275-SW152-USP18/(bE77-TUBA8)-AMC/CNTN1-SW904/SW1094*-telomere. Furthermore, to reduce the frequency of *AMC*-carriers, a diagnostic genetic marker test, comprising the microsatellites *bE77* and *SW904*, was developed. The markers were analyzed to predict the presence of the *amc*-allele for the disease of 80 Large White (LW) boars currently used for artificial insemination (AI) in Switzerland. The marker alleles strongly associated with the disease in the experimental herd indicated a higher risk of susceptibility to AMC for 17 boars (21.3%). The test was also applied to 41 piglets from 14 commercial farms, believed to be affected with AMC. The results confirmed the previous diagnosis in 34 cases (83%). In conclusion, we established a powerful and reliable marker system to discover *AMC* carriers and, thus, to reduce the incidence and spread of the disease and to minimize economic losses in the Swiss pork industry.

D420

Fine-mapping of a pork tenderness QTL on porcine chromosome 2 (SSC2)

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A QTL located on SSC2 and exhibiting large effects on both Instron shear force and taste panel tenderness was detected within the Illinois Meat Quality Pedigree (IMQP). Comparative analysis of SSC2 indicates that the QTL region is orthologous to a segment of HSA5 in which there appears to be only one major candidate gene, calpastatin (*CAST*). *CAST* polymorphisms have recently been shown to be associated with meat quality characteristics; however, for a number of reasons, the possible involvement of other genes and/or molecular variation in this region can not be excluded, thus requiring fine-mapping of the QTL. To initiate fine-mapping, a minimal tiling path (MTP) of regional BAC clones was selected using the high-resolution porcine WG-RH and BAC physical maps. Marker density within the ~30 Mb region surrounding the most likely QTL position was increased through the use of four publicly available microsatellites as well as 10 novel informative microsatellite markers, including two within the *CAST* gene, isolated from relatively evenly spaced MTP clones. Phenotypic and marker data were analyzed using the outbred F2 analysis servlet of QTL Express. In-depth analyses of individual half-sib families suggest the presence of two QTL within this region, each affecting both Instron shear force and taste panel tenderness. Further fine-mapping of the QTL interval will involve an iterative process of continued development and combined linkage/linkage disequilibrium analysis of new markers from MTP clones until maximal refinement has been achieved.

D422

Identification of single nucleotide polymorphism for the adipocyte fatty acid binding protein (FABP-4) and its SNPs is associated with marbling score in Hanwoo steers

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The adipocyte-fatty acid binding protein (FABP4) transports fatty acids into the cell, the uptaken fatty acid is processed *de novo* lipid biosynthesis as well as has a function as signal molecule of adipogenesis. The bovine FABP4 locus is mapped a region between BMS 740 and BMS 1304 marker on the bovine chromosome 14 and this region has been reported as a quantitative trait loci (QTL) affecting marbling score in Hereford × Brahman sire. A total of 17 SNPs was found in 4.2kb of 4.2 kb FABP4 genomic sequences of 24 unrelated Hanwoo steers and classified into three sets of SNPs : 2821 nt (G to C), 3520 nt (A to T), and 3678 nt (A to G). The three SNPs were genotyped on 574 Hanwoo individuals and 7 different foreign cattle breeds by PCR-RFLP. The Hanwoo, Charolais, Simmental, Angus and Limousin in the *bos taurus* species had a same allele frequency at any position; however, Hereford, Brahman and Brown swiss breeds considerably differed from Hanwoo allele frequency. By using GLM association analyse with EBV and phenotypic data of Hanwoo steers, significant associations ($P<0.05$) were found between FABP4 three SNP genotypes and marbling score. Therefore, these SNP polymorphisms in the FABP4 might have a potental value for marker-assisted selection of marbling trait in Hanwoo cattle.

D439

Origin of fishy taint in brown egg layers

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The fish-odour syndrome in humans, cattle and chicken is characterized by an odour reminiscent of rotting fish, caused by abnormally high amounts of excreted trimethylamine (TMA). Normally the liver enzyme *flavin-containing mono-oxygenase 3* (*FMO3*) is catalysing the oxidation of dietary derived TMA to odourless trimethylamine N-oxide. The tainting is observed only under certain feeding regimes, which overload the TMA oxidation process. We have shown that both egg-taint and chicken *FMO3* gene map to the same location on GGA8. A missense mutation in the chicken *FMO3* gene was found to be associated with elevated levels of TMA and fishy taint in the egg yolk of commercial brown egg layers. This particular mutation changes an evolutionary highly conserved amino acid at the position of 329, occurring within an evolutionary conserved pentapeptide motif -*FATGY*- that has been speculated to be a substrate recognition pocket of mammalian flavin containing monooxygenases. The mutation was found in several brown layer breeds. We have analysed the sequence surrounding the *T329S* mutation and present a phylogeny of the different haplotypes of chicken *FMO3*. We analysed 2690 bp of the sequence of the *FMO3* gene on both sides of the *T329S* mutation from 9 chicken breeds or lines. The mutation was found in 6 of them (ISA Brown, TETRA, Lohmann Brown, Transylvanian naked-neck, Green-legged partridge and Marans). In total, 17 haplotypes were identified from the sample. A phylogeny of the most common haplotypes shows grouping of all haplotypes containing the amino acid S variant. Moreover, a similar haplotype of *FMO3* was identified in all three commercial brown lines, indicating a common origin for the taint in present-day commercial brown layers. In addition, we present a method to identify carriers and possible tainters by direct testing of *FMO3* genotype using feather samples. The gene test does not depend on the age, environmental factors, or gender of the tested individuals.

D450

Genetic characterization of the Chiapas sheep and its relationship with Spanish breeds

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Sheep is one of the principal means of subsistence for the Mayan Tzotzil native groups in southern Mexico. Sheep were introduced in America by the Spaniards in the XVI century. The objective of this study was to genetically characterize the Chiapas sheep breed and assess the intra-breed biodiversity of three varieties (or lines) of the breed (Chiapas, Café, and Chamula). An additional objective was to ascertain the putative spanish origin of the Chiapas sheep breed by comparing it to populations from Spain (Spanish Merino and Churra) and the Canary Islands (Canaria and Palmera). Three hundred eighty eight animals were sampled for this study and 27 microsatellites were used to characterize the breeds. The mean number of alleles in the Chiapas breed was 7.53. Most markers were in Hardy-Weinberg equilibrium. Analysis of molecular variance (AMOVA) showed that: Fis= 0.041, Fst= 0.049, and Fit= 0.089. Results indicate that 4.9% of the variation was between populations, the variance between individuals within populations explained 4.1% of the variation, and the variation between individuals was 8.9%. This information supports the existence of three varieties of Chiapas sheep breed. Genetic distance analysis indicated the Merino and Palmera as the most differentiated breeds. The closest breed relationships were between Café and Chamula. The Churra breed had the closest relationship to the Chiapas sheep. No evidence supported the influence of the Merino, Canaria and Palmera breeds on the Chiapas sheep breed.

D452

Inherited diseases in Australian dairy cattle

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Single nucleotide polymorphism (SNP) assays for inherited diseases in dairy cattle, were included in a high density SNP panel incorporating 10,000 random markers and 5000 targeted markers (Raadsma et al these proceedings) and were screened across 1494 Australian dairy as part of a large dairy research program. The targeted disorders deficiency of uridine monophosphate synthase (DUMPS), complex vertebral malformation (CVM), citrullinaemia and bovine leukocyte adhesion deficiency (BLAD) were chosen as they are caused by single point mutations and have been identified in Holstein Frisian cattle.
The BLAD mutation was not successfully transformed into a SNP assay. Carrier frequencies for CVM and citrullinaemia in the tested population are 12.47% and 2.45%, respectively. These results essentially confirmed commercial DNA testing records in the Australian Dairy Herd Improvement Scheme (ADHIS) database and identified several new carriers in the group of previously untested bulls. As expected, no homozygous affected animals were detected for these lethal disorders. The DUMPS mutation was not detected and has to our knowledge not previously been reported in the Australian dairy population. In addition to detection of known inherited disorders, analysis of extreme segregation distortion clusters in the whole genome scan is used to identify genome areas that are likely to carry embryonic lethal genes or genes with strong negative selection response.

D454

Fine mapping of QTL affecting mastitis resistance in Nordic dairy cattle

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Mastitis is a prime candidate for genetic improvement using marker assisted selection. This is because it is the most costly disease in dairy production, phenotypic information is difficult to obtain, and genetic progress with traditional breeding is restricted because the heritability is low. The extensive records on clinical mastitis in the Nordic countries were used to finemap QTL affecting mastitis and develop tests for marker-assisted selection. A granddaughter design of 34 families with 1098 bulls from three related Nordic red breeds (Finnish Ayrshire, Swedish Red and White and Danish Red) was used first to confirm previous genome scan results and then to fine-map a QTL region. Initially, six candidate chromosomes were examined using a new common marker map, and the two chromosomes with the best evidence for a QTL with major effect were selected for fine-mapping. Dense marker maps were developed for the QTL regions and genotyped across the families. Information on linkage disequilibrium within and between breeds was exploited to finemap the QTL using single- and multiple-trait LA/LD models. A QTL on BTA9 affecting clinical mastitis was mapped within a 2 cM region. Haplotypes that are predictive for differences in mastitis incidence in the general population were identified. These marker haplotypes associated can be used in marker-assisted selection in the Nordic red breeds. This work was funded in part by EC FP5 project MASTITIS RESISTANCE (QLK5-CT-2002-01186).

D465

Mapping QTLs for resistance to coccidiosis (*Eimeria tenella)* in an experimental F2 cross and validation in commercial lines in chicken.

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The production of healthy animals is a major request from the breeders and the consumers, for economical reason and increasing concern about food safety and animal welfare. In chicken, coccidiosis is an important parasitic disease. Genetic resistance is an attractive complementary approach to classical control ways. Strategy to identify quantitative trait loci (QTL) linked to resistance to coccidiosis (*Eimeria tenella)* in chicken is presented. Screening of outbred chicken lines for resistance to *E. tenella* identified two lines showing extreme resistance vs susceptibility (Fayoumi vs Leghorn). After testing the optimal inoculation dose, an F2 cross was produced from the Fayoumi and Leghorn lines and challenged for *E. tenella* (n=860). Resistance phenotypes were body weight gain, plasma coloration, hematocrite, lesion scores, rectal temperature and mortality. Selective genotyping was applied by typing the 15 % most resistant and 15% most susceptible birds within each F1 father families, using 139 microsatellites on 22 chromosomes. In the F2, 13 QTLs chromosome-wide significant ($P<0.05$) were found on 9 regions of 7 chromosomes (1, 2, 3, 6, 10, 15 and 23). In 4 regions, QTLs affecting 2 resistant traits shared the same position. For validation, 8 regions were tested in two different commercial « label » lines (L1, n=395; L2, n=540). Two regions were validated in L1 and in L2, on chromosomes 2 and 6, whereas two different regions of chromosome 1 were validated either in L1 or L2. Validation from F2 to commercial lines was observed for either the same resistance trait or not. In experimental and commercial challenged animals, resistant traits were significantly correlated. Further research will address refining the regions of interest and integrate functional studies.

D468

Quantitative trait loci for abdominal fat weight and feed conversion in chicken

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To reduce the body fat deposition is one of the current purposes of poultry breeding programs, because the fatness synthesis has a high energy cost, which depress the feed conversion. Molecular markers information can be very important for poultry breeding programs, due the expensive fatness evaluation. This study aimed to map QTL in 350 F2 chickens from the EMBRAPA resource population (broiler x layer cross). Abdominal fat weight was measured at 42 d of age and feed conversion between 35 and 41 d. Here we report results from analysing chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 11, and 13 with 91 microsatellite markers, covering a total of 1659.5 cM. The data were adjusted after an analysis of the factors by stepwise regression. The fixed effects of sex and hatch and the random infinitesimal genetic value were adjusted for both traits, while the covariate body weight at 42 d of age was adjusted only for abdominal fat weight. Qxpak v.13 (Pérez-Enciso and Mizstal, 2004) was used for QTL mapping. Two QTLs for abdominal fat weight were found between MCW0297 and LEI0146 (86 cM) in GGA1 (Nominal P-value = $9x10^{-9}$), and between LEI0029 and ADL0371 (122 cM) in GGA3 (Nominal P-value = $1x10^{-5}$). For feed conversion a QTL was mapped between LEI106 and ADL183 (317 cM) in GGA1 (Nominal P-value = $5x10^{-6}$). The bivariate analysis of those traits did not indicate any pleiotropic QTL in those regions. The QTL for abdominal fat weight in GGA1 confirms that reported by Jennen et al (2005), although they found only suggestive linkage. Many QTLs mapped for abdominal fat weight by others authors, were not confirmed here, which probably due to age differences. Our population was evaluated with six weeks, therefore before the beginning of the largest adipocyte hypertrophy phase.

D469

Association of transferrin variants and microsatellite alleles with the production of equine chorionic gonadotrophin.

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The production of equine Chorionic Gonadotropin (eCG) by the endometrial cups derived from the fetus appear to be necessary for the establishment and maintenance of normal pregnancy. The development of endometrial cups produce a maternal cell mediated immune response to the fetal-placental unit, which is related with cups survival. Transferrin gene (Tf) has been associated with fertility and was considered as a key marker in reproduction traits. In the present study, the level of eCG production was determined by Enzyme Immune Assay (EIA) in 179 mares, during four consecutive years. In addition, polymorphisms of the transferrin gene and five microsatellite markers linked to the Equine Lymphocyte Antigens complex (ELA) were analyzed. The studied microsatellites were the following: LEX 14, LEX 64, LEX 52, UM 011 and VIASH 64. The relationship between a particular genetic profile and eCG production was assessed by the ANOVA test using the algorithm implemented in the software STATISTICA 5.5 for Widows. Significant associations were found between Tf, LEX 52 and UM 011 and production level. The obtained results could assist in the development of genetic testing that might improve the eCG production.

D472

Quantitative Trait Locus Affecting Susceptibility to *Mycobacterium paratuberculosis* Infection Identified on BTA20 in US Holsteins

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Our objective was to identify QTLs affecting susceptibility to *Mycobacterium paratuberculosis* infection in US Holsteins using a daughter design. Twelve paternal half-sib families were phenotyped based on their large number of daughters in production and low relationships among sires. Serum and fecal samples from 4233 daughters of these 12 sires were obtained for disease testing. Sampling focused on cows in second or third lactation because these animals are more likely to test positive for infection. Disease testing was performed with serum ELISA and fecal culture in parallel. Case definition for an infected cow was ELISA S/P ratio $\geq$ 0.25 and/or a positive fecal culture. Apparent prevalence of infection across families was 0.079. Three families were selected for genotyping based on their high apparent prevalence (0.068 - 0.098) and number of daughters tested for disease (252 - 570). A genome-wide scan was performed within these 3 families using selective DNA pooling. Selective DNA pooling was used to narrow the number of chromosomal regions to test with interval mapping. Cows testing negative for infection could be resistant to infection, susceptible but not sufficiently exposed to *M. paratuberculosis*, or false negatives (infected but test-negative). To account for this heterogeneity among test-negative cows, infected cows (positive pool) were matched with two noninfected herdmates in the same lactation (negative pool). Selective DNA pooling identified nine chromosomal regions putatively linked with susceptibility to *M. paratuberculosis* infection using a t-test ($P < 0.01$). Interval mapping has been completed for four of these chromosomal regions (BTA 10, 15, 20, and 25). Average number of markers and spacing per region was 4.5 and 7.06 cM, respectively. Cows included in DNA pools were individually genotyped. Interval mapping was performed with web-based QTL EXPRESS, which uses linear regression methodology. Significance levels were defined with chromosome-wide permutation tests (10,000 iterations). Interval mapping has confirmed presence of a QTL affecting susceptibility to *M. paratuberculosis* infection near the centromere of BTA 20 (chromosome-wide $P < 0.05$).

D475

Identification of markers potentially associated to QTLs for performance traits in a Brazilian chicken F_2 resource population

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Two F_2 chicken populations (TCTC and CTCT), especially designed for QTL mapping, were developed in Brazil from the reciprocal crossbreeding between a broiler (TT) and a layer (CC) line. In TCTC, QTLs have been mapped for performance and carcass traits using interval mapping, whereas the first few results for CTCT are coming out. The objective of this study was to identify markers potentially associated to QTLs for performance traits in CTCT and to compare the results with those from TCTC. A total of 360 F_2 individuals from four full-sib families, males and females, were produced over 13 hatches and genotyped with 17 microsatellite markers from chicken chromosomes 1, 3 and 4 that flanked QTLs mapped in the TCTC population. Body weight at 41 d (BW) and feed intake from 35 to 41 d (FI) were recorded for these chickens; weight gain (WG) and feed efficiency from 35 to 41 d (FE) were calculated. A single-marker analysis of variance was employed, considering the fixed effects of hatch, full-sib family (F), sex (S), genotype (G) and the GxF and GxS interactions, assuming $P<0.10$. Associations between LEI0160 and FE; LEI0079 and BW, WG and FI; MCW0145 and FI were detected on chromosome 1. On chromosome 3, associations between *LEI0161* and BW; *MCW0116* and BW and FI were identified. On chromosome 4, associations were found between *LEI0122* and *MCW0240* and BW, WG and FI; *LEI0085* and each of the four traits; *MCW0174* and BW and WG. Two possible QTL regions were identified on each chromosome: 309 cM and 413 to 455 cM on chromosome 1, 113 and 310 cM on chromosome 3, and 132 and 205 to 252 cM on chromosome 4. The QTL regions pointed out in this study corroborate the results from the TCTC population, suggesting that the QTLs may be the same for the two populations. Future studies involving a higher number of markers and interval mapping will be carried out to precise the location and effects of the QTLs. In addition, differences in QTL effects between these two populations might allow the investigation of maternal, cytoplasmic, imprinting and sex-linked effects.

D479

A bovine QTL map for growth and carcass traits in Japanese Black cattle.

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The main objective of livestock quantitative trait loci (QTL) analyses is to elucidate economically important traits loci for applying marker-assisted selection in a breeding program. Here we describe a bovine QTL map for growth and carcass traits in Japanese Black cattle. Fifteen paternal half-sib families, each consisted of more than 190 offspring, were constructed from a commercial population of purebred Japanese Black cattle. Genome scanning were performed independently, and 60 QTL were detected for six traits at less than 1% chromosome-wise significant level with less than 0.1 of false discovery rate: 15 for beef marbling score, 9 for cold carcass weight, 9 for body weight at slaughter, 9 for longissimus muscle area, 5 for rib thickness, and 13 for subcutaneous fat thickness. Each QTL accounted for 2-13% of the total variance in the family. The QTL were distributed on 23 chromosomes with some replicated regions. Haplotype analyses in replicated regions revealed shared *Q* haplotypes as hypothetical identical-by-descent (IBD) alleles for two carcass and body weights QTL and one beef marbling score QTL. The abundant and replicated QTL information will enhance the opportunities for efficient breeding using marker-assisted selection.

D486

QTL for testis size in ram lambs as an indicator of ovulation rate in Indonesian Thin Tail (ITT) x Merino backcross sheep.

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Literature suggests that in sheep, indirect selection for testis size in males may lead to correlated changes in female ovulation rate, an important component of fecundity. This study is the first to investigate male fertility traits in a sheep resource. A multi family QTL analysis was conducted on all autosomal chromosomes for testis weight with skin (TWS), testis weight without skin (TW) and for live weight (LW), in a multi sire flock of 9 ITT x Merino backcross families (male nmax = 269). An analysis was also conducted for the two testis weight traits after adjustment for live weight. The study revealed 13 QTL that were significant at the chromosome wide level. Five were for LW (F-value range: 2.23-2.69, OAR6: 4cM distal to CP125, OAR10: 3cM proximal to TGLA441, OAR14: 2cM distal to BMS2213, OAR18: 6cM proximal to VH54, OAR25: 4cM distal to MCM200). Four QTL were for TW (F-value range: 2.43–2.93, OAR10: 3.5cM proximal to TGLA441, OAR18: 6cM distal to VH54, OAR25: 16cM distal to MCM200) and TWS (F-value: 2.51, OAR18: 2cM proximal to VH54), all apparently driven by LW. Two similar QTL were found for each of the two corrected testicular traits (F-value range: 2.02-2.76, OAR20: 0cM proximal to INRA132, OAR24: 8cM distal to JMP29, for each trait). Maximum absolute allelic effects reported per family per trait ranged from 1.01 to 2.76 residual standard deviations, but values should be interpreted with caution, given small sample sizes per family. Confidence intervals for location of the QTL essentially cover the entire chromosome. In sheep, regions associated with female prolificacy include OAR6, OAR-X and OAR5. None of the present QTL for testicular weight mapped to these known QTL regions. In cattle, chromosomes of interest for male reproductive traits include BTA5 and BTA29, while many chromosomes are of interest for female reproductive traits. Of these, BTA23 is largely syntenic with OAR20, and thus OAR20 may be consistent with a QTL previously identified in cattle for both ovulation and twinning rate.

D489

Whole-genome LD mapping of bovine QTL that control superovulatory response of ovary using microsatellite markers.

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It is important to predict ovulation induction responsiveness for increasing the reproductive rate of valuable females in cattle. Since it is difficult to prepare a family pedigree large enough for quantitative trait loci (QTL) analysis, whole-genome linkage disequilibrium (LD) mapping was conducted for follicle-stimulating hormone (FSH) -induced ovulation rate QTL using Japanese Black cattle (Wagyu) herd. According to the collected number of eggs after decreasing doses of FSH administration for three days, 96 individuals of high-phenotypic value (14.6 or more) and 96 individuals of low-phenotypic value (7.5 or less) were chosen from 703 cows (average; 13.2, standard deviation; 8.2, range; 0.0 to 57.0) for a primary scan. Up to five paternal half-sibs were included in each group. The genotypic data consisted of 1073 microsatellite markers along BTA 1 to 29 and BTA X (3-cM interval in average). Haplotype frequencies of two adjacent markers (pair-wise) were estimated using the expectation-maximization algorithm and followed by a Fisher exact test using a 2xn-contingency table in which haplotypes of minor frequency (less than 0.05) were ignored. Seventeen loci were mapped at significance level of $P < 10^{-3}$; seven of them were at $P < 4.8 \times 10^{-5}$ which is equivalent to Bonferroni-correceted $P = 0.05$ (1047 tests). The results were confirmed at the four loci among six loci where additional markers were genotyped. Interestingly, the genes involved in synthesis of prostaglandins, which have been reported to concern oocyte maturation, ovulation, and litter size in mice reside close to two of highly significant loci. We successfully mapped for the first time QTL of a livestock animal by whole-genome LD mapping using microsatellite markers. These data provide the first molecular genetic markers for bovine reproductive QTL that control responsiveness to FSH. These markers will enable a more accurate prediction of ovarian responsiveness to FSH and provide selection criteria for reproductive performance in cattle.

D516

Haplotypes in the bovine leptin gene associated with serum leptin, feed efficiency, growth and carcass merit

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Leptin is an endocrine hormone product of the obese gene secreted from adipocytes at concentrations proportional to body fat content and acts principally on the hypothalamus to modulate energy balance and feeding behavior. The bovine gene has been mapped to chromosome 4 and several polymorphisms related to economically important traits have been identified in the coding and promoter regions. This study relates haplotypes of three previously reported single nucleotide polymorphisms (SNP) namely UASMS2, E2FB and A59V to serum leptin, feeding behavior, feed intake and efficiency, growth and carcass merit in 464 composite cattle sired by Angus, Charolais and Hybrid bulls. The three SNP have been reported to be independently associated with the traits. The UASMS2 SNP is a C-T substitution at position 528 of the leptin promoter; E2FB SNP is a C/T transition at position 73 of exon 2 and the A59V SNP is a C/T substitution at position 321 of leptin exon 3. Haplotypes (UASMS2-E2FB-A59V) were constructed using the haplotype procedure of SAS/genetics while the mixed procedure of SAS was used for haplotype association analysis. Overall, eight haplotypes were obtained, three of which were excluded from subsequent analysis (frequency less than 1%). Five haplotypes C-C-C (n =199, 25.4%), C-C-T (n =134, 16.7%), C-T-T (n =294, 39.5), T-C-T (n =140, 15.9%) and T-T-T (n = 18, 2.4%) were retained. Animals with the T-T-T haplotype had significantly higher gain and final ultrasound marbling as well as higher gain and final ultrasound back fat ($p =<0.05$). Animals with the C-C-C haplotype had significantly higher carcass rib eye area, higher lean meat yield and higher final ultrasound ribeye area as well as lower serum leptin levels, lower final ultrasound back fat ($p =<0.05$). Animals with a C-C-T haplotype tended to have lower feed conversion ratio ($p = 0.058$) while those with a C-T-T haplotype had lower flight speed ($p = 0.0033$). These results are consistent with individual SNP analyses.

D526
Linkage disequilibrium mapping of meat quality QTL

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Previous studies based on linkage analysis have identified broad areas in the bovine genome associated with meat quality. Linkage disequilibrium (LD) analyses have the potential to identify narrower regions and point towards candidate genes. Tenderness and marbling were chosen to be evaluated in a fine-mapping project exploiting LD of maternally inherited alleles. Two paternal half-sib families, where linkage effects were known and accounted for, were chosen to evaluate LD. Each family was composed of more than 500 offspring produced by mating two *Bos taurus* x *Bos indicus* crossbred sires with *Bos taurus* crossbred dams from the Germplasm Evaluation Project Cycle IV (MARC, USDA, Clay Center, NE). Phenotypes for meat tenderness, measured as Warner Bratzler shear force at 14 days postmortem (kg), and marbling score were adjusted within family for fixed effects (sex, days on feed, dam line and year) and the probability of inheriting alternative QTL alleles from the sire at the given chromosomal region. Offspring of each sire were ranked using residuals from the models and equal amounts of DNA from 7% of the animals with the highest, and 7% with the lowest values were combined to form pools of high and low values for each trait. DNA was hybridized to a microarray containing approximately 10,000 SNPs (Affymetrix®), and the normalized allele intensities were used to define allele frequencies in the pools. Allele frequency difference between high and low pools was calculated and used to generate point-wise significance. Chromosome maps, containing point-wise values and running averages of five consecutives SNPs, were plotted. Regions with significant markers (nominal P<.01), with significant running averages (nominal P<.05), and corresponding to areas where QTL were identified previously were considered as candidate regions. Regions on BTA 2, 5, 9, 14 and 29 showed preliminary association with marbling, and a region on BTA 5 was associated with tenderness. Significant markers in those areas have been chosen for individual genotyping to validate pool typing results.

D538
Detection of QTL influencing fleece trait on chromosome 2 in Angora goats.

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Previous studies have indicated the presence of gene or gene families involved in fleece traits in different species. The aim of this study was detect QTL on CHI2 using a set of microsatellites in Angora goats. A total of 636 kids were analyzed from 14 Angora bucks. The number of half-sib offspring per buck ranged between 21 to 84 kids. Eight phenotypic fleece traits were recorded: Average Fiber Diameter (AFD; µm), Coefficient of Variation of AFD (CVAFD; %), the percentage of fiber with diameter over 30 µm (F30), percentage of kemp fiber (KEMP; %), percentage of Continuous Medullated Fibers (CONT; %), percentage of Discontinuous Medullated Fibers (DISC; %), Staple Length (SL; mm) and the Average Curvature of Fiber (ACF; deg/mm). An interval analysis was performed under a half-sib model using the *QTL Express* program. The fixed effects included in the analysis were: sex, year of birth (2000, 2001, 2002, 2003 or 2004), birth type (single or twin) and flock (8 levels). Appropriate *F*-statistic thresholds for chromosome wise type 1 error rate were generated by permutation test of 10,000 iterations. Significant evidence (P<0.05) was found in three families for the ACF trait. New families, increased number of kid in extant families and a fine mapping on the candidate regions will be the next steps to carry out.

D544
Genome-wide association test for bovine twinning rate QTL

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A genomewide scan for twinning rate quantitative trait loci (QTL) was conducted in North American Holstein dairy cattle. The sires used were from 16 paternal-half sib families and were chosen for having twinning rate PTAs (predicted transmitting abilities) of high accuracy and values in the upper and lower tails for the distribution within family. In addition, sons were selected for balanced representation of maternal grandsires within family relative to twinning rate PTA. A total of 201 sires were genotyped using the Affymetrix GeneChip Bovine Mapping 10K SNP kit. 9,919 SNP markers obtained from bovine genome sequencing project were typed, though only 3,197 informative SNPs were selected for mapping analysis. The remaining SNPs were not utilized because of low minor allele frequency (MAF), missing genotypes, non-Mendelian inheritance and unknown genomic location (Bos taurus genome assembly as of 03/10/2005). Due to incomplete bovine genome sequence assembly over 5,000 SNP loci were excluded from this initial analysis because of uncertain genomic location. Markers with MAF below 5 % or > 20 % missing genotypes were also excluded from the genome scan. Though marker loci were not evenly distributed across the genome, average distance between two loci was < 1 Mb with informative markers. Association between marker locus and twinning rate was evaluated with a model including effects of sire and SNP genotype. Statistical thresholds for a genome wide search were determined accounting for multiple comparisons. A comparison-wise error rate of 5×10^{-5} yields an experiment-wise error rate of approximate 0.05. Significant marker-trait associations were detected on BTA 2, 5, 11, 14, and 16 based on the genome-wide 0.05 threshold. Associations on chromosomes 5 and 14 are strongly supported by previous linkage mapping studies.

D545
Fine mapping of twinning rate QTL on bovine chromosome 14

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QTL affecting ovulation and twinning rates have been previously identified on BTA14 in a twinning cattle herd at the USDA Meat Animal Research Center (MARC) and a North American Holstein family, respectively. The objective of the current study was to refine QTL map location by genotyping multiple microsatellite markers and SNPs in the putative QTL region on specific Holstein sires. The sires used were from 16 paternal-half sib families and were selected for having twinning rate PTAs of high accuracy and values in the upper and lower tails of the distribution within family. In addition, sons were selected for balanced representation of maternal grandsires within family relative to the twinning rate PTA. A total of 238 individuals were genotyped with six fluorescently labeled microsatellite markers which span the region between 67 and 83 cM on BTA14. Based on genotypic data, haplotypes of individuals were inferred by identity by descent of marker allele within family using Genoprob software. Association between haplotype and twinning rate was evaluated with models that included effects of sire, paternally inherited QTL allele and maternally inherited haplotypes. Probability of paternal allele inheritance was calculated using markers informative in sires. Two significant (P<0.01) effects of maternally inherited haplotypes were observed for two adjacent marker brackets. Combining analysis of paternal and maternal haplotype effects narrowed QTL location to a region between 77 cM and 83 cM (37 Mb ~ 40 Mb) on chromosome 14 which should help in identifying candidate genes. Additional SNP markers will be genotyped in this region to further refine QTL location.

D557

Fine-mapping of a bovine QTL for marbling on BTA 4 using association study

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Marbling is one of the most important economical traits in beef cattle. We located a marbling QTL on BTA 4 (35-85 cM) at 0.1% chromosome significance level in a purebred Japanese Black cattle population using a paternal half-sib family of Sire A (Mizoguchi *et al*., 2006). The *Q* (a superior haplotype) was derived from its dam, thereby the origin of *Q* is not known, but the *Q* should be present in the local population. In fact, similar QTL have been detected in three additional half-sib families (Takasuga *et al*., ISAG 2006). These four sires are related to each other, suggesting that the marbling QTL on BTA 4 might be derived from a common ancestor. However, an apparent identity by descent (IBD) was not identified among the four sires. To confirm and further narrow down the QTL origin, we analyzed several half-sib families of Sire A's related bulls with a higher marker density and compared their haplotypes. We detected a significant marbling QTL on BTA 4 (30-80 cM) in a half-sib family of Sire B, a half-brother of sire A (n=341). Haplotype comparison in BTA 4 between Sire A and Sire B revealed that the *q* (an inferior haplotype) chromosome, not *Q*, of Sire A was identical to that of Sire B. We compared *Q* haplotype between Sire A and Sire B, and found a putative IBD spanning 2 cM composed of 12 polymorphic microsatellites. Next we performed association study using two extreme groups regarding marbling (116 and 191 cattle) in the local population. The apparent IBD region was associated with the Q alleles (p =0.0058), indicating the presence of a gene responsible for marbling.

D569

Utilization of microsatellites markers developed for bovine for the valuation of genetic variability in bubaline (*Bubalis bubalis*) in North of Rio de Janeiro state.

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The knowledge of genetic variability is essential in the elaboration of strategy of genetic improvement in bubaline (*Bubalus bubalis*). In properties with reduction in population effective, is necessary some care to do not jeopardize the genotypes because of reduced genic flux. The aim of this work is to verify if the microsatellites developed for bovines can be used to valuate genetic variability in bubaline. There were collected bubaline blood samples from thirty six animals in three different properties in the North of Rio de Janeiro state. The extraction of DNA was done using BLOODCLEAN DNA Purification Kit from Human Blood Samples®. For the variability's valuation, were used the following microsatellites developed for bovines: ETH225, ETH10, ETH3, BM2113, BM1824, TGLA227, TGLA126 (676), TGLA122, TGLA53, SPS115 and INRA023. Agarose gel analysis determined that some microsatellite had similar allele size for both species, in other microsatellite new alleles were found in bubaline when compared to previously found in bovine. The success rate in converting bovine markers for buffalos is seven in eleven tested microsatellites, that are ETH225, ETH3, BM2113, BM1824, TGLA126 (676), SPS115 and INRA023. In this way was concluded that the microsatellite developed for bovine show potential usefulness in the analysis of the genetic variability in bubaline.

SECTION E
Genome Technologies and Bioinformatics

E219
The Mouse Genome Informatics Database as a resource for mammalian phenotype and gene expression data.

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The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) provides free access to extensive information on the genetics, genomics and biology of the laboratory mouse. Core data include gene and mutant descriptions, DNA and protein sequences, and mapping data. Integrated with this core information are phenotypic and expression data and comparative data on orthologous genes and human disease models. Over 14,000 phenotypic alleles, including spontaneous, targeted, transgene, conditional mutants and QTLs are described. Researchers can also query gene expression data detailing when and where a gene is expressed in specific tissues and developmental stages. To facilitate these searches, structured vocabularies for phenotypes and mouse anatomy are provided. Comparative mapping data are curated for orthologous genes between mouse and 19 other mammalian species, including human and agriculturally important mammals, and are linked to the appropriate species-specific databases. MGI is updated with new data nightly and continually adds new datasets and enhances the user interface. MGI's dedicated User Support group is available at: mgi-help@informatics.jax.org.

E246
Improved annotation of the porcine Affymetrix GeneChip® and functional comparison to QIAGEN-NRSP8 oligonucleotide array data

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The Affymetrix GeneChip® is currently the largest expression profiling tool available for pig, containing 23,935 probe sets. As of the December 2005 NetAffx update, 9,937 probe sets lack any annotation, thus our purpose was to provide additional annotation for all probe sets. A blast analysis using the Affymetrix consensus sequence as a query to refseq_rna from NCBI resulted in a total of 17,193 probe sets with a blast hit with an E-value $\leq e^{-10}$, of which 4,192 were previously un-annotated. Blast analyses using the Affymetrix consensus sequences as queries to refseq_protein from NCBI were then run. Using an E-value $\leq e^{-5}$ as criterion, 11,424 probe sets had a hit; 143 were previously un-annotated. In additional probe set investigation, we used tissue expression pattern across similar experiments to validate probe set data. Using three different pig liver profiling experiments, it was possible to compare the Affymetrix platform with itself, and to the other wide-coverage porcine expression profiling tool, the 13,309 probe QIAGEN array. Pairings between the Affymetrix and the QIAGEN arrays were generated using blast: the 70-mer QIAGEN sequence as the query against the Affymetrix consensus sequence. Requiring an alignment length $\geq$ 67 nucleotides with $\geq$ 97% identity, a total of 8,317 cross-platform pairs were found: 7,765 of the QIAGEN probes and 7,706 of the Affymetrix probe sets. Using for expression criteria an FDR q-value of 0.01 on QIAGEN data and $\geq$ 75% agreement for MAS5 present/absent calls on Affymetrix arrays, 7,188 pairs were compared. These analyses found 82% agreement across all three experiments. In summary, we improved annotation for a large portion of previously un-annotated Affymetrix probe sets, developed a cross-platform comparison method and showed a high agreement in liver gene expression between platforms.

E260
Statistical tools to aid domestic breeds' management.

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Domestic breeds play a determining role on local economies. There are still many communities that depend on these breeds for their subsistence. For instance, the use of the horse has changed throughout times but horse breeds still have their importance within the agricultural environment. Genetic variability of domestic breeds is usually under very strict local management systems and suffers from the negative effects of selective pressure and cross breeding. For this reason, special attention must be paid to genetic erosion symptoms. If genetic identity of native breeds must be preserved, a delicate balance must be maintained between gene flow and genetic isolation of local populations. New clustering and assignment methods are important statistical aids to address this problem. Bayesian methods and frequency based methods were tested, in the present work, for their efficacy in allocating individuals to their populations of origin and detecting genetic structure within populations. We have genotyped 50 individuals form each of the three Portuguese native horse breeds as a case study and we compared *Structure*, *GeneClass2* and *Whichrun* for their efficiency *versus* computing time. We also compared the performance of clustering methods opposing genetic distance measures to a Bayesian method implemented in *Structure*. *Whichrun* was the most effective allocation tool whereas genetic distance methods did not perform as well. We were able to confirm internal genetic structure within two of the tested breeds using *Structure*. Such genetic structure was not detected with traditional genetic distance methods, which could not differentiate between individuals of these two breeds. We have genotyped 50 individuals from each of the three Portuguese native horse breeds for 23 horse specific microsatellite markers (AHT4, AHT5, ASB2, HMS1, HMS2, HMS3, HMS6, HMS7, HTG10, HTG14, HTG4, HTG6, HTG7, HTG8, LEX20, LEX23, LEX36, LEX41, NVHEQ18, UCDEQ405, UCDEQ425, UCDEQ5 and VHL20) and we have used a Li-Cor 4200 Series automated sequencer with 6% poly-acrylamide gels to run and visualize the PCR products.

E266
A porcine model of T-cell lymphoma

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Non-Hodgkin's lymphoma (NHL) is a disease of increasingly serious proportions. The highest incidence rate in the world is seen in the United States and Canada, with forty to fifty thousand new cases per year in the U.S. NHL is the sixth most common cancer and the sixth most common cause of cancer death, accounting for 4% of all cancers and 4% of cancer-related deaths. There is a clear need for relevant animal models of lymphoma that provide preclinical tools. Our objective is to create a porcine model of lymphoma analogous to the human disease. Genetically engineered porcine tumors may prove to be invaluable for: (1) determining the efficacy of anti-cancer drugs; (2) studying the process of tumorigenesis; and (3) producing cancer in a genetically compliant animal model that is physiologically more similar to humans than rodents. We have previously shown that solid tumors could be readily induced in immunosuppressed pigs via the expression of proteins disrupting the p53 tumor suppressor pathway, and activating c-Myc and Ras pathways, all of which are commonly corrupted in human cancers. In an attempt to induce a less immunogenic tumor with a defined phenotype, retroviral vectors encoding four genes (Cyclin d1, CDK 4, c-Myc, and H-Ras) were constructed and injected SC into the mammary area and/or behind an ear, or IV via the ear vein. Both challenge routes induced T-cell lymphoma (n = 6 / 6) in the absence of immunosuppression. The effects of vector dose responses are currently being evaluated and future studies will attempt to induce tumors in cloned animals, thus producing tumors that can be transferred to any number of identical animals to study the process of tumorigenesis and cancer phenotypes. This work was supported in part by USDA/NRI-CSREES grant AG2001-35205-11698 and USDA-ARS AG58-5438-2-313.

E337
A Genetically defined porcine model of tumorigenesis

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Given the limitation of using mouse models for preclinical studies in radiation, hyperthermia, and photodynamic therapies, we sought to exploit the pig as an experimental and preclinical model for human cancer. Pigs offer the advantage of having a similar size, diet, metabolism, and anatomy to humans. To this end, we have shown that porcine cells can be genetically converted to a tumorigenic state and produce tumors when returned to the host animal. However, this growth depended on suppression of the immune system, likely owing to the reliance on expressing human genes for this tumorigenic conversion. Future studies will determine whether modified porcine genes or *in vivo* tumor induction methods can be employed to drive cells to malignant fates and overcome the need for immunosuppression. In short, we have developed a rapid, reproducible, and genetically malleable method to induce tumors of sizes similar to those treated clinically in humans in a large mammal, which should provide a robust preclinical cancer model for studies of imaging as well as hyperthermia, radiation and photodynamic therapies.

E341
Representative whole genome amplification of ovine DNA isolated from senescent nuclear donor cells.

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Gene targeting in the mouse using embryonic stem (ES) cells has been extremely successful. In livestock, in the absence of proven ES cells, the alternative route involves nuclear transfer of gene-targeted somatic cells. At present, there are several reports describing gene targeting in fetal fibroblasts from sheep, pig and cattle. A disadvantage of using primary fibroblasts in this kind of experiments is that they have a limited proliferation potential and eventually enter replicative senescence. However, to identify targeted cells prior to nuclear transfer, a certain number of cell divisions would be desirable in order to provide enough DNA for genetic testing (PCR and Southern blot analysis). To deal with this problem, we evaluated the reliability of whole genome amplification procedures when applied to ovine DNA from senescent fibroblasts. First of all, we analysed the efficiency of isothermal multiple strand-displacement amplification (IMDA) technique with ovine genomic DNA isolated from an early passage of fetal fibroblasts. DNA was mixed with random hexamer primers, dNTPs and Phi 29 DNA polymerase, and incubated at 30 ºC overnight. This way, microgram quantities of high-molecular-weight DNA were produced from as little as 1 ng of ovine DNA input. The amplified material was representative of the whole genome as demonstrated by microsatellite analysis from nine different chromosomes and genotyping at the well characterized *PRNP* gene. Afterwards, DNA lysates from senescent fibroblast clones were used as starting material. The amplification was equally efficient and representative. PCR analysis and Southern blot of chosen genes (β-casein, β-actin, α1-procollagen) was performed and similar results were obtained with DNA plus and minus IMDA treatment. These results indicate that IMDA could be used as a tool to facilitate the identification of targeted nuclear donor cells, what points out to a promising application of this technology in livestock.

E353
Detection of single nucleotide polymorphisms on porcine chromosome 7 associated with ultrasonic backfat in a segregating Meishan x White Cross population using a model with polygenic effects.

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Multiple genome scans have indicated the presence of QTL for backfat deposition on porcine chromosome 7 (SSC7). The objective in this study was to determine if genotypes of 14 SNP positioned between 50 and 69 cM were associated with variation in ultrasonic measures of backfat. Genotypic and phenotypic data were collected on 298 gilts, evenly split between the F8 and F10 generations of the USMARC Meishan resource population and 4 and 6 generations after the herd was closed and random-mated (¼ Meishan composition). Backfat phenotypes were recorded from three locations along the back using an A-mode Renco Lean-Meter probe at approximately 210 and 235 days of age in generations 4 and 6, respectively. Gilts were also genotyped for a *TBG* SNP thought to influence backfat. The three ultrasound measures were averaged for the SNP analysis. Regressors for additive, dominant, and imprinting effects o*f each SNP were calculated using genotypic probabilities derived by allelic peeling algorithms in GenoProb. The association model included fixed effects of scan date and TBG genotype, covariates of weight and SNP regressors, and random additi*ve polygenic effects to account for genetic similarities between animals not explained by known genotypes. Variance components for polygenic effects and error were estimated using MTDFREML. Each SNP was fitted separately due to potential multicollinearity between regressions of closely linked markers. Power to detect imprinting effects was weak so it w*as eliminated from the model. Across all analyses, TBG genotype was significant (P<0.05) with an additive effect of approximately 1.1 mm of backfat. Additive effects (P<0.05) between 0.7 and 1.1 mm of backfat were observed for markers 13438.1h, 11807.1h, and 17281.1h at locations 50.1, 63.0 and 63.0 cM, respectively. These associations likely represent the same causative mutation; no* significant effects were detected if these SNP were fitted simultaneously because the total effect was partitioned across markers. Genetic improvement programs for lean yield could benefit from inclusion of these marker haplotypes.

E434
microRNA Prediction Using Sequence Mutation Features and Support Vector Machine from Genome Pair-wise Alignments

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MicroRNA (miRNA) is recognized as one of the most important families of non-coding RNAs. There are several software packages available for prediction of miRNAs from genomic sequences. miRNAs must fold to extended hairpin structures, which are basic requirements for miRNA function and basic features used for miRNA prediction. Our analysis showed that mutations in real miRNA can locate not only in the loop region but also in the stem region, both of which slightly affect the stability of the miRNA secondary structure. However, mutations in pseudo miRNAs can dramatically decrease the stability of the secondary structure. In addition, the existing packages using multiple alignments that detect miRNA candidates may lose real miRNAs that are less conserved or only conserved between two species. Here we describe a computational miRNA prediction approach designed for genome-wide, pair-wised sequences from two related species. This tool consists of 3 basic components: *(1)* an algorithm to measure the ability to folding a hairpin secondary structure. *(2)* a measurement of sequence divergence, which is characterized by high mutation frequency for genome pair-wised sequences but low frequency for the miRNA pair-wise sequences, and *(3)* an algorithm to measure how the mutations will change the ability to folding a hairpin secondary structure. We combined all three measurements by performing an SVM (support vector machine) classification which enabled us to efficiently and accurately detect the miRNAs in pair-wised sequence alignments. The rules and parameters used in the method were evaluated and optimized by statistical analysis of the prediction results using experimentally confirmed miRNAs. We demonstrate that the software has both reasonable sensitivity and improved speed by effective recognition of 15 known miRNAs among 270 candidates from a pair-wise genome alignments of *D. melanogaster* / *D. pseudoobscura* chromosome *2L*. We implemented the procedure and algorithm in a software suite called *miRFinder*.

E449
NRSP-8 Bioinformatics: Databases and Resources

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Our focus for the NRSP-8 bioinformatics program has been on the development of tools and databases. Over the last couple years, we have developed and maintained a number of public database resources and web functions to facilitate the community effort for livestock genome research. The tools include a blast server for livestock sequences, computer programs for designing gene-specific primers using consensus sequence information, and based on homologous sequence information from two species. An online ToolBox was set up to share bioinformatics tools, such as reverse-complement sequence converter, sequence alignment diagram drawing tool, and gene ontology classification counter, etc. Our significant progress is on the development of the Pig QTL Database (PigQTLdb). In particular, we have integrated existing related information from various databases, such as linking QTL information to the NCBI Gene database, to pig clone finger printed contig (FPC) maps, to radiation hybrid (RH) maps, and comparative maps to human genome, etc. In addition, we maintain a mirror site for TheArkDB (Chick,Cow, Horse, Pig, Salmon, Sheep, and Tilapia). Opportunities for further development of our database system into one that can serve multiple species are discussed.

E507
Enhanced mapping tools for the sheep genome – BACs, SNPs and the virtual map

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Experiments are in progress in sheep that aim to identify loci influencing traits of economic importance, such as wool, meat, and milk production, as well as resistance to acquired diseases. The sheep is also an important model organism for humans for physiological development traits and for genetic diseases. Hence, there is a need for a sheep genome sequence map to assist with the genetic mapping of these traits. However, the sheep genomics community currently lacks the resources for such an enterprise. A project is underway to produce a virtual sheep genome map using a range of information including the sheep genetic map and public GenBank sequence information, such as the bacterial artificial chromosome (BAC) end sequences from the 12x CHORI-243 library and expressed sequence tag (EST) sequences. The virtual map is being built based on a framework of genetically mapped marker sequence links between the sheep genetic map and the sequence maps of cattle, humans, dogs and other species, together with sequence alignments among these species. The map will be able to be viewed from a sheep perspective, and will include information on the likely positions of genes, BAC clones, BAC contigs, EST and RefSeq sequences, and both actual and potentially polymorphic microsatellite markers. The map will also include information from the current International Sheep Genome Consortium single nucleotide polymorphism (SNP) discovery project. It is planned to also integrate positional information from the sheep radiation hybrid map once information for this becomes available. Viewing tools for the sheep virtual genome will be made available via rubens.its.unimelb.edu.au/~jillm/jill.htm and www.livestockgenomics.csiro.au/sheep.shtml.

E524
Resources for genomics and functional genomics research in Atlantic salmon

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The Norwegian Salmon Genome Project has constructed a genetic map, a BAC library, cDNA libraries form various tissues, EST sequences, a microarray chip and a bioinformatics infrastructure. All data and resources can be accessed through our web-site: www.salmongenome.no. A genetic map has been constructed containing over 450 markers representing an average of one marker for every 2-3 cM. A total of 1,227 microsatellite sequences have been developed and submitted to GenBank. In addition, over 2,500 SNPs have been identified from clustered ESTs. We constructed cDNA libraries from 15 different tissues and 160,000 primary clones were picked and stored in 384-well microtiterplates. In addition, all libraries have been amplified and permanently stored. Nearly 68,000 ESTs have been sequenced from these libraries and 55,118 sequences have been submitted to GenBank. In addition, over 1,100 cDNAs have been sequenced in full by primer walking. A microarray chip has been developed in collaboration with groups in the UK. The chip consists of approx. 17,000 different cDNAs in duplicate and contains clones from SGP, the EU funded SALGENE and the UK TRAITS consortium (www.abdn.ac.uk/sfirc/salmon/). An Atlantic salmon (*Salmo salar*) BAC library has been constructed from DNA prepared from sperm of a single male. The work was done in collaboration with BACPAC Resources at Children's Hospital Oakland Research Institute and the Canadian project Genetic Research on Atlantic salmon Project (GRASP). The library consists of 313,000 clones, representing 19-fold genome coverage. The bioinformatics infrastructure includes a database, data processing tools and pipelines as well as three computers installed at USIT, University of Oslo. Currently our database system stores and provides tools for processing and access to data including source files (raw data), data on clusters, SNP data, annotations data, the genetic map and genetic markers. The web-based SGP data management system has two major components: a relational database and a web site. The site provides public Blast and sequencing data processing service.

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