# XXVIII International Conference on Animal Genetics in Göttingen - Germany

# ISAG

International Society for Animal Genetics

## Proceedings 2002

**ISBN 3-00-010597-2**


Applied
Biosystems


Deutsche
Forschungsgemeinschaft
DFG


AGOWA


EDP
SCIENCES


Biozym


G.A.G BioScience
Genomics and Genetagging


Blackwell
Publishing


eppendorf


IBA


d-bird
Bundeshybridzuchtprogramm


ITAS

# Contents



**These proceedings are published under:**

## ISBN 3-00-010597-2

**Georg-August-University** **City of Göttingen**

*Dear colleagues,*

*on behalf of the Local Organizing Committee, the International Society for Animal Genetics and the Georg-August-University of Göttingen, we would like to welcome all delegates and accompanying persons to the 28th International Conference on Animal Genetics in Göttingen. It is a great pleasure and honor for us that a conference of the ISAG will take place again in Göttingen after 17 years.*

*The conference will be a unique blending of individual sessions and themes designed to provide you with an exceptional experience. The conference gathers a cadre of international experts in molecular genetics, molecular biology and related fields. These world-class presenters bring together a wealth of experience and knowledge for your benefit. Add to this the ability to share ideas and interact with a global network as well as to view the latest results in Animal Genetics from around the world and you have a conference that cannot be missed. The ideal forum to increase your personal knowledge and professional capabilities, the **ISAG2002** conference will sure exceed your expectations.*

*We wish you all pleasant, inspiring and motivating days in one of the oldest and most attractive University cities in Germany. Because of its central geographical position in Germany, Göttingen is also an extremely good starting point for pre- and post conference tours.*

*We hope to see you in Göttingen at the International Conference and look forward to welcoming you to the "City of Science".*

Bertram Brenig Jan-Nikolaus Meyer

## Greeting from the Minister for Science and Culture of Lower Saxony,

**Thomas Oppermann**

Niedersachsen

Only 17 years ago the term Animal Genetics was in public at most connected with the attempt to increase the milk production through breeding. Today it can be found in almost any news media, related to a variety of issues.

"Dolly", the name of the first ever sheep that had been cloned is just as well the label for a milestone in the history of modern science. For weeks on end "Dolly" made headlines and triggered controversial discussions about genetic engineering and biological sciences. But another problem just as well represents the new requirements Animal Genetics is facing today: The public world-wide was alarmed when BSE spread among the cattle all over Europe. Suddenly, everybody was looking at this field of veterinary medicine, at Animal Genetics, as a source of hope and wishes as well as fear and worries.

Considering this growth in importance, I am especially happy the 28th International Conference for Animal Genetics is taking place in Göttingen again after 17 years. Surely triggered by Animal Genetics, genetics has undergone quite an astonishing development during this time and is becoming one of the most important science branches in the 21st century. The genetic mapping of animals has progressed so far that in the years to come there could be enormous breakthroughs concerning the resistance to illnesses in animals and breeds. As well, the existing methods of animal breeding are going to change drastically due to the progress in Animal Genetics.

The 28th International Conference for Animal Genetics is approaching the complexity of these issues from several sides. I am especially pleased that in addition to the participation of several veterinary and biological branches of science, bioethical aspects will be discussed as well. The general public will be expecting the new biological sciences to put up a critical reflection on this issue in particular.

I'd like to thank the International Society for Animal Genetics (ISAG) for being host to this conference and the Institute of Veterinary Medicine and the Institute of Animal Breeding and Genetics of the Georg-August-Universität Göttingen and the Chairmen of the local organizing committee, Professor B. Brenig and Dr. Jan Meyer for preparing it and I wish all participants of this conference good progress and stimulating discussions.

## *Local Organizing Committee 2002*

Sabine Bramsmann
Bertram Brenig
Claus-Peter Czerny
Gabriele Hörstgen-Schwark
Christoph Knorr
Ute Margan
Jan-N. Meyer
Burchhard Möllers
Andreas Müller-Belecke
Ina Pfeiffer
Henner Simianer
Helge Täubert
Clemens Wollny
Secretary: Kaarina Hillmer
Katharina Kurth
Treasurer: Monika Werst

Institute of Animal Breeding and Genetics, Albrecht-Thaer-Weg 3
D-37075 Göttingen, Germany
Institute of Veterinary Medicine, Groner Landstraße 2,
D-37073 Göttingen, Germany

Göttingens most famous monument „Gänseliesel“

| Guest Speakers | Topic |
|---|---|
| Prof. Dr. Adriano Aguzzi (Switzerland)<br>adriano@pathol.unizh.ch | The immunobiology of prion diseases |
| Prof. Dr. Choy Leong Hew (Singapore)<br>dbshead@nus.edu.sg | Biotechnology and Transgenesis in fish |
| Dr. Björn Ingemarsson, PhD (Sweden)<br>bjorn.ingemarsson@pyrosequencing.com | Comprehensive DNA analysis using Pyrosequencing™ techn. |
| Prof. Dr. Dr. Bernhard Irrgang (Germany)<br>irrgangb@rcs.urz.tu-dresden.dc | Ethical Issues of Genetic Manipulation of Lifestock |
| Prof. Dr. Joan Lunney (USA)<br>jlunney@anri.barc.usda.gov | Can we use genomics to select for healthier swine? |
| Prof. Dr. Paula Schneider (Brazil)<br>paula@ufpa.br | Biodiversity and Conservation in Amazon |
| Prof. Dr. Hans-J. Thiesen (Germany)<br>hans-juergen.thiesen@med.uni-rostock.de | Proteomics |
| Dr. Alain Vignal (France)<br>vignal@autan.toulouse.inra.fr | SNP technology |

**Adriano Aguzzi** studied medicine at the University of Freiburg (Germany) form 1980-1986. He received his Ph.D. in 1986. From 1986 to 1989 he was resident in Neuropathology at the University Hospital of Zürich (Switzerland). Since 1997 he is full professor of Neuropathology and Director of the Institute of Neuropathology at the University of Zürich. He is president of the Swiss Society of Neuropathology and member of several international Societies. His research interests are in the area of prion diseases with special focus on their pathogenesis.

**Choy Leong Hew** is Head of the Department of Biological Sciences at the University of Singapore. He received his Ph.D. in 1970 at the University of British Columbia. From 1972 to 1974 he was a C.H. Best fellow at the Banting & Best Dept. of Medical Research of the University of Toronto (Canada). He was then working at the Memorial University of Newfoundland as assistant professor until 1983. From 1983 to 1999 he was a visting professor at a variety of Universities in Canada and China. His main research areas are biology and biotechnology of antifreeze proteins, transgenic fish, molecular endocrinology and proteomics.

**Björn Ingemarsson** is Director of Pyrosequencing AB's technical and scientific support function based in Uppsala. Prior to joining Pyrosequencing 3 years ago, he worked as a specialist in automated DNA analysis at Amersham Pharmacia Biotech. He aquired his Ph.D. in plant physiology at the Stockholm University, where he thereafter continued research on the physiology, biochemistry and molecular biology of nitrogen assimilation in plants for several years.

**Bernhard Irrgang** studied Philosophy, Theology and German Philology at the University of Würzburg (Germany), Passau (Germany) and Munich (Germany). He received his Ph.D. in Philosophy in 1982 and Theology in 1991. From 1982 to 1992 he worked at different Universities in Germany. Currently, he is Director of the Institute for Philosophy and the Centre for Interdisciplinary Technical Research at the Technical University of Dresden (Germany). Bernhard Irrgang main interests are in the area of medical and technical ethics, consequences of technology and philosophy of technology.

**Joan K. Lunney** studied Chemistry at the John Hopkins University (Baltimore, USA) and received her Ph.D. on studies on the regulation of serum glycoprotein homeostasis in 1976. From 1976 to 1979 she was guest postdoctoral research worker in the Immunology Branch (NCI, NIH, Bethesda, MD). From 1983 until 1995 she was working in different position in the Helminthic Diseases Laboratory at the USDA, ARS (Beltsville, MD). Since 1995 she is GM15 Research Leader and Supervisory Research Immunogeneticist at the Immunology and Disease Resistance Laboratory, USDA, ARS (Beltsville, MD).

**Maria-Paula Cruz Schneider** is Director of the Laboratory of DNA Polymorphisms at the University of Belém (Para, Brasil). She received her Ph.D. 1988 at the Federal University of Rio Grande do Sul (Brasil) working on protein polymorphisms in primates of the Amazon region. Her main interests are in the area of genetics and molecular genetics in domestic species.

**Hans-Jürgen Thiesen** studied medicine at the University of Hamburg (Germany) from 1976 to 1982. He received his Ph.D. in 1983 for studying the physiology of parathyroid hormone. From 1985 to 1987 he was working at the EMBL followed by a fellowship the Basel Institute of Immunology. In 1996 he received a Chair in Immunology at the University of Rostock (Germany). His main interests are focussed on technologies of functional genomics in a clinically-oriented environment. Hans-Jürgen Thiesen is member of the Proteome-Center at which complex human diseases are analysed by microarray analysis.

**Alain Vignal** received his B.Sc. in Agronomic Sciences in 1981. From 1982-1987 he studied biochemistry, cellular genetics and molecular biology at the University of Paris. In his Ph.D. thesis at the INSERM (Laboratory of Jean-Pierre Carton) Alain Vignal analysed the human glycophorin A and B gene family. Between 1991 and 1993 he had a postdoctoral fellowship at the Genethon (Laboratory of Jean Weissenbach). During this period he was involved in the human genome project, genotyping the CEPH reference families. Since 1994 he is at the INRA in Toulouse (France). He is responsible for the development of structural genomics in chicken and expert in the field of QTL mapping.

# Plenary Session:
# Invited Speakers

**P001:**

### Comprehensive DNA analysis using Pyrosequencing™ technology

Björn Ingemarsson
*Pyrosequencing AB, Uppsala, Sweden*

Pyrosequencing™ is a fast and accurate technology for analysis of short to medium length DNA sequences. It is a non-electrophoretic sequencing method based on luminometric real-time detection of pyrophosphate released upon nucleotide incorporation by DNA polymerase. Samples are sequenced and analyzed in a standard microtiter plate format, without any need for labeled primers or nucleotides.

The technique is well established for rapid genotyping of single nucleotide polymorphisms (SNPs). High accuracy is achived by polymerase-catalyzed incorporation of nucleotides at the polymorphic positions as well as adjacent nucleotides. The non-variant positions serve as internal controls and allow for automatic quality assessment of each analyzed sample. Single well multiplex genotyping on pooled simplex or multiplex PCR products can be accurately performed for SNPs and insertion/deletion polymorphisms.[4]

The close correlation between nucleotide incorporation/pyrophosphate release and light detection, makes Pyrosequencing technology suitable for quantitative applications such as, allele frequency determination, assessment of gene copy number, analysis of splice mutations and CpG methylation, genotyping in mixed cell populations or polyploidic genomes, as well as for determination of viral load

The technology can also be used for sequence identification/verification and *de novo* sequencing of 20–50 nucleotides in less than one hour. Hence, it can be used for a number of applications including, analysis of mutation hot spot areas, cDNA re-sequencing, virus and bacteria identification/typing, as well as resistance typing.

Pyrosequencing AB has developed complete systems for low to high troughput demands, which include instrument, optimized reagent kits and dedicated software.

**P002:**

## SNP technology

Alain Vignal
*Laboratoire de génétique cellulaire, Inra, chemin de Borde-Rouge, Auzeville BP 27, 31326 Castanet-Tolosan cedex, France.*

Although the highly informative, multi-allelic microsatellite markers have dominated for now over ten years the field of molecular genetic studies in human and in animals, either used as model organisms or studied for their interest in agriculture, an increasing importance is now given to SNPs (Single Nucleotide Polymorphisms), that are merely di-allelic base substitutions of lower heterozygosity. This is due to several reasons, amongst which the main are that SNPs can be found at very high densities throughout the genome (1 SNP every 1300 bp, when two random human chromosomes are compared), and that they can be found in exons of genes. The very high densities of SNPs in the genome make them ideal for association and short range haplotype studies, although this usually means that far more markers will have to be used than for microsatellites. The coding SNPs, sometimes referred to as cSNPs, are often studied as candidate polymorphisms when they imply an amino acid change, although it is not always easy to distinguish between the real implication of the polymorphism in the biological phenomenon studied and a simple allelic association.
However, contrariwise to microsatellites, for which the genotyping technology has only evolved from manual scoring of alleles, seen as PCR products of varying sizes on acrylamide gels, to a semi-automated method using sequencing machines, the situation is not so clear concerning the genotyping of SNPs, for which a broad range of methodologies are available. Amongst these are: direct hybridisation of allele-specific oligonucleotides (ASO) on membranes or glass arrays containing PCR products of individuals to genotype; hybridisation of PCR products on chips containing oligonucleotides; single base primer extensions (SBE) whose products can be separated by various means such as gel electrophoresis, tagged arrays, tagged beads or matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF); oligonucleotide ligation assay (OLA); monitoring the exonuclease degradation of an internal allele-specific oligonucleotide during PCR; real time sequencing...
For small scale studies, the major concerns when choosing an SNP genotyping technology, will be the possibilities of using existing platforms available at hand, such as an automatic sequencer or a DNA spotter. For larger scale studies, according to the number of SNPs and of individuals to be genotyped, the choices will vary. Indeed, some technologies, such as those involving glass arrays or chips, will enable to study high numbers of SNPs in parallel, but only on a low number of individuals, and others, such as MALDI-TOF separation of SBE products, will be more efficient with a high number of samples, but will need more effort put into the development of each individual SNP. Efforts are still underway, to develop a very high throughput genotyping system, capable of generating the very high numbers of genotypes needed for the genome-wide association studies, such as envisaged in human genetics.

P003:

**Biotechnology and Transgenesis in fish**

Choy L.Hew, Xiaobo Zhang, Canhua Huang, Qingsong Lin
*Department of Biological Sciences and Tropical Marine Science Institute*
*National University of Singapore, Singapore and*

Garth Fletcher
*Ocean Science Center, Memorial University of Newfoundland, St John's, Canada*

Food security will be a major challenge facing mankind as we enter the new millennium. The challenge for the agricultural sector is to double food production by year 2025 and triples it by year 2050. It is anticipated that aquaculture and mariculture will become a major driving force to increase food production. However, new technologies will needed to be employed to improve the efficiency and many bottleneck issues facing the industries.

Numerous platform biotechnologies that include molecular biology, genomics, cloning technologies, DNA chips and proteomics can improve the industry's output substantially. In this presentation, we will describe two specific approaches from our and other laboratories.

A.Transgenesis. Transgenic technology is the transfer of a foreign gene into a host organism enabling the host to acquire a new and inheritable trait. The technology is specific for the properties of the candidate gene and appears to be better than the traditional selective breeding methods. We have attempted to generate transgenic fish that are freeze-tolerant, faster growing and disease resistant using the antifreeze protein, growth hormone or the pleurocidin genes from different fish species respectively. We have demonstrated that these transgenes can be stably integrated, inherited and expressed. There is also a strong correlation between genotypes and phenotypes. The faster growing salmon (GH transgenic) is now being commercialized. Some of the issues dealing with food safety and ecology will be commented.

b. Proteomics. Proteomics provides a global and dynamic profiling of protein activities within a cell or organisms and is now a popular method in functional genomics to examine cellular activities as well as for biomarker identifications. We have used this approach successfully to characterize the viral proteins from shrimp white spot syndrome virus. More than 15 novel proteins have been isolated and these proteins are potential candidates for vaccine development to prevent this pandemic disease in the shrimp industry.

(Supported by NSERC, Canada and A*STAR, Singapore)

**P004:**

## Biodiversity and Conservation in Amazon – Prospects of Habitat Fragmentation Studies

Maria Paula Cruz Schneider
*Departamento de Genética, Universidade Federal do Pará, Caixa Postal 8609, CEP 66750-900, Belém-PA, Brazil. E-mail: paula@ufpa.br.*

Brazil is the country with the largest undisturbed Tropical rain forest in the world, with almost half of the 6.5 million $km^2$ of the Amazon river basin being found in its territory. Tropical rain forests are also found in Central America, Africa and Southeast Asia, and they are supposed to play a central role in environment and climate stability worldwide. Besides the forest, the Amazon region is formed by a complex of ecosystems which includes cerrado and other types of vegetation totaling 5,029 millions $km^2$, comprising around 10 to 20% of all animal and plant species of the planet. However, such biodiversity has been threatened by an accelerated rate of damaging and destruction of various habitats.

Official reports alerted to the high level of deforestation and decline of biodiversity due to commercial logging, burning, construction of dams for hydroelectric plants, cattle ranching, and agriculture expansion. Thus the Brazilian Government, through its Ministry of Environment, provided incentives, by way of grants and scientific meetings, in order to elaborate a national policy for biodiversity based on the intentions of the 1992 UN Conference on Environment and Development (Earth Summit). The granted proposals would identify environmental, social and economic conditions for conservation and sustainable use of animal and plant species, as well as partition of the benefits resulting from biodiversity usage. Priority areas for conservation and inventory of species were also planned to be defined.

The research conducted by our group in Belém was always related to biodiversity and conservation issues. One of our main interests during two decades was the study of genetic polymorphism and molecular phylogeny of Neotropical primates1- (3). The aim of assessment of the diversity of New World primates is to have at hand a list of Primates as a basis for conservation measures, and to stimulate further research into the systematics an taxonomy of the group. In fact, without the formal structure of names and an agreed system of usage, there can be no understanding of what exists to be conserved (4,5). More recently we focused our research on the effects of fragmentation of some Amazonian habitats on populations of silvery marmoset (Mico argentatus), howler monkey (Alouatta belzebul) and birds species (guará, Eudocimus ruber, and some species of migrating shorebirds). Studies of habitat fragmentation has clearly and easily evaluated effects on parameters such as species diversity, population density, and behavioral patterns, but there are as yet few data available on its influence on genetic variation in free-ranging primate populations (6,7). Inbreeding and loss ofgenetic variability are generally presumed to be the primary results of the fragmentation of continuous populations, although outbreeding depression may also be relevant in many cases (8,9).

Preliminary data on the variability of DNA microsatellite markers in silvery marmoset populations at four sites in central Amazonia (10), representative of different degrees of habitat fragmentation, found a surprisingly high genetic variability among populations even in the smallest ones. However, the level of heterozygosity indicates that all populations are subject to inbreeding or genetic drift. Despite the preliminary nature of the data, the results show that the remnant populations of M. argentatus are quite genetically distinct. Direct evidence of inbreeding depression has been recorded in fragmented populations of golden lion tamarins (Leontopithecus rosalia), a species belonging to the same family as the silvery marmoset (Callitrichidae) (11), although no clear indications of this phenomenon were found in the M. argentatus population from the right bank of the Tapajós river.

Whatever the exact effect on genetic parameters, habitat fragmentation, in particular the isolation of relatively small subsets of original populations, is likely to have highly deleterious implications for the long-term viability of remnant populations (12). A systematic analysis of complementary ecological factors will be required before definitive guidelines can be drawn up for the management of remnant populations of silvery marmosets (10).

Another research currently under way by our group aims to provide ecological and genetic data about the long-term effects fragmentation habitats created after building of a hydroelectric plant dam. For

this purpose we use a wide range primate species, the howler monkey (Alouatta belzebul), whose habitat was radically altered through the formation of thousands of islands along the Tocantins river banks after the flooding of 2,500 $km^2$ due to the construction of the Tucuruí Hydroelectric plant (1984/1985). In order to obtain a more precise picture of how the genetic variability changed through time in this species, DNA microsatellite markers are being used in recent collected samples of the species, as well as in samples obtained 18 years ago, before the dam was built. Hence, the data gathered in that study may help to define viable population size and management in A. belzebul, as well as in other mammal populations, after habitat fragmentation.

Acknowledgements
The studies herein described were financed by the National Program for Biodiversity (PRONABIO) of the Brazilian Ministry of Environment, through BIRD and CNPq. Additional support was received from IBAMA/Santarém and the Kapok Foundation. IBAMA authorized the collection of specimens through special licences. We also thank to MCT/PPG7 and to ELETRONORTE for financial support of the Tucurui Project.

REFERENCES

1.Schneider& Rosenberger, 1996. Molecules, morphology, and Platyrrhini systematics. In: Adaptative Radiations of Neotropical Primates, M. A. Norkonk, A.L. Rosenberger and P.A. Garber (Eds.), pp.3-19. Plenum Press, N.Y
2.Schneider, H., Schneider., M. P. C., Sampaio, I., Harada, M. L., Stanhope, M., Czesluniak, J. and Goodman, M. 1993. Molecular phylogenetics of the New World monkeys (Platyrrhini, Primates) Molecular Phylogenetics and Evolution 2 (3): 225-242.
3.Schneider, H., Sampaio, I., Harada, M. L., Barroso, C. M. L., Schneider., M. P. C., Czelusniak, J. and Goodman, M. 1996. Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two unlinked nuclear genes: IRBP Intron 1 and epsilon-globin sequences. Am. J. Phys. Anthropology. 100: 153-179
4. Collar (1997), N. J. 1997. Taxonomy and conservation: Chicken and egg. Bull. B. O. C. 117 (2):122-136.
5.Rylands, A. Schneider, H., Lannguth, A. Mittermeier, R. A., Groves, C., Rodrigues-Luna E. 2000. An assessment of the diversity of New World primates. Neotropical Primates 8 (2).
6.Pope, T. R. 1996, Socioecology, population fragmentation, and patterns of genetic loss in endangered primates, in: J. C. Avise and J. L. Hamrick, eds., Conservation Genetics: Case Histories from Nature, Chapman and Hall, New York, pp. 119-159.
7.Pope, T. R. 1998, Genetic variation in remnant populations of the woolly spider monkey (Brachyteles arachnoides). Int. J. Primatol. 19: 95-109.
8.Templeton, A. R., 1986, Coadaptation and outbreeding depression, in: M. E. Soulé, ed., Conservation Biology. The Science of Scarcity and Diversity, Sinauer Associates, Sunderland, pp. 105-116.
9.Dudash, M. R. and Fenster, C. B., 2000. Inbreeding and outbreeding depression in fragmented populations, in: A. G. Young and G. M. Clarke, eds., Genetics, Demography and Viability of Fragmented Populations, Cambridge University Press, Cambridge, pp. 35-53.
10.Gonçalves, E. C., Ferrari, S. F., Silva, A., Coutinho, P., Menezes, E., Schneider, M. P. C. 2002.Effects of habitat fragmenttion on the genetic variability of silvery marmosets, Mico argentatus. In L. K. Marsh, ed., Primates in fragments, Plenum Press, New York, in Press.
11.Dietz, J. M., Baker, A. J. and Ballou, J. A. 2000, Demographic evidence of inbreeding depression in wild golden lion tamarins, in: A. G. Young and G. M. Clarke, eds., Genetics, Demography and Viability of Fragmented Populations, Cambridge University Press, Cambridge, pp. 203-211.
12.Lacy, R. C. 1997, Importance of genetic variation to the viability of mammalian populations. J. Mammal. 78: 320-335.

**P005:**

**Can we use genomics to select for healthier swine?**

Joan K. Lunney
*USDA, ARS, ANRI, Immunology and Disease Resistance Laboratory, Beltsville, MD USA*

Genome mapping efforts in swine have been targeted mainly to production traits, reproductive issues and meat quality. Early efforts did result in identifying genes encoding resistance to specific bacterial infections, e.g., K88R. Recent work has identified swine with improved, not complete, resistance to specific viral infections. However, identifying genes encoding resistance for each of the large number of viral and bacterial infections that impact swine production is virtually impossible. Therefore our research has been aimed at defining immune properties that result in swine with improved production traits and survival capacity due to improved disease responses, targeting cells and genes associated with type 1 immunity. Edfors-Lilja, Andersson, et al. mapped QTL associated with 'stress' (mixing and transport) induced alterations of porcine immune functions. Mallard, Wilkie, et al. selected swine for high immune response. Delineation of traits most appropriate to targeting for "enhanced immune properties" associated with disease resistance as well as vaccine and immune responsiveness will be discussed. Functional genomic tools, real time PCR and microarray analyses, as well as SNP studies, will help identify relevant genes, their regulators and best alleles. Major collaborative studies that integrate analyses for enhanced immune properties along with production traits are required to confirm the potential of using these genomic approaches to select for healthier swine.

***Nomenclature:*** Functional genomics, disease resistance, immune responsiveness.

**P006:**

## Proteomics

Hans-J. Thiesen
*Institut für Immunology, Schillingallee 70, D-18055 Rostock*

With the advent of complete genome sequences the analysis of gene functions is one of the scientific challenges in the post-genome-era. Great efforts have to be put forward on the analysis of complex gene and protein networks describing cellular as well as intercellular interactions. Corresponding research projects take advantage of novel enabling technologies i.e. chip technology and mass spectrometry.

### Comparative Genomics

Research projects studying processes in developmental biology and cellular differentiation, demonstrated in the past as exemplified by the functional analysis of homeobox-genes how informative the comparative analysis of protein domains from drosophila to human sequences turned out to be (Comparative Genomics). The functional analysis of protein domains manifests that protein functions can be correlated with structural domains. Based on sequence information on evolutionarily conserved structural elements, proteins can be grouped, classified and assigned to functional groups enabling phenotype-genotype comparisons.

### Functional genomics

Currently, multiple genomes have already been completely sequenced. Nowadays, the elucidation of individual gene functions becomes one of the prominent enterprises in research. Hereto, new research disciplines evolved such as transcriptomics, proteomics and metabolomics that are combined by integrative bioinformatic platforms (Figure I). Each of these disciplines is based on enabling technology platforms that requires specific instrumentation, expertise and the establishment of standard operating procedures (SOPs). How these technologies can be successfully integrated in one centre, has recently been realized by the Proteome Centre Rostock (www. proteome-alliance.de).

### Definition Proteome:

The expression "Proteome" firstly coined by Marc Wilkins in 1994 describes the attempt of describing the composition of all proteins in a qualitative and quantitative manner present within one entity (tissue, body fluid, organism) at specified physiological states and time points.

### Comparison Genome and Proteome

In contrast to a genome, the proteome is dynamic and presents specific physiological states under specified environmental conditions at specific time points. Furthermore, the proteome determines the functional state of living organisms. The genome just ensures that the proteome is functional. How the proteome of a cell operates, is based on environmental factors ie. such as light, energy, nutrition and physical activities. In multicellular organisms, the interplay and turn-over of cells within one organism has to be taken into account as well. Obviously, the proteome determines how the genetic information is being used (Transcription factors regulate the retrieval of genetic information). Thus, one might even say the proteome determines under evolutionary aspects how the corresponding genome has been assembled. The genome probably ensures that the proteome remains operational in living organisms. In general, protein compositions have to be stabilized by replacing proteins according to their specific half-lives. Furthermore, perturbations of the proteome can then be introduced by changing the composition or the rate of protein renewal.

### Proteome-analysis:

With the development of the 2-dimensional gel electrophoresis by Patrick O'Farell and Joachim Klose in 1975, the basis for proteome analysis had been established. This technology was then extended by the engineering of sophisticated mass spectrometry tools such as MALDI-TOF- (Matrix-Assisted-Laser-Desorption-Ionisation-Time-Of- Flight-) and electrospray mass spectrometry. In general, proteome analysis starts with the sampling of biological material under standardised conditions and ends with the computer analysis of mass spectrometric data that finally leads to the assignments of

identified proteins to pathways displaying functional activities. In particular, biological samples are collected under standardized conditions (SOPs) and prefractinated i.e. using gradient centrifugation, free-flow-electrophoresis, multidimensional protein identification technology (mudPIT) followed by 2-dimensional gel electrophoresis. Either chromatographicly separated protein fractions are directly subjected to liquid chromatographic mass spectrometry (Nano-electrospray-MS) or are further separated on 2D-gel-electrophoresis (2DE) using immobiline strips or ampholyte gel electrophoresis. Proteins identified in 2DE to be differentially expressed are isolated by picking robots and digested by enzyme digestion (i.e. Trypsin). The obtained peptide fragments are then subjected to MALDI-TOF-MS analysis for protein identification by mass finger printing.

**Proteome analysis**
Initially, global proteomic approaches were performed followed by the analysis of subproteomes ( Organelles and protein complexes). Hereto, Cellzome (Heidelberg) developed technologies for determining protein-protein interaction maps. MELTEC (Magdeburg) developed protocols to analyze the toponome of cells and tissues coined toponomics and Biovision in Hannover concentrates on studying the peptidome (peptidomics).

**New Technologies**
Recently, fluorescence dyes have been introduced to visualize differentially expressed proteins (Pharmacia), or to meet the sensitivity of silver stains (SyproRuby). Microfluid-Chips are expected in the future to replace 2DE gels, multidimensional protein identification technology (MudPIT) has been successfully developed to approach proteins that do not separate well in conventional 2DE gels., ICAT methods are used for quantification purposes.

**Bioinformatics**
Laboratory-Information-Management-Systems (LIMS) are essential to enable high-throughput-analysis. Hereto, the Proteome-Center Rostock has established the "Proteobase-Data-Management-System", in which clinical data, SOPs, RNA and protein expression data are going to be integrated.

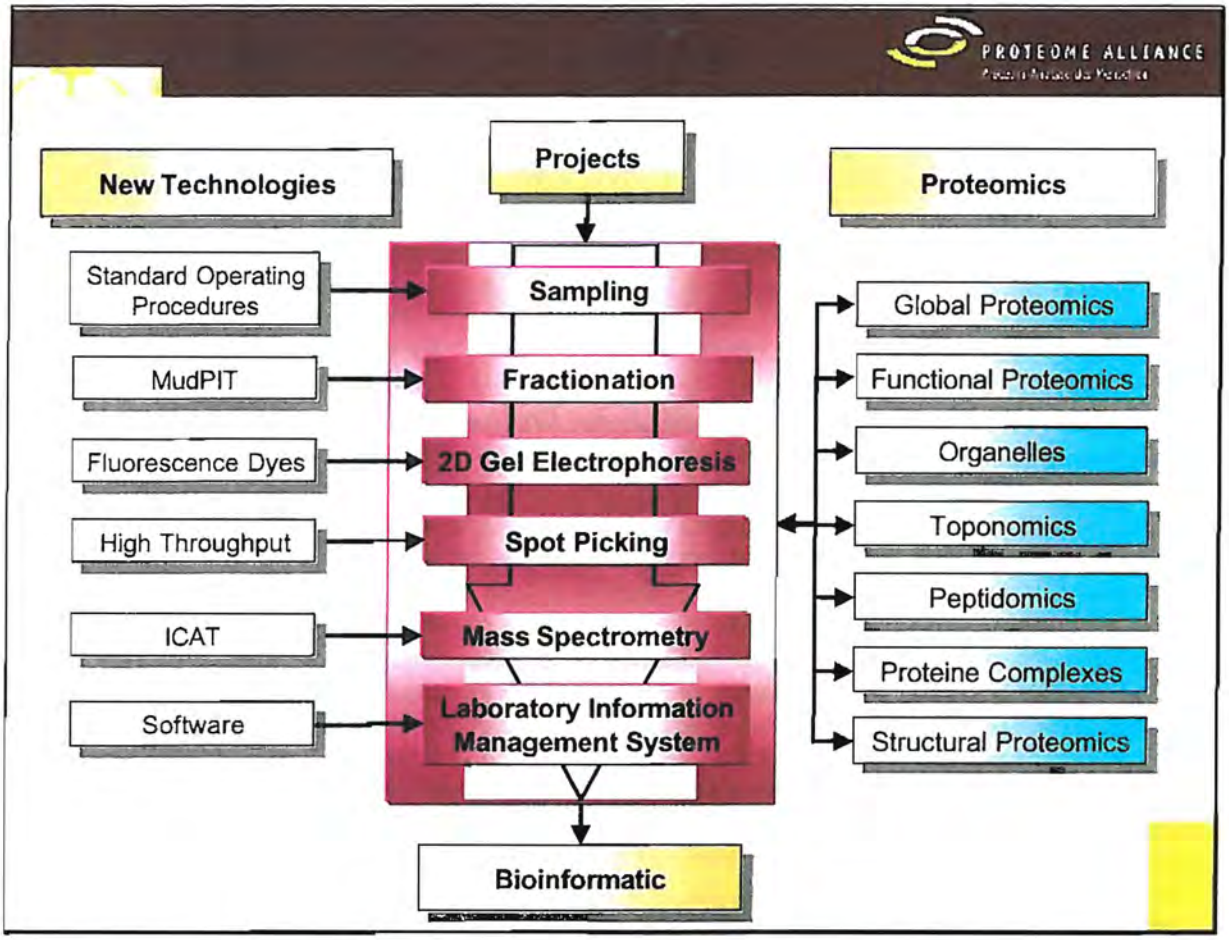
**The BMBF-Leitprojekt:**
In the BMBF-Leitprojekt "Proteom-Analyse des Menschen" (www.proteome-alliance.de), genome-, transcriptome- and proteome analyses are combined to elucidate common and disease specific mechanisms involved in the pathophysiology of autoimmune diseases. For instance, only a correlation coefficient of 0.2 was obtained once RNA and protein expression levels of synovial tissues were compared. That RNA- and protein expression levels are not well correlated is not surprising since numerous kinds of transcriptional and translational regulation are known to exist in the cell. In addition, mRNA molecules and proteins vary in their propensities to become degraded. This comparative analysis underlines the importance of determining RNA and proteome expression levels in order to validate the relevance of RNA and protein expression profiling experiments.

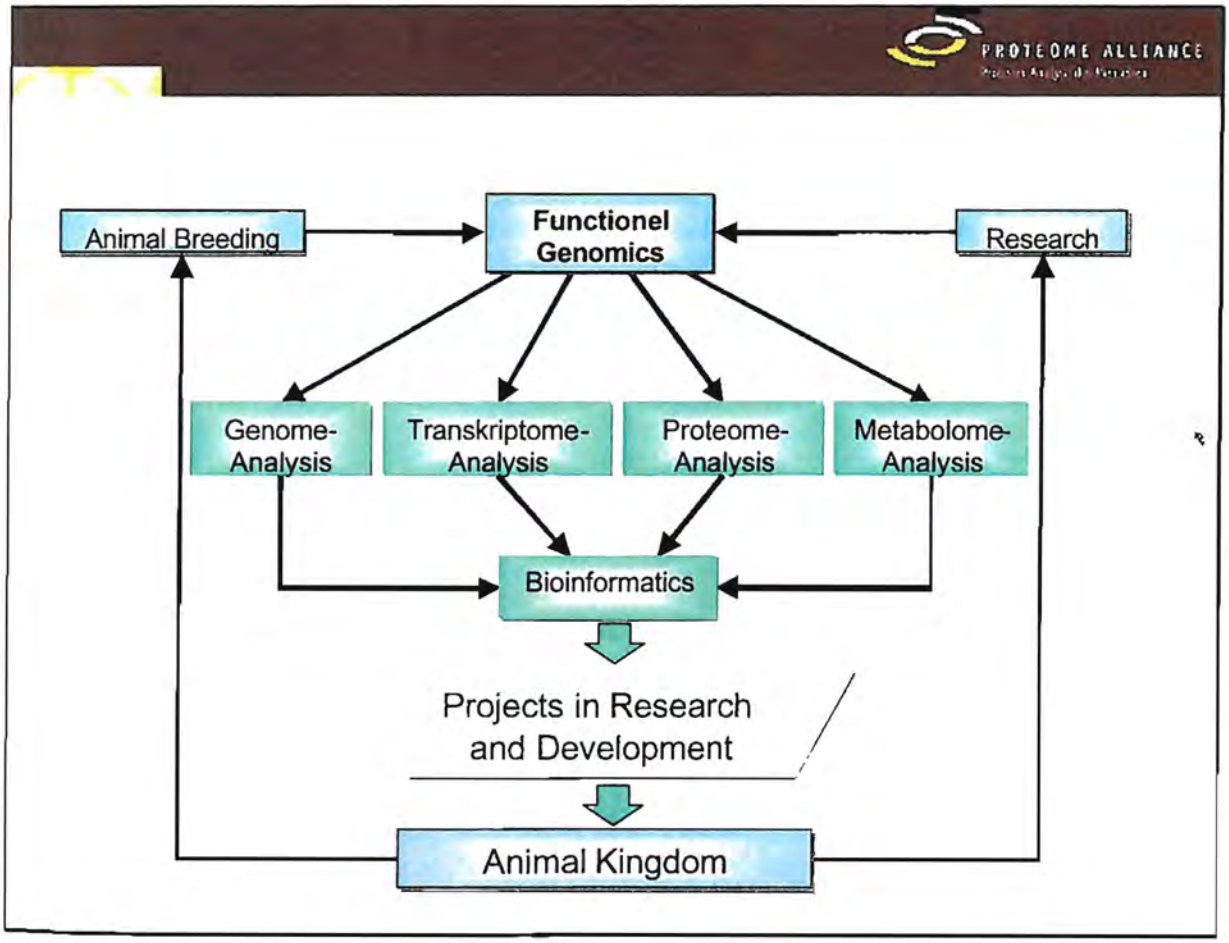
**Services**
The Steinbeis-Transfer-Center for Proteome-Analysis offers services in protein and transcriptome analysis (http://www.stw.de/stz/424.htm) by making use of our Affymetrix Service / custom-made chip approach as well as the technology platform in proteomics run by Prof. Dr. M.O. Glocker.

**Perspectives**
By bringing experts of experimental research together with mathematicians and engineers, a new discipline is about to be born coined **system biology** with the aim of developing new theoretical approaches and computational tools in order to analyze, to model, and to predict network behaviours of proteomes.

**Acknowledgement:**
This work is supported by the BMBF FKZ 01GG9831/3. We thank our experimental and clinical collaborators for their support.

**URLs:**
http://dip.doe-mbi.ucla.edu/
http://www.cgr.harvard.edu/research/biological.html
http://yeast.cellzome.com/browsec.php


PROTEOME ALLIANCE
New Technologies
Projects
Proteomics
Standard Operating Procedures
MudPIT
Fluorescence Dyes
High Throughput
ICAT
Software
Sampling
Fractionation
2D Gel Electrophoresis
Spot Picking
Mass Spectrometry
Laboratory Information Management System
Global Proteomics
Functional Proteomics
Organelles
Toponomics
Peptidomics
Proteine Complexes
Structural Proteomics
Bioinformatic


PROTEOME ALLIANCE
Animal Breeding
Functionel Genomics
Research
Genome-Analysis
Transkriptome-Analysis
Proteome-Analysis
Metabolome-Analysis
Bioinformatics
Projects in Research and Development
Animal Kingdom

**P007:**

## The immunobiology of prion diseases

Adriano Aguzzi
*Institute of Neuropathology, University Hospital Zurich, CH-8091 Zurich, Switzerland*

Mice deficient in the normal prion protein are resistant to exposure to prion infectivity, and expression of the normal prion protein by neurons is necessary for the development of histological damage[1]. But how do prions reach the brain after entering the body from peripheral sites? The first portal of entry in the gut may be represented by M-cells[2]. Neuroinvasion, i.e. the process by which prions march through the body of the host towards the brain, is dependent upon expression of the normal prion protein in a non-hematopoietic extracerebral site[3]. We therefore developed the hypothesis that neuroinvasion takes place in two distinct steps: first the lymphoreticular system is diffusely colonized by the agent, while at a later time infectivity progresses from lymphoreticular organs to the central nervous system[4], probably via sympathetic nerves[5,6]. There is an absolute requirement for B-lymphocytes in peripheral prion pathogenesis[7]. Surprisingly, the presence of the normal prion protein is not necessary on B-lymphocytes to enable them to support this process[8]. The mechanism of action of B lymphocytes may consist of presentation of lymphotoxin-ß to follicular dendritic cells[9]. This paves the way to post-exposure prophylaxis strategies[10] that exploit the anti-prion effect of soluble lymphotoxin-ß receptors. Why do follicular dendritic cells accumulate prions? We tested the hypothesis that prion uptake may be complement-mediated. Indeed, certain components of the complement system (C1q, CR1/2) proved to play an important role in pathogenesis[11]. Finally, we have found that transgenic expression of an anti-PrP antibody heavy chain suffices to confer to mice antiprion protection – a finding that may be relevant to the development of antiprion vaccines[12].

1. Brandner, S., Isenmann, S., Raeber, A., Fischer, M., Sailer, A., Kobayashi, Y., Marino, S., Weissmann, C. & Aguzzi, A. Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* 379, 339-43 (1996).
2. Heppner, F. L., Christ, A. D., Klein, M. A., Prinz, M., Fried, M., Kraehenbuhl, J. P. & Aguzzi, A. Transepithelial prion transport by M cells. *Nat Med* 7, 976-7 (2001).
3. Blättler, T., Brandner, S., Raeber, A. J., Klein, M. A., Voigtländer, T., Weissmann, C. & Aguzzi, A. PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* 389, 69-73 (1997).
4. Aguzzi, A. & Weissmann, C. Prion research: the next frontiers. *Nature* 389, 795-798 (1997).
5. Glatzel, M., Flechsig, E., Navarro, B., Klein, M. A., Paterna, J. C., Bueler, H. & Aguzzi, A. Adenoviral and adeno-associated viral transfer of genes to the peripheral nervous system. *Proc Natl Acad Sci U S A* 97, 442-7 (2000).
6. Glatzel, M., Heppner, F. L., Albers, K. M. & Aguzzi, A. Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion. *Neuron* 31, 25-34. (2001).
7. Klein, M. A., Frigg, R., Flechsig, E., Raeber, A. J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R. M. & Aguzzi, A. A crucial role for B cells in neuroinvasive scrapie. *Nature* 390, 687-90 (1997).
8. Klein, M. A., Frigg, R., Raeber, A. J., Flechsig, E., Hegyi, I., Zinkernagel, R. M., Weissmann, C. & Aguzzi, A. PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nat Med* 4, 1429-33 (1998).
9. Montrasio, F., Frigg, R., Glatzel, M., Klein, M. A., Mackay, F., Aguzzi, A. & Weissmann, C. Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* 288, 1257-9 (2000).
10. Aguzzi, A. & Collinge, J. Post-exposure prophylaxis after accidental prion inoculation. *Lancet* 350, 1519-20 (1997).
11. Klein, M. A., Kaeser, P. S., Schwarz, P., Weyd, H., Xenarios, I., Zinkernagel, R. M., Carroll, M. C., Verbeek, J. S., Botto, M., Walport, M. J., Molina, H., Kalinke, U., Acha-Orbea, H. & Aguzzi, A. Complement facilitates early prion pathogenesis. *Nat Med* 7, 488-92. (2001).
12. Heppner, F. L., Musahl, C., Arrighi, I., Klein, M. A., Rulicke, T., Oesch, B., Zinkernagel, R. M., Kalinke, U. & Aguzzi, A. Prevention of Scrapie Pathogenesis by Transgenic Expression of Anti-Prion Protein Antibodies. *Science* 294, 178-182 (2001).

**P008:**

## Ethical Issues of Genetic Manipulation of Lifestock

Prof. Dr. Dr. Bernhard Irrgang,
*TU Dresden, Institute of Philosophy, G-01062 Dresden;*
*E-Mail: irrgangb@Rcs1.urz.tu-dresden.de*
*Tel.. 0049-351-46336001*
*www.tu-dresden.de/phfiph/dozenten/irrgang.htm*

I would like to answer the question of ethical general conditions for genetic manipulation of life in two steps:
(1) First it needs to be clarified why we have moral obligations towards live and how we can point them out;
(2) Then the question of necessary special regulations for genetic manipulation needs to be answered.
These issues should be discussed concerning the positions of Biocentric and "Deep Ecology".

1) About the attempt of an Ethics of nature

Regarding the question of man's use of live in a morally acceptable way, various attitudes are possible: (1) Anthropocentric, (2) Pathocentric, and (3) Biocentric. From the ethical point of view it is important to know why we have the obligation of moral behaviour (for example consideration) towards living organisms. The classical Anthropocentric (I. Kant) considers cruel treatment of animals as an act against man's duties towards himself because through becoming insensible, morality will be weakened ore destroyed. For those who follow Pathocentric (P. Singer and M. St. Dawkins) in the tradition of Utilitarism, the ability of suffocation is the central criteria. Following Bentham's thesis they also include animals because of their ability of suffocation within the weighing of interests. But it is difficult to find out empirical criteria for animal suffocation for example the phenomenon of stress (measurement of heartbeat) ore the behaviour of an animal in painful situations. The scoop of interpretation is large. The members of Biocentric (A. Schweitzer, G. M. Teutsch, T. Regan) with their claim for an attitude of "respect for live" avoid this problems. Modern biocentrical positions rely on the consciousness of animals and their interests. Many philosophers of Biocentric consider the difference between the human and the animal consciousness as gradual and not essencial, a problematic premise. They demand an equality of man and animal, as extensive as possible. O. Höffe takes a mediate position with his though of a (regarding the organisation of the sense organs and the central nervous system) hierarchical solidarity between man and animal. Interpreted as a priority criteria this leads to the demand of equal treatment of man and animal in comparable situations. Based upon this urgency criteria concrete demands for the use of nature ore animals can be made (Irrgang 1997, 172-182).

Within the field of Philosophy especially since Descartes the animal-man-difference and since Darwin the animal-man-transition is being discussed. The question of man-animal-comparison (Teutsch 1985, 133-135) is of primary importance for the personal status but also difficult: Until today there has been no real success in the exact definition of difference between man and animal (Brockhaus 1975, 110). The consideration would be widely extended for those animals to which we attribute the personal status and the individual interest of survival. Peter Singer discusses such a personal status for chimpanzees, gorillas, dolphins and whales. Traditionally the animal-man-difference and the personal status is considered to be located in the rationality and morality of man. If personality is being connected to criteria like feelings of pleasure and reluctance ore consciousness ore individuality, it is allowed to ask the question weather there can be something like an animal-person.

In deed, some common ideas about animals and their consciousness will have to be corrected. Griffin could make this plausible in many cases (Griffin 1990, 28-30). But there is no reliable criteria for ascribing consciousness to animals (Griffin 1990, 68). Nevertheless it is possible to learn something about their thinking from their ability to learn, to adjust and their communication behaviour. Even though I do not consider a animal-person from the ethical point of view, ethically relevant criteria for the consideration of animals are pain, their repertoire of behaviour, especially their communication

behaviour and the possibility of consciousness. Therefore a close inspection is necessary. In this sense I do not know any position which claims that the acting of animals is morally responsible. Therefore it would have to be proofed that a least some animals do act morally responsible and give reasons for their objectives.

The most radical ethical duties towards nature and live are being claimed by the "deep ecology". Already in 1972 when the Norwegian analytical Philosopher Arne Naess introduced the expression "Deep Ecology", it was the central objective to make a revolutionary change in the anthropocentric orientation of western Ethics and Politics. The utopical character of many of the deep ecology objectives is obvious. The basic attitude of society should be overcome with the interpretation constructions "the nature" and especially the system as a whole like landscape, ecosystems and biological species all of which are nothing purely existing but constructions which include human perception, cultural interpretation and scientific conventions (Birnbacher 1997, 12). Deep Ecology does support many of the objectives of the Reformism but it is also revolutionary and seeks a new Metaphysics, Epistemology, Cosmology and Environmental Ethics for the man-earth-system (Dervall 1997, 17). Following the current paradigm, nature is only a supply of resources which has to be produced in order to meet the permanently growing needs of the permanently increasing number of people. Science and technology work hand in hand. Technology develops techniques to conduct natural processes. Changes become an end in itself. The new is higher regarded than the old, the currant generation higher than the future one i (Dervall 1997, 19f). Deep ecology puts it's main effort into finding and discussing alternatives to the conventional way of thinking of the modern West. That includes modern natural sciences and technology, especially genetic engineering and it's full technological handling of live. But did modern science bring such a big change into our relation to nature?

2) The claim for Biocentic and "Deep Ecology"

Because of the demoralisation of the definition of live in modern biology, modern biocentric positions ore the so called "deep ecology" rather follows the romantic – organic definition of live which is especially for Schopenhauer and in the philosophy of live connected to a (cultural-) pessimistic thinking. This background is also being established in the basic attitude of respect for every live as it has been formulated in the Ethics of Albert Schweitzer. But if one tries to live consequently following this attitude, huge problems come up. In it's close sense it is not possible to realise. Also, respect is a religious ore at least no specific moral attitude. One can have respect for virus but fight them with good reasons without guiltiness if they harm ore endanger man. Attitudes towards live which are similar to Biocentric can be found in Jainism (Religion of he Jaina, India), in Buddhism (Eastern Asia) and in a slightly different way in Hinduism (India) in the Ahimsa-Commandment (prohibition of harming any life).

Biocentric position demands protection of life by itself. Interventions in nature are actually prohibited and need to be justified. Günther Altner claims: "The possibility of a self-recreational evolution for nature has to be preserved because this is it's freedom" (Altner 1987, 216). Like in the definition of the animal-person, the living nature is being personalised in order to ascribe moral values and moral rights to it. The first claim of biocentric positions is the protection of species: "The self-value of non-human creatures shows itself in the unique developed nature, in it's natural interplay which again expresses itself in the typical community of species (biotopes) and in the relatively stable ecological balance" (Altner 1991, 217). A living nature is being imputed in which man does not exist. To give reasons for it's protection, nature is being personalised. But in the other hand, a more correct method would be the use of definition of species in the sense of ecological adjusting as an ethically relevant empirical criteria for the protection of species.

Further, biocentric positions ascribe consciousness to animals and often demand a general prohibition of killing animals. Tom Reagan regards the difference in consciousness of animal and man as only gradual and not essential. He ascribes a certain autonomy to animals because they can express preferences especially interests of wellbeing. The definition of interests is connected to the definitions: selfishness, use in general, survival, egoism, protection of benefits and avoiding of damages, though calculated benefits on the basis of reason and needs as action motivation can most probably only be

ascribed to man, natural efforts towards damage prevention and protection of ones own life can be found also with other organisms.

3) Fairness towards every living being

The following critics against Biocentric and "Deep Ecology" have to be done: Although the fact of one origin of every living being, including man, gives a certain respects towards all forms of life although not all of them are fundamentally equal. The needs of man are more differentiated than the ones of animals. As well animals do not have the reflective consciousness which enables man to make experiences and act responsible, to make claims ore judgements (Frey 1980, 120). Moral acting cannot be ascribed to animals. From the ethical point of view the self-value of nature cannot be the same as the one of man (Irrgang 1997, 211f). This puts the personification of nature but not the use of biology in the sense of an ethically relevant empirical criteria into question. In the end, not the use of empirical results like certain proofs of live but the use of ethical principals like justice ore damage prevention gives reasons for claims like prohibition of direct harming of animals (animal prevention) and the protection of species (biodiverstiy).

That means that the principal of justice of life is fundamental for Bioethics of life. It applies to all living beings. Nobody seriously claims that animals can act morally responsible. At the most a moral-like behaviour of animals is being described (Brockhaus 1975, 111). Although Bioethics does not justify the concept of a morally responsible acting animal-person it does develop a ethically relevant empirical criteria for the consideration of animals: Ability to feel pain, behaviour repertoire, communication behaviour and the possibility of consciousness.

In order to avoid the naturalistic fallacy from evolutionary justified "similarities" to moral obligations (Irrgang 1997, 163-182), a gradualism has been developed on the base of an ecological conception of justice. The gradual urgency criteria of consideration of the concerned acting persons (Irrgang 1997, 204-212), considers as commandment of fairness ore justice with growing grade of urgency the balance and cycles of nature which are fundamental for other general coherences of life, as well as issues of species protection not only for vertebrates, emotionality and ability to feel pain of organisms and the attempt of higher developed animals to lead a live without suffering, especially if we have a special responsibility for them as useful animals and that means not only to consider them as genus but as individuals and also the urgency criteria does ascribe a very high consideration to highly developed mammals like monkeys ore certain sea mammals (Irrgang 1997, 205).

This way a differentiated Ethics of protection of life on the basis of an ecological anthropocentric can be justified. Animals are to be protected in coherence to their communication ability and competence, pain and forms of consciousness. The unjustified harming of any parts of nature and especially highly developed animals does brake the ethical principal of justice. In order to measure weather we are harming animals, ethically relevant criteria are needed. Obligations towards animals ore towards nature cannot be shown apodictically but they need an argument of convergence which considers most of the mentioned criteria as possible. In general these are ethically relevant empirical criteria which help to assess in which cases the obligation of the protection of animals does ore does not apply.

4) Biodiversity – an absolutely obligatory criteria ?

Many people consider Biodiversity as diversity of life – in German mentioned as diversity of species – as an indicator which they suppose to know from direct [empirical] experience within a nature which is intact and meets the needs of humans. But it is unclear what diversity of life means and through which criteria and basis this diversity has to be seen. The problem already begins with the attempt to find a clear definition for the classical objects which are supposed to show the diversity of life, these are "species ", "gene" and "ecosystem". The differences in the terminology are signs for divergences regarding the in biological sciences recognised research procedures and their corresponding research objects. All together, throughout the whole earth age, it turned out that the paleo-biodiversity has neither through background events nor through exogenous catastrophes been extinguished nor damaged with lasting effect. Although living beings have died out not only as individuals but also as families ore orders but this has always been an accompanying phenomena for the development of life

on earth in which the decreasing of diversity was always been followed by an increase. Even though some species, families ore orders have disappeared from earth forever, it did not lead to a long-term damage of the world of organisms in total.

The micro-biological diversity research showed that only a very small number of currently living micro-organisms have been isolated given a valid description. Regarding this it has to be mentioned that micro-biologists do not demand the protection of single species but the protection of locations for the taking of specimen. Procedures of molecular-biology put main emphasis on genetic information. This is especially suitable as a base for procedures of proving of biodiversity. Significant parts of the biodiversity of plants cannot be just morphologically characterised on the genetic level. The objects of the biodiversity debates like gene, species ore ecosystem are no purely natural ore scientific objects but are also determined by giving practical purposes (Janich et al. 2001, XXI till XXIX). The reasons for the widespread dying of species are shield tectonic, sea level and changing of magnetic fields as well as volcano and asteroids (Janich et al. 2001, 70-99). The value of species can be differentiated as a self-value and an instrumental value. Biodiversity does have a certain production value, although the problem of value assessment of biodiversity has to be regarded in relation to the problem of prevention of nature.

Biodiversity and prevention of species are ethical claims which can be justified, especially considering our non-knowledge about most of the biological species and considering the caution regulation, but it is no absolute commandment. Above all a prohibition of an inter-species gene transfer cannot be justified on this base. In order to answer this question it needs the reflection of the specification of genetic acting in comparison to all other technological acting. Special regulations are only obligatory, if the genetic praxis does fundamentally differ from all the other forms of technical praxis.

5) Genetic Engineering as Laboratory Praxis

The praxis of genetic engineering does basically not differ from other technological praxis. The structure of technological acting gives reasons for the unavoidable trying out. This trying out can never be fully calculated although certain possibilities can in advance be excluded as being practically irrelevant through calculation. Technological acting is no blind use of nature ore objects. It is no accidental process but it is lead by an organised ore at least a heuristic process of searching and finding. Useful animals and plants are no objects in the close sense but in order to deal with them, man first of all uses the natural processes of reproduction. Useful animals are no technological means, no tools ore machines in the average sense but they also are no classical objects. The expanded definition of technology as handling of both technological and natural processes can avoid a to close understanding of technology in the sense of tool and object. It constitutes a concept of technological acting both from the point of view of the participant and of the observer (about the concept of instrumental understanding see Irrgang 1998a, 75-120, about technological acting see Corona/Irrgang 1999, 166-212). The technological handling of a single and concrete technological problem takes place on the basis of a (implicit) technological regulation knowledge with the aid of means and / ore technological knowledge. This regulation knowledge depends on frameworks ore paradigms which often cannot be shown explicitly. But on the basis of a reflected handling knowledge they can be understood, put as subject and be criticised. I explained this more detailed in my book "Technische Kultur" (Irrgang 2001a).

In the praxis of breeding organisms of one and the same kind are being crossed with a combination of genetic marks without having an exact knowledge of the genetic information. The definition of species can be regarded as a human interpretation construction, which although it might give some kind of information about the biological reality it does give reasons for doubt.
The gene transfer changes one ore several genetic marks of an organism although it does normally not change the species which in theory would be possible. The differences between technical and technological praxis that is differences between breeding and genetic engineering are in general the inserted laboratory procedures. These lead to better information about details and causal paradigm of biological processes but not to information about general coherences. This is the difference between breeding praxis on farms and breeding research in labs, whereas the modern seed breeding companies are differing from university ore MPI- laboratories. The procedures of genetic engineering in the sense

of a technological praxis – a reflected strategy of attempts and errors, ethical evaluation included – does correspond with a concept of technological acting which reduces technology to objects. Technological acting which opens new technological possibilities opens a new range of possibilities using them in praxis which often were regarded as technological imperatives (this is possible and is sometimes being done) but actually it is the opposite: only after trying out you know what is possible and you can decide which action options one wants ore wants not to take. Technology as technological acting can be seen under several aspects: objectives, technological means, consequences ore interventions in nature can also be relevant. Especially the last point is of interest. Technology is not to be regarded as practical experimental science. On one hand experimental science also needs to give things a trial but on the other hand a lot of things work out in theory but not in praxis. Asking for the possibilities of trying out ore making experiments – at the end there are no other ways to handle technological action than to give technologies a try in praxis. The meaning of technological experiences can never be reduced to the pure observer position. Possibilities of making mistake and of making innovation depend on each other. Organised and rational experiments also includes responsibility and not to give it a try no matter what happens. This also has consequences for ethical evaluation of genetic engineering. Nevertheless, every form of selection includes certain risks of failure. Risks in the releasing of trans-genetic organisms cannot be totally excluded. No form of technological acting is without risks. Although, they do not depend on the method of gene transfer but on the nature of the changed organism and ecosystems into which it is being released, which means they depend on the way of technological acting. A special Ethics for genetic engineering such as "Gene-ethics" is not necessary, but a differentiated ethical reflection within specified areas for a specialised form of technological practice is needed.

Literature:


Altner, Günther 1987: Die Nutzungsziele der Gentechnologie unter der Perspektive von Umwelt- und Sozialverträglichkeit; in: V. Braun, D. Mieth, K. Steigleder (ed.); Ethische und rechtliche Fragen der Gentechnologie und der Reproduktionsmedizin; Munich, 213-223

Altner, Günther 1992: Naturvergessenheit. Grundlagen einer umfassenden Bioethik; Darmstadt

Bayertz, Kurt 1989: Entmoralisierung des Lebendigen; in: Matthias Gatzemeier (ed.): Verantwortung in Wissenschaft und Technik; Mannheim, Vienna, Zurich, 220-238

Birnbacher, Dieter 1997: Ökophilosophie, Stuttgart

Brockhaus, Wilhelm 1975: (Ed.) Das Recht der Tiere in der Zivilisation. Eine Einführung in Naturwissenschaft, Philosophie und Einzelfragen des Vegetarismus, Munich

Corona, Nestor; Irrgang, Bernhard 1999: Technik als Geschick? Geschichtsphilosophie der Technik; Dettelbach

Dervall, Bill 1997: „Tiefenökologie" – Eine ökologische Ganzheitsphilosophie; in: D. Birnbacher, Ökophilosophie, Stuttgart, 17-59

Frey, R. G. 1980: Interests and Rights. The case Against Animals; Oxford

Griffin, Donald R. 1990: Wie Tiere denken. Ein Vorstoß ins Bewußtsein der Tiere; translation by Elisabeth M. Walther (1984); Munich

Irrgang, Bernhard 1991: - Transgene Tiermodelle im biomedizinischen Experiment. Forschungsethische Überlegungen ; in: Forum für interdisziplinäre Forschung 4 (1991), issue 1, 54-66

Irrgang, Bernhard 1992: Ethische Aspekte der Biotechnik; in: H. Wilhelm Schaumann-Stiftung (ed.): Biologisch-technische Entwicklungen in der Tierproduktion (14. Hülsenberger Gespräche); Hamburg, 36-46

Irrgang, Bernhard 1996: Handbuch der angewandten Ethik; ed. by Julian Nida-Rümelin; article Gen-Ethik; Stuttgart 1996, 510-551

Irrgang, Bernhard 1997: Forschungsethik Gentechnik und neue Biotechnologie. Grundlegung unter besonderer Berücksichtigung von gentechnologischen Projekten an Pflanzen, Tieren und Mikroorganismen; Stuttgart

Irrgang, Bernhard 1998a: Praktische Ethik aus hermeneutischer Perspektive; Paderborn

Irrgang, Bernhard 1998b: Wozu können Klonierungsverfahren dienen: ethische Bewertungskriterien; in: Johannes S. Ach, Gerd Brudermüller, Christa Runtenberg (ed.): Hello Dolly? Über das Klonen; Frankfurt/M., 72-89

Irrgang, Bernhard 1998c: Lexikon der Bioethik; ed. by Wilhelm Korff, Lutwin Beck, Paul Mikat; Gütersloh 1998; art. Biozentrik (vol. I, 402-404); art, Pathozentrik (vol II. 2, 834-835); art. Physiozentrik (vol. III, 28-30); art. Theozentrik (vol. III, 526-528); art. Tierschutz (vol. III, 561-567)

Irrgang, Bernhard 1998d: Am Ende der Anthropozentrik? Wie lassen sich unsere Verpflichtungen gegenüber der Natur begründen; in. Gotthard Fuchs, Guido Knörzer (ed.): Tier, Gott, Mensch. Beschädigte Beziehungen; Frankfurt 1998, 13-32

Irrgang, Bernhard 1999a: Gentechnik in der Tierzüchtung; in: Thomas Hausmanninger, Scheule, Rupert (ed.): ... geklont am 8. Schöpfungstag. Gentechnologie im interdisziplinären Gespräch; Augsburg, 227-242

Irrgang, Bernhard 199b: - Nachhaltigkeit als Leitbild für Grüne Gentechnik? in: Dirk Harreus (ed.) Gentechnologie. Fakten und Meinungen zum Kernthema des 21. Jahrhunderts; Berlin 1999, 146, 195-210, 241f

Irrgang, Bernhard 2000: Ethische Betrachtungen der Anwendung bio- und gentechnologischer Verfahren in der Tierzucht; in: Deutsche Gesellschaft für Züchtungskunde e.V. (ed.) Bio- und Gentechnologie in der Rinderzucht – Risiko oder Chance? DGfZ-Schriftenreihe 17; Bonn, 56-66

Irrgang, Bernhard 2001a: Technische Kultur. Instrumentelles Verstehen und technisches Handeln; (Philosophie der Technik vol. 1); Paderborn

Irrgang, Bernhard 2001b: Gefangen in Sachzwängen? Zur ethischen Dimension der Gestaltbarkeit der Biotechnologie; in: St. Heiden et al. (ed.): Biotechnologie als interdisziplinäre Studie; Heidelberg / Berlin, 83-96

Irrgang, Bernhard 2002: Technische Praxis. Gestaltungsperspektiven technischer Entwicklung; (Philosophie der Technik vol. 2); Paderborn 2002

Irrgang, Bernhard et al. 2000: Gentechnik in der Pflanzenzucht. Eine interdisziplinäre Studie; Dettelbach

Janich, P. et al. 2000 (ed.): Biodiversität. Wissenschaftliche Grundlagen und gesetzliche Relevanz; Berlin et al.

Lenk, Hans; Maring, Matthias 2001: Advances and Problems in the Philosophie of Technology; Münster

Teutsch, Gotthard M. 1985: Lexikon der Umweltethik; Göttingen

Zimmerli, W. Ch. 1993: Die Bedeutung der empirischen Wissenschaften und der Technologie für die Ethik; in: A. Hertz, W. Korff, T. Rendtorff, H. Ringeling (ed.): Handbuch der christlichen Ethik; Freiburg, Basel, Vienna, Aktualisierte Neuausgabe vol. I, 297-316

# Section A: Genome technologies

## A001
### Mb resolution radiation hybrid map of the canine genome

RICHARD GUYON[1], LISA KIM[2], CHRISTOPHE HITTE[1], TRAVIS LORENTZEN[2], EDOUARD CADIEU[1], HEIDI G. PARKER[2], PASCALE QUIGNON[1], JENNIFER K. LOWE[2], CORINNE RENIER[1], BORIS GELFENBEYN[2], STEPHANIE GLOUX[1], FRANÇOISE VIGNAUX[1], GREGORY MAHAIRAS[3]; CATHERINE ANDRÉ[1], FRANCIS GALIBERT[1], ELAINE A. OSTRANDER[2]

*[1]UMR 6061 CNRS, Génétique et développement, Faculté de Médecine, 35043 Rennes, France; [2]Clinical and Human Biology Divisions, Fred Hutchinson Cancer Research Center, Seattle WA 98109-1024, USA; [3]Department of Genome Sciences, University of Washington, Seattle WA,USA*

The purebred dog population consists of over 300 genetic isolates termed breeds, representing a unique resource for dissecting the genetic basis of both simple and complex mammalian traits. Toward this end, we developped a comprehensive radiation hybrid map of the canine genome composed of 3400 markers. The map was constructed using the RHDF5000-2 whole-genome radiation hybrid panel and computed using the Multimap and TSP/CONCORDE programs. This map provides an average inter-marker distance of 1Mbase and a nearly complete coverage of each of the 40 chromosomes composing the canine karyotype. The inclusion of 1000 dog genes and ESTs allows refined numbering and mapping of conserved segments between human and dog. Moreover, of the 1,700 mapped microsatellites, approximately 800 have HET or PIC values $\geq$0.5, thus providing a well characterized resource of highly polymorphic markers suitable for genome wide scans in genetic linkage studies with pedigrees of interest. Finally, the 700 mapped BAC-ends constitute an initial framework of clones for anchoring a physical map and provide a format for positional cloning studies. Overall this work defines a powerful resource for genetic studies in the canine system, and underscores the emerging power of canine genomics in the pursuit of the genes causing human variation and disease.
http://www-recomgen.univ-rennes1.fr and http://fhcrc.org/science/dog_genome/dog.html

## A002
### Disruption of the *PLC-β1* gene in a *βLG* transgenic line affects growth and fertility in mice

MARIA BALLESTER[1], ELENA IBÁÑEZ[2], RAMONA N. PENA[3], JOSEP MOLIST[2], JOSEP SANTALÓ[2], ARMAND SÁNCHEZ[1], JOSEP M FOLCH[1]

*[1]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Departament de Biologia Cellular, Fisiologia i Immunologia, Facultat de Ciències, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain and [3]Division of Gene Expression and Development, Roslin Institute, Roslin EH25 9PS, Midlothian, UK*

Eight lines of transgenic mice for the goat *β-lactoglobulin* gene *(βLG*), comprising a 410 bp-long promoter, were generated to study the effect of promoter size on transgene expression, resulting in high levels of expression in a position independent manner. Homozygous animals for the transgenic line Tg56 showed a distinct phenotype consisting in retarded growth, high mortality after birth and infertility in females. This line contained 22 copies of the transgene integrated in a single genomic position and expressed the goat *β-LG* at high levels in the mammary gland. In the present work, we have analysed the transgene integration site to verify if the phenotype was produced by the disruption of a specific mouse gene. The "Vectorette system" (Genosys), which is based on the Anchored PCR technique, was used to isolate and sequence the flanking regions of the transgene integration site. The 22 copies of the goat *βLG* are integrated in the intron 30 of the *Phospholipase C-β1* gene (*PLC-β1*). This gene is normally expressed at high levels in the mouse brain and its protein is implicated in the transmembrane signal transduction, catalyzing the hydrolysis of phosphoinositide 4,5-biphosphate. An expression analysis of the *PLC-β1* gene in the Tg56 line by RT-PCR and sequencing, showed that an hybrid mRNA is transcribed. This hybrid mRNA contains a truncated mouse *PLC-β1* gene (from exon 1 to 30) fused to all seven exons of the goat *βLG* gene (including the polyadenylation signal).

## A003
### Cytogenetic studies on Assam local cattle(Bos Indicus) and Jersey bulls(Bos Taurus)

ANJANA CHOUDHURY, R.N. GOSWAMI, D. DAS

*Animal Husbandry & Veterinary Department, Govt. of Assam; Guwahati, Assam, India*

Studies on chromosomal architecture, karyotyping of chromosomes and chromosomal abnormalities , if any in 30 Assam Local cattle ( 15 males and 15 females ) and 12 Jersey bulls were undertaken.

The diploid number of chromosomes was 60 irrespective of genetic groups and sexes. All the 58 autosomes were acrocentric and X-chromosomes were submatacentric in both the genetic groups. Existances of a distinct morphological difference in Y-chromosome between Assam Local male and Jersey bulls was confirmed. The mean relative length of chromosomes with their standard errors and coefficient of variation for both local cattle and jersey bulls were determined. No major chromosomal abnormalities could be detected in animals of either genetic groups under study.

For Correspondence:
Dr.(Ms.) Anjana Choudhury C/O. Dr. B.N.Saikia ,Assoc. Prof., Deptt. Of Animal Nutrition,
College of Vety.Science, AAU, Post Box No.15, Khanapara, Guwahati-781022

**A004**

**Study of expressed sequences during somite and limb development in chicken (Gallus gallus)**

ERIKA C. JORGE, CLARISSA S. SILVA, CLAUDIA B. MONTEIRO-VITORELLO, MATEUS PATRÍCIO, LUIZ L. COUTINHO
*Animal Production Departament – ESALQ - USP - Piracicaba – Brazil*

Embryonic development is marked by an intricate succession of events that controls cell division, determination and differentiation. We are particularly interested in the processes that control myogenesis. Expressed Sequence Tags (ESTs) allow determination of gene expression profiles in any particular tissue under different conditions or status. In order to identify genes important for muscle formation, two cDNA libraries from somite and limb were prepared. Somites, containing neural tube and notochord, were dissected from 290 stage 15 (E15) H&H embryos. Limb tissue was dissected from 60 embryos in three different stages of development (E21, E24 and E26, H&H). mRNA was selected from total RNA and converted into double-stranded cDNA by SuperScriptRT II and oligo (dT) primer. A directional library was constructed and cloned in pSPORT 1 vector. Clones were sequenced from its 5' end, using Big Dye Terminator Kit (Applied Biosystems - Perkin Elmer) and T7 primer. Sequences were analyzed with Phred/Phrap/Consed and Cap3. A total of 2328 clones from the limb library resulted in 527 singletons and 485 contigs while the 2383 clones from the somite library resulted in 891 singletons and 411 contings. Chicken ESTs are highly homologous (over 70% identity at protein level) to humans and rodents. Our data allowed identification of growth factors (TGF-β, HGF, FGF-receptor), transcription factors (Hox genes, Pax3), cell cycle regulators (cyclins) and several muscle specific genes.

**A005**

**Recombinant Bovine Growth Hormone and Peptidylglycine Monooxygenase Activity**

PETER CREIGHTON, JOHN DONLON
*Department of Biochemistry, National University of Ireland, Galway, Ireland*

Carboxy-terminal amidation is a frequent post-translational modification required for biological activity and stability of many bioactive peptides. About half of all known neuropeptides and bioactive peptides are C-terminally activated. Peptide amidation, in vivo, is a two-step process with hydroxylation of a glycine-extended peptide followed by its dismutation to release glyoxylate with retention of the α-carbon glycine as the amide nitrogen. Peptidylglycine monooxygenase (PGM) is the enzyme involved in the first step of the amidation process and the second step is catalysed by the enzyme peptidylamidoglycolate lyase (PAL). Previous studies have shown that PGM isolated from bovine pituitary is identical to somatotrophin or growth hormone (GH). Since commercially produced recombinant bovine growth hormone does not show amidation activity, we set out to express and purify recombinant bovine growth hormone using non-denaturing methodologies that allow retention of the enzyme activity of GH. Our studies confirm that recombinant bovine growth hormone expressed in *E.coli* shows peptidylglycine monooxygenase activity.

**A006**

**Sampling gene expression from mammary tissues by EST sequencing of ORESTES cDNA libraries from Holstein and Gir cattle.**

ADILSON F. DA MOTA[1,2,3], TAD S. SONSTEGARD[1], CURTIS P. VAN TASSELL[1], ERIN E. CONNOR[1], ANTHONY V. CAPUCO[1], MARIA APARECIDA P. BRITO[2], MARCO A. MACHADO[2], MARIO L. MARINEZ[2], LUIZ L. COUNTINHO[2]
[1]*United States Dept. Of Agriculture, Agricultural Research Service, Beltsville, USA;* [2]*EMBRAPA Dairy Cattle Center, Juiz de Fora, Brazil;* [(3)]*University of Sao Paulo, ESALQ, Piracicaba, Brazil*

Even though more than 230,000 bovine expressed sequence tags (EST) are available in public databases, additional EST are needed. The objective of this study was to generate EST to better characterize the mammary gland transcriptome. Construction of cDNA libraries was performed using the open reading frame EST

(ORESTES) method to avoid the transcript bias inherent to mammary and to maximize discovery rate of novel EST. ORESTES relies on arbitrarily primed RT-PCR to generate a partial expression profile that can be shotgun cloned. In a preliminary study, six libraries were constructed using mRNA from pre-pubertal mammary and primers that amplify the estrogen receptors. Sequencing of 576 clones generated 455 GenBank quality submissions. Sequence assembly was used to assess rate of clonal redundancy relative to each RT-PCR primer and provide tentative consensus (TC) sequences for BLAST analysis. A total of 64 TC sequences were assembled from three libraries leaving 178 singletons and an average redundancy rate of 47%. BLAST analysis of the 242 unique sequence elements revealed 81 sequences (17%) that had no match to GenBank nt and 147 (32%) were novel relative to cattle. Because ORESTES produced a high rate of novel sequences, this method will be exploited to generate mammary-derived EST from Holstein- and Gir-derived libraries. The resultant breed specific polymorphisms and novel sequences will be a valuable resource for understanding gene expression differences between breeds.

**A007**

**Sequencing and comparison of total brain cDNA Libraries of White Leghorn chicken and Red Jungle fowl**

CAROLYN J. FITZSIMMONS[1], PETER SAVOLAINEN[2], GÖRAN HJÄLM[1], JOAKIM LUNDEBERG[2], LEIF ANDERSSON[1]
*[1]Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala Sweden; [2]Royal Institute of Technology, Department of Biotechnology, Stockholm, Sweden*

We report the sequencing and comparison of two total brain and two testis cDNA libraries from White Leghorn chicken (WL) and Red Jungle fowl (RJ). The aim of the project has been to generate a large number of sequenced cDNA clones that can be used for gene expression analysis using cDNA arrays. The detection of new SNPs supplements our ongoing QTL mapping project which consists of a cross between the White Leghorn and Red Jungle Fowl. A total of 25824 clones were sequenced from all 4 libraries, and after removal of low quality sequence, E. coli, vector, and mitochondrial contamination, 16667 sequences were assembled in Phrap. 6209 singlets were identified, and 10458 sequences were assembled into 3055 contigs (9264 unique sequences). Of these contigs, 581 were single reads. 746 SNPs and 37 insertions/deletions were tentatively identified in 479 contigs, and it was calculated that on average 1.4 SNPs were found for every 1000 bp of good quality, aligned sequence. BLAST analysis of the 9264 unique sequences against the UK chicken EST database (http://www.chick.umist.ac.uk/), using a significance threshold of E = e30, revealed 7070 hits (76.3%) and 2191 no hits (23.7%). The 2191 sequences that did not have a significant hit in the UK database were BLASTed against NCBI nr and est databases, and it was found that 660 had a significant hit (E = e30), while 1531 sequences (16.5% of the total unique sequences that were originally BLASTed), remained unidentified.

**A008**

**Determination of RNaseH accessible sites along the Sus scrofa *FUT1* and *FUT2* RNA sequences for design of efficient antisense oligonucleotides**

ANGELIKA GABLER, STEFAN KREBS, MARTIN FÖRSTER
*University of Munich, Institute of Animal Breeding, Munich, Germany*

Diarrhea in suckling piglets is supposed to be in part due to adhesion of Escherichia coli F18 to antigenic structures on the surface of small intestine mucosa cells engineered by the products of the *FUT* genes. Animals lacking specific *FUT* alleles are thought to be resistant to E.coli F18 colonization. Temporary blockade of the *FUT* gene product synthesis should therefore circumvent E.coli F18-related intestinal troubles. Thus we looked for a strategy to find targets for antisense oligonucleotide annealing along the *FUT* RNA sequences. To do so, RNA was transcribed in vitro and incubated with short, denatured DNA fragments obtained after DNase-digest of PCR-generated fragments corresponding to the RNA sequences. Then RNase H was added in order to cleave the RNA at sites where DNA-RNA-hybrids have formed. The resulting RNA fragment pattern was analysed by MALDI-MS. The masses of all possible *FUT* sequence segments have been calculated in theory and compared to the measured peak masses from MALDI-MS. Because multiple fragments matched these conditions the analysis was further refined by subsequent RNase T1 digest of the initially generated RNA fragments. As RNase T1 cleaves RNA after each G the cleavage pattern of each of the possible *FUT* sequence segments could be calculated. Comparing these data to the measured RNase T1 digest spectra allows for determination of the underlying sequence segment. Thus by knowing the sequences of RNase H cleavage sites efficient antisense oligonucleotides can be constructed.

**A009**

**Validation of sperm sexing in Bos taurus by dual color Fluorescence in situ Hybridization (FISH)**

FELIX A. HABERMANN[1], INGRID OLSAKER[2], PETER REICHERT[3] , RUEDI FRIES[1]

*[1]Technical University of Munich, Chair of Animal Breeding, Freising-Weihenstephan, Germany; [2]The Norwegian School of Veterinary Science, Department of Morphology, Genetics and Aquatic Biology, Oslo, Norway and [(3)] Big-X AG, Seewen-Schwyz, Switzerland*

Separation of X- and Y-bearing sperm cells and artificial insemination with sex-specific semen makes it possible to pre-determine the sex of calves. This has the potential to considerably improve cattle breeding, genetic resource management and particularly the efficiency of dairy and meat production. The broad use of sexed semen, however, will depend on availability, price, fertilizability and the actual sorting purity of sperm doses. To validate the accuracy of sperm sexing in *Bos taurus* a simple, fast and reliable dual color FISH test has been developed: Y-bearing spermatozoa are identified by hybridizing a DNA fragment that recognizes a repetitive DNA block on the bovine Y chromosome. Simultaneously a second DNA probe identifying a repeat block on the bovine autosome 6 is used as indicator of sufficient accessibility of the sperm DNA and adequate hybridization efficiency in each spermatozoon. Both DNA probes can be amplified and labeled effectively by PCR and provide specific and clear signals even after short incubation without the need of competitor DNA. The procedure was evaluated using the established Beltsville sperm sexing technology that separates X- and Y-bearing sperm cells by flow-sorting according to their DNA content.

**A010**

**Development of a characterized cDNA resource in Atlantic salmon (*Salmo salar*)**

LARS-ERIK HOLM[1], FRANK PANITZ[1], BJØRN HØYHEIM[2], HEIDI HAGEN-LARSEN[2], JOHN TAGGART[3], MARGARET CAIRNEY[3], GRACE C. DAVEY[4], SARAH A. MARTIN[4], NICOLE C. CAPLICE[4], RICHARD POWELL[4], CHRISTIAN BENDIXEN[1]

*[1]Danish Institute of Agricultural Sciences, Department of Animal Breeding and Genetics, Tjele, Denmark; [2]Norwegian College of Veterinary Medicine, MGA - Genetics, Oslo, Norway; [3]University of Stirling, Institute of Aquaculture, Stirling, Scotland; [4]National University of Ireland, Department of Microbiology, Galway, Ireland*

Atlantic salmon is one of the most important aquacultural fish species with a yearly production of more than 600,000 tons (year 2000) in the European countries. The importance is reflected in the increased interest in the molecular evolution and genetics of the species. Genetic maps of several salmonid species are currently being constructed. However, the number of known gene sequences in the public databases is highly limited. The aim of the current project (SALGENE, EU FAIR-CT98-4314) was to increase the number of known Atlantic salmon ESTs considerably. Non-subtractive and subtractive cDNA libraries from nine different tissues (kidney, liver, gill, intestine, brain, muscle, testis, ovary, and spleen) were constructed. A total of more than 15,000 EST sequences has been obtained, adding substantially to the number of known ESTs in Atlantic salmon. All obtained sequences were analysed by the BLASTN/BLASTX programs against the public genomic and EST databases to identify the corresponding genes. Also, more than 400 candidate SNPs were identified. The cDNA clones comprise a valuable resource for the future construction of cDNA microarrays with the purpose of gene expression analysis to enable identification of differentially expressed genes from individuals exposed to different treatments. This will facilitate the future identification of genes underlying potential important QTLs in Atlantic salmon.

**A011**

**Diagnoses of blood chimerism between twins in cattle using quantiative PCR**

HANNES W. JOERG[1], MIKA ASAI-COAKWELL[1], FREDI JANETT[2], DARIA A. GRAPHODATSKAYA[1], DAGMAR STEIGER[1], GERALD F. STRANZINGER[1]

*[1]Swiss Federal Institute of Technology, Institute of Animal Sciences, Zurich, Switzerland; [2]University of Zurich, Faculty of Veterinary Medicine, Zurich, Switzerland*

In cattle, genomic DNA for parentage control is commonly extracted from blood samples. However, in cases of blood chimerism, the detected alleles may interfere with parentage control. Blood chimerism is also associated with freemartinism, where a female calf twinned to a male calf is sterile. In cattle, 92% of females born as a twin of a male calf are freemartins. Using a multiplex TaqMan® assay, DNA from 6 pairs of chimeras of both sexes were analysed through quantitative PCR. The relationship between the amplification of a SRY fragment and an autosomal gene (MSHR) fragment was used to calculate the male cell proportion. Our results showed amplification

of SRY with differing quantities in all 6 females. The efficiency of the MSHR and the SRY amplification was different, so that a correction by difference of the mean values was performed. Nevertheless the quantification of the proportion of male cells showed high variation and even proportions above 100%. Seventeen samples of leukocyte cultures of male twins were analysed through quantitative PCR and compared with their corresponding male and female metaphase spread counts. The correlation between the results of the quantitative PCR and the chromosome typing was 0.3. To quantify the proportion of male to female cells in different samples is not very accurate, especially in samples with few female cells. Therefore it is difficult to use quantitative PCR systems to detect chimerism in random samples.

**A012**

**Toward a comparatively anchored, sequence-ready whole genome physical map of the cattle genome**

DENIS M. LARKIN[1], JACQUELINE SCHEIN[2], CHERYL GREEN[1], THOMAS R. DEKOJ[1], SHARON BACHMANN[1], PETER SCHWEITZER[1], MARK REBEIZ[1], ANNELIE EVERTS-VAN DER WIND[1], STEVEN JONES[2], IAN BOSDET[2], CARRIE MATHEWSON[2], NATASJA WYE[2], READMAN CHIU[2], STEPHEN MOORE[3], BERNHARD BENKEL[4], JOHN W KEELE[5], STEVEN M. KAPPES[5], MARCO MARRA[2], PIETER DE JONG[6], JAMES E. WOMACK[7], HARRIS A. LEWIN[1]

*[1]University of Illinois at Urbana-Champaign, Urbana, IL, US; [2]British Columbia Cancer Agency Genome Sciences Centre, Vancouver, BC, Canada; [3]University of Alberta, Edmonton Alberta, Canada; [4]Agriculture and Agri-Food Canada, Lethbridge Research Center, Lethbridge, Alberta, Canada; [5]USDA-MARC, Clay Center, NE, USA; [6]Children's Hospital Oakland Research Institute, Oakland CA, USA, [7]Texas A & M University, College Station, TX, USA*

An international consortium has been formed in order to create a comparatively anchored bacterial artificial chromosome (BAC) map of the cattle genome. To date >130,000 clones have been fingerprinted from the CHORI-240 and RPCI-42 cattle BAC libraries. A total of 14,844 contigs have been identified. BAC-end sequencing has been performed on >10,000 ends. The fingerprinting and end-sequencing results are being integrated to produce a comparatively-anchored whole-genome physical map. The cattle BAC-end sequences are being anchored to the human genome by BLASTN similarity search against the draft human genome sequence. Approximately 20% of BAC-ends have significant BLASTN hits against non-repetitive segments of the human genome. These sequences are termed comparatively anchored sequence tagged sites (CASTS). The COMPASS III program is used to predict the chromosome map location and position of CASTS in the cattle genome. For each CAST the predicted cattle genome position is determined computationally in a look-up table that contains the current cattle-human comparative map. This table relates all positions in the human genome to all known positions in the cattle genome on the basis of a previously published whole-genome radiation hybrid-based cattle-human comparative map. Using this approach it is possible to scaffold up all of the contigs with multiple CASTS on the cattle chromosomes thus enabling the creation of a whole genome contig that reveals fine details of chromosome rearrangements.

**A013**

**Production and Characterization of Chicken-Duck Chimeras**

ZANDONG LI, JIN SHA, SHENGHUA JIANG, MENG JIANG, CHUNHAI LIU, JINSONG HUANG, NING WANG

*Department of Biochemistry and Molecular Biology, College of Biology, China Agricultural University, Beijing 100094, P. R. China*

Avian blastodermal cells at stage X are pluripotent, they are capable of differentiating and developing into various cells including germline cells.

In this study, blastodermal cells isolated from fertilized White Leghorn eggs at stage X were labeled or not with PKH26, and microinjected respectively into subgerminal cavities of Maya Duck eggs at the identical stage. In the PKH26-labeled group, gonads of 8-days-old embryos were examined under a fluorescent microscope, and PKH26 positive cells could be observed in 30 out of 71 embryos (42.3 %). In the unlabeled group, brains and main visceral tissues isolated from the treated embryos, were sectioned and detected by in situ hybridization using W chromosome-specific repetitive sequences of chicken as DNA probe, the donor-derived cells could be found in all these tissues, with the chimeric rate highest in liver at 40.9 %, lesser in muscular stomach, kidney, heart and brain, and lowest in gonad at 11.4 % (5/44).

The results showed that the exogenous chicken blastodermal cells could incorporate into the development of duck embryos, especially in the germinal system. It suggested it was possible to yield the germline chimera by the method of blastodermal cells transplantation.

**A014**

**Construction of a 3.5X genome-coverage physical map of the porcine genome by BAC fingerprinting**

BRANDY M. MARRON AND JONATHAN E. BEEVER

*University of Illinois, Department of Animal Sciences, Urbana, Illinois, USA*

The identification of genes that influence economically important traits or ETL, has been the driving force for the development of genome maps in our agricultural species. To this end, the genome map of the pig contains sufficient genetic markers to conduct genome scans for these ETL. As a result of these studies, we are finding an increasing need to fine-map regions of the genome for the eventual identification of the underlying gene or polymorphism causing the effect. Restriction endonuclease fingerprinting of large-insert clones, such as BACs, has been utilized in the production of high-resolution genome-wide physical maps in organisms such as *C. elegans*, humans and mice. The RPCI-44 BAC library, consisting of approximately 10.2X genome equivalents, was used to construct a physical map of the porcine genome by fingerprinting. DNA from approximately 70,000 BAC clones was isolated and digested with *Hin*d III, followed by separation of the fragments by agarose gel electrophoresis and visualization by fluorescence imaging using SBYR Green I nucleic acid stain. Gel images were analyzed using IMAGE and fingerprints were assembled into contigs using FPC. This high-resolution physical map will provide the foundation for fine-mapping of ETL and is the initial step towards a complete high density, sequence ready map of the porcine genome.

**A015**

**A BAC/PAC contig of the porcine *RYR1* gene region on SSC 6q1.2 and comparative analysis with HSA 19q13.13**

FLÁVIA MARTINS-WEß[1], RODJA VOß-NEMITZ[1], CORD DRÖGEMÜLLER[1], BERTRAM BRENIG[2], TOSSO LEEB[1]

[1]*School of Veterinary Medicine Hannover, Institute of Animal Breeding and Genetics, Hannover, German;* [2]*University of Göttingen, Institute of Veterinary Medicine, Göttingen, Germany*

To generate a detailed physical map of the ryanodine receptor 1 gene (*RYR1*) region on SSC 6q1.2 a porcine bacterial artificial chromosome (BAC) and a P1 derived artificial chromosome (PAC) library were screened resulting in a sequence-ready ~1.2 Mb BAC/PAC contig. For the library screenings several heterologous probes from the extensively characterized human syntenic region on HSA 19q13.13 were used as starting points. By a chromosome walking strategy gaps between clones could then be closed and a single contig of 59 clones was obtained. During the construction of the contig 54 new sequence tagged site (STS) markers were generated. Detailed physical mapping of this gene-rich region allowed the assignment of 17 porcine genes orthologous to known human chromosome 19 genes to this contig. Except for the relatively well characterized porcine *RYR1* gene the other 16 genes represent novel chromosomal assignments and 14 genes have been cloned for the first time in pig. Comparative analysis of the porcine BAC/PAC contig with the human HSA 19q13.13 map revealed a completely conserved gene order of this segment between pig and human. The compilation of a detailed porcine-human comparative map might help to resolve existing discrepancies between the currently available human HSA 19 maps.

**A016**

**A putative gene therapy vector for prevention of transmisible spongiform encephalopathy (TSE)**

JAVIER MIANA-MENA[1], JESÚS CIRIZA[1], JULIO POZA[1], INMACULADA MARTÍN-BURRIEL I.[1,2], MARIA JESÚS MUÑOZ[3], CLEMENTINA RODELLAR[1], PILAR ZARAGOZA[1], ROSARIO OSTA[1]

[1]*Universidad de Zaragoza, Laboratorio de Genética Bioquímica y Grupos Sanguíneos, Zaragoza, España;* [2]*Centro Nacional de Encefalopatías Espongiformes, Zaragoza, España;* [3]*Universidad de Zaragoza, Departamento de Farmacología, Zaragoza, España*

The Transmissible Spongiform Encephalopathies (TSEs) are fatal, neurodegenerative diseases for which no effective treatments are available. Several studies have suggested that gene therapy with mutant PrP may be effective in preventing TSE diseases. Moreover certain antibodies anti-PrP have recently been shown to block *in vitro* prion replication and disemination. We have considered that specific systems to deliver this therapeutic agents to the neurons should be developed. *In vivo* gene transfer has been explored as an invasive method to deliver enzymatic activities to the brain using viral vectors, which are efficient in transducing cells. However safety concerns regarding the use of virus in humans make non viral delivery systems an attractive alternative. Fragment C of tetanus toxin (TTC) retains specific nerve cell binding and transport properties of the holotoxin, lacking any toxicity. At this work we have used the naked DNA encoding ß-galactosidase-TTC

hybrid protein to transfect muscle cells *in vivo*. An intramuscular injection of naked DNA resulted in a selective gene transfer of the enzymatic activity to the motoneurons and their connections. Labelling in the motoneurons and motor cortex was observed from 4 days postinjection whereas the β-galactosidase expression was maintained for 60 days. We propose a gene therapy approach based on TTC hybrid proteins which appears to be a feasible method of delivering a biological function to the CNS for TSE therapy.

**A017**

**Development of a novel homologous promoter for expression of a recombinant glycoprotein in Chinese Hamster Ovary cells.**

CATHY NOONE, TERRY SMITH, MICHAEL CAIRNS
*National University of Ireland, Galway*

Globally there is widespread use of CHO cell culture in the production of recombinant proteins. The advantage of the CHO system is its ability to achieve authentic glycosylation of products, unlike bacterial, fungal or yeast systems. Without this post-translational modification recombinant products do not mimic the natural molecule. The aim of this project is to improve upon current high-level expression systems by the replacement of viral promoters with homologous CHO promoters. A CHO genomic library was screened with a number of different probes derived from cDNA's of genes known to be highly expressed in CHO cells. One clone of 7Kb has been fully sequenced and analysed by web-based promoter analysis programs (e.g. TESS). Promoter fragments have been cloned into the pGL3-Basic reporter vector and characterization of these, in comparison to viral SV40 and CMV promoters, has been carried out using a luciferase reporter assay. The promoter is currently being incorporated into a two-vector system expressing the bovine follicle stimulating hormone genes, with the aim of producing and purifying recombinant FSH, which can be used for superovulation treatment of cows.

**A018**

**Construction of a cDNA library from preimplantation cattle embryos**

INGRID OLSAKER[1], KRISTINE G. GAUSTAD[1], WENCHE FARSTAD[2], ELISABETH KOMMISRUD[3]
[1]*Department of Morphology, Genetics and Aquatic Biology, The Norwegian School of Veterinary Science, Oslo, Norway,* [2]*Department of Reproduction and Forensic Medicine, The Norwegian School of Veterinary Science, Oslo, Norway,* [3]*GENO, Hamar, Norway*

A group of *in vivo* produced preimplantation cattle embryos frozen during 1992-1993 by GENO, the Norwegian cattle breeding and AI association, was in 1999 released for research. Twenty of these embryos, representing the stages from morula to expanded blastocyst were chosen for construction of a cDNA library. The pool of 20 embryos constituted about 5000 cells in total. This yielded a fairly low amount of mRNA and the library was constructed by utilizing the „PCR cDNA Library Construction Kit" from Stratagen. Obtained average insert size is 800-900 basepairs. Initially 8000 clones were gridded in microtiterplates, and 2300 clones have at present been sequenced. About 30% of the sequenced clones are unique and the rest (70%) are represented by on average, 4-5 copies. Preliminary homology searches (BLAST) with a subset of the sequences revealed that 20% contains SINE and / or LINE repeats and 30% gives match to known genes and / or ESTs (mainly human and bovine). The rest seems to represent not yet described genes, but homology searches against the human genome sequence has not yet been performed. This library should constitute a valuable resource for identification of genes involved in the early stages of cattle development. Updated results will be presented.

**A019**

**Generation of dominant-negative murine myostatin alleles**

DIMITRI PIROTTIN, LUC GROBET, DOMINIQUE PONCELET, LUIS ROYO, BENOÎT BROUWERS, MICHEL GEORGES
*Department of Molecular Genetics, Faculty of Veterinary Medicine, University of Liege, B43, 20 Boulevard de Colonster, 4000, Liege, Belgium*

Loss of myostatin function in the mouse and in several cattle breeds leads to a dramatic increase in skeletal muscle mass. Myostatin is a member of the TGF-beta superfamily of proteins which are secreted as disulfide-bounded polypeptides and characterized by a proteolytic processing site thought to mediate cleavage of the bioactive C-terminal domain from the N-terminal latency-associated fragment. All loss of function mutations identified in cattle are recessive. Our aim is to produce potentially dominant-negative variants of myostatin and to test them by a transgenic approach using homologous recombination in ES cells. Four regions of the protein were targeted : the proteolytic cleavage site, the putative glycosylation site, a residue potentially involved in receptor binding and a

highly conserved sequence of 8 amino-acids in the latency associated fragment. In order to increase the efficiency of the recombination events, we have generated a murine ES cell line carrying a floxed myostatin allele allowing repeated Cre-recombinase mediated cassette exchange. However, because of the relatively high efficiency of homologous recombination at the myostatin locus compared to a very inefficient recombinase mediated cassette exchange, all mutated ES cell clones were generated by classical homologous recombination. Germline chimeras have been produced from ES cell-diploid embryo aggregation experiments for two of the four variants until now and phenotypes of F2 mice are being analysed.

**A020**

**A second generation EST radiation hybrid comparative map of the Porcine genome**

ANETTE RINK[1], ELISABETH M. SANTSCHI[2], KIM A. VONNAHME[3], KATIE E. EYER[1], BENJAMIN M. ROELOFS[1], KATHLEEN J. SHARKEY[1], SUDKAMON LEKHONG[1], ERIN F. MURPHY[1], STEPHEN P. FORD[3], MARTINE YERLE[4], DENIS MILAN[4], CRAIG W. BEATTIE[1]

*[1]Department of Animal Biotechnology, University of Nevada, Reno, NV 89557, USA, [2]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, USA; [3]Department of Animal Science, University of Wyoming, Laramie, WY 82071, USA; [4]Institut National de la Recherche Agronomique, Laboratoire de Genetique Cellulaire, Castanet-Tolosan, France*

Expressed Sequence Tag (EST) based radiation hybrid maps are powerful tools for QTL identification or positional candidate approaches. We have constructed a second-generation EST radiation hybrid comparative map of the porcine genome by ordering >2,000 ESTs from immune tissues and placentae on the $IMpRH_{7000}$ panel and identified the respective location for each human orthologue. Chromosomal maps were constructed with a 2pt LOD of 6 or 8. The number of observed/expected markers per chromosome ranged from 0.34 for SSCX to 2.06 for SSC12, respectively. Average marker retention frequency was 30.3%. Annotated ESTs represent 47% of the markers, 30% ESTs showed homology to ESTs from a variety of species, 20% represent novel sequences (ns) with no significant match in any nucleotide or protein database, 3% of the ESTs only identify open reading frames (ORFs) in human genomic sequence (HGS). More than 20% of the ESTs assigned to the physical map of the pig did not find a homologous sequence in V28 the most recent human genome assembly (Dec 24th, 2001). This is indicative of assembly gaps in the HGS. The comparative map covers 3,290MB, or 98% of the presumed size of the human genome. Six porcine chromosomes, SSC2, 5, 6, 7, 12 and 14 are syntenic with the three gene-richest human chromosomes, HSA17, 19 and 22. Pig chromosomes 1, 8, 11 and X display a low DNA/marker ratio are syntenic with the 'genome deserts' HSA18, 4, 13 and X.

**A021**

**Construction of a canine BAC-library compatible with PCR analysis**

CLAUDE SCHELLING[1], JÖRG SCHLÄPFER[2], ALAIN BILLAUT[3], KARINA GUZIEWICZ[2], BENITA PINEROLI [1], ANDREAS GMÜR[2], IRIS KATMANN[2], OLIVER RICKLI[2], CHRISTINE WITTWER[2], ANGNIESZKA PIASECKA[1], BRIGITTA COLOMB[2], GAUDENZ DOLF[2]

*[1]Federal Institute of Technology and Faculty of Veterinary Medicine, Department of Animal Sciences, Zurich, Switzerland, [2]University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, Switzerland; [3]Molecular Engines Laboratories, Paris, France*

A canine Bacterial Artificial Chromosome (BAC) library was constructed from a male white shepherd. Approximately 96'768 clones were stored in 96-well microtiter plates and aliquots were organized in a three-dimensional pooling system to prepare DNA ready for screening by PCR. The 42 superpools each contained 24 plates of 96 clones, that is, a total of 2'304 clones. Each superpool is represented by 8 row pools, 12 column pools and 24 plate pools. The screening of the library by PCR requires a total of 86 reactions plus appropriate controls. This two-step procedure involves an initial screening of 42 superpool DNAs, followed by screening of 24 plate pool DNAs, 8 row pool DNAs and 12 column pool DNAs of the initially identified superpool. The library was screened with 111 microsatellites representing all canine chromosomes. Eighty-six primer sets (85 %) yielded between 1 and 7 positive clones. In order to increase the genomic coverage the library will be expanded by adding another 30 superpools representing different canine genomic insert sizes. Interested researchers can access the library following the rules published at http://www.dogmap.ch.

**A022**
**Recombineering of Porcine BACs for Somatic Cell Genomics**

MARGARITA B. ROGATCHEVA[1], LAURIE A. RUND[1], JONATHAN E. BEEVER[1], CHRISTOPHER M. COUNTER[2], LAWRENCE B. SCHOOK[1]
[1]*University of Illinois, Department of Animal Sciences, Urbana, Illinois;* [2]*DukeUniversity Medical Center, Department of Pharmacology and Cancer Biology, Durham, North Carolina, USA*
In current model species, functional genomics is supported by the ability to develop individuals possessing specific modifications either by deletion or substitution of genes. In pigs, the tools required to create such animals are lacking. Thus, there is a need to develop *in vitro* correlates that will allow the validation of hypotheses with respect to allelic variation and gene-gene interactions associated with multigenic traits. It also would be ideal that such an experimental system allowed the introgression of "alleles" into germplasm. Using the recombigenic *E. coli* strain DY380, we have rapidly generated multiple genetic modifications in BAC clones containing the porcine myostatin gene. Single-stranded oligonucleotides and PCR-derived DNA fragments were used for targeting and generation of a point mutation, a 68-bp deletion, and a 24-bp in-frame insertion of the sequence encoding the FLAG octapeptide into exon 3 of pig myostatin. Modified BACs are identified by allele-specific PCR amplification from pooled bacterial cells. Individual targeted clones were identified within positive pools and sequenced to confirm the mutations. The observed rate of modification is one per 170 to 400 surviving cells. Constructs generated by this approach can be used to develop specifically modified somatic cells for gain-of-function [knock-in including either new functions or allelic substitution] or loss-of-function [knock-out] studies. The promise of such an approach in cell culture [without the complexity of whole animal models] is the ability to link genetic variation with rigorous genetic analyses [e.g., microarray expression profiling] to assess gene function.

**A023**
**Establishment of the procine radiation bybrid panel and construction of the framework map**

HIDEAKI SUZUKI[1]; AYUMI MIKAWA[1], TAKEYA MOROZUMI[1], TADAYOSHI MITSUHASHI[2], NORIYUKI HAMASIMA[1]
[1]*STAFF-institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki, 305-0854, Japan,* [2]*National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602, Japan*
We are carrying out EST analysis produced from porcine back fat cDNA. Assignment of those ESTs to the porcine map will facilitate comprehensive mapping and construction of a comparative map based on the human information. Radiation hybrid (RH) panel is the most convenient and efficient tool for mapping non-polymorphic markers, such as EST. By fusing normal porcine aortic endothelial cells (Cell Systems Corp. WA, USA), which were irradiated with 7000 to 8000 rads, with recipient mouse cells, L-M (TK-)(ATCC; CCL1.3), we established 110 hybrid cell lines. Among 1091 microsatellite (MS) markers investigated for construction of framework map, we could type 842 markers (77 %) based on the 110 clones. According to the typing data, we built a framework map including 390 MS markers with RHMAPPER-1.22 Z-extension program (Stein, Kruglyak, Slonim, Lander, 1995, Whitehead Institute/MIT Center for Genome Research). After integrating additional 240 MS markers to the framework map (placement map), this RH maß was comprised of 630 MS markers. The estimated average retention frequency of the RH panel was 30.6 %. We are presently mapping porcine back fat ESTs on the framework map.

**A025**
**Construction and characterization of a 15 fold redundant BAC library of *Salmo salar* (Atlantic salmon)**

JIM THORSEN[1,4], WILLIAM S. DAVIDSON[2], BEN KOOP[3], BJØRN HØYHEIM[1] & PIETER J. DE JONG[4]
[1]*Norwegian School of Veterinary Sciences, Oslo, Norway,* [2] *Simon Fraser University, Burnaby, Canada* [3]*University of Victoria, Victoria, Canada* [4]*BACPAC Resources, Children's Hospital of Oakland Research Institute, Oakland, USA*
BAC (Bacterial Artificial Chromosome) libraries have been an important and essential resource for genome studies, such as sequencing of whole genomes, physical mapping and in reconstruction and positional cloning of genomic regions. The salmon BAC library was constructed using high molecular weight DNA prepared from sperm of a single anonymous male. The genomic DNA prepared was partially digested with EcoRI in the presence of EcoRI Methylase and separated by pseudo-double size fractionation using PFGE (pulsed-field gel electrophoresis). Size selected DNA was then electro-eluted and ligated to the EcoRI sites in the pTARBAC2.1 vector. Ligation products were transformed into electro-competent T1-resistant DH10B cells. A total of 300.000 clones, representing 15 fold genome coverage, have

been generated and arrayed into 384-well microtiter dishes, and spotted onto 22x22cm nylon high-density colony filters. Each hybridization membrane represents over 18,000 distinct salmon BAC clones, stamped in duplicate. The clones in the library have an average insert size ranging from 170 kb to 197 kb with a none-insert rate of 2-3%. More than 20 oligo probes, including micro-satellites and ESTs (expressed sequence tags), have been chosen for screening of the high-density filters to obtain BACs for use in further characterization, e.g. finger-printing analysis and contig assembly. The BAC library will be publicly available from BACPAC Resources.

**A026**
**Strategy for efficient isolation of microsatellites**

TERUAKI TOZAKI[1], SUGURU MASHIMA[1], SHUN-ICHI NAGATA[1], KEI-ICHI HIROTA[1], MASAHIKO KUROSAWA[1], TELHISA HASEGAWA[2]
*[1]Laboratory of Racing Chemistry, Utsunomiya, Japan; [2]Equine Research Institute, Japan Racing Association, Utsunomiya, Japan*

The linkage maps will serve as very useful tools for tracing genes governing economically significant traits. It is necessary to isolate many microsatellites for the purpose. In this study, we draw the strategy for efficient isolation of microsatellites. The strategy contains (I) construction of an enrichment library, (II) high speed sequencing, and (III) genotyping using so-called "pig tail PCR" with an adaptor sequence designed newly. The library was constructed by following; capture microsatellites from genomic DNA by hybridizing to biotin-conjugated probes, subsequent extraction with magnetic beads coated with streptavidin, nucleotide substrate-biased polymerase reaction, and amplification by PCR followed by cloning. Isolated microsatellites were treated from culture to sequencing with 96-well format. The genotyping method used three primers of a sequence-specific forward primer with the adaptor sequence at its 5' end, a sequence-specific reverse primer, and a fluorescent-labeled adaptor primer, and was performed with one reaction. In this study, we adapted the strategy to equine genome, resulting in isolation of 624 novel microsatellites. About 20% of the microsatellites showed some homologous groups, such as *ERE-1* and *ERE-2*. *MLRE* (*microsatellite-linked repetitive elements*) was characterized as a novel homologous group. The results proved the efficiency of our strategy in this study. Once applied, the strategy will accelerate construction of linkage maps in many species.

**A027**
**ChickRH6: a chicken radiation hybrid panel, and its use for improving the resolution of GGA7 and GGA14 comparative maps**

MIREILLE MORISSON, CARINE JIGUET-JIGLAIRE, THOMAS FARAUT, SUZANNE BARDES, KATIA FÈVE, MARTINE YERLE, ALAIN VIGNAL
*INRA, Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France*

Previous reports on the possibilities of generating a chicken whole genome radiation hybrid mapping panel by fusion with rodent recipient cells, were rather pessimistic due to low retention frequencies of chicken chromosome fragments in most clones. However, we were successful in developing a panel of 90 clones, by applying a drastic selection based on marker retention tested with 46 markers chosen genome-wide, over 452 hybrids produced by fusing female chicken fibroblasts irradiated at 6,000 rads to HPRT-deficient hamster cells. The average retention frequencies in this 90 clones panel, are of 22.0% for the whole genome, 20.1% for macrochromosomes and 25.7% for microchromosomes. To estimate the validity of the panel for mapping, we are currently constructing radiation maps of macrochromosome 7 (GGA7) and microchromosome 14 (GGA14). According to the state of the art of comparative maps, human genes located in the corresponding human regions (HSA2q, HSA16q13-3) are used to search, on a sequence similarity basis, for candidate orthologous chicken ESTs. These ESTs, likely to map to GGA7 and GGA14, are used to design primers for the mapping experiment on the radiation hybrid panel. This computer assisted primer design is part of a computer tool, ICCARE (Interspecific Comparative Clustering and Annotation foR ESTs), developed in our laboratory.

**A028**
**BAC map of the chicken genome**

JAN AERTS, RICHARD CROOIJMANS, SANDRA CORNELISSEN,
KAVEH HEMMATIAN, JAN VAN DER POEL, MARTIEN GROENEN
*Wageningen University, Animal Breeding and Genetics Group, Wageningen, The Netherlands*

The Wageningen chicken BAC library contains approximately 50,000 BACs, representing a 5.57-fold redundant coverage of the chicken genome. A physical map of BAC contigs is created using the fingerprinting technique. The BAC DNAs are digested with the *Hin*dIII restriction enzyme. The band patterns are scanned and loaded into the

Image program (Sanger Institute) to perform the band calling. To build contigs of these BACs, the resulting band data are imported into the FPC program (FingerPrinted Contigs, Sanger Institute). As in the Human Genome project, the resulting number of contigs is high. Therefore, the fingerprinting results are combined with the results of a marker screening of the BAC library. A large repository of microsatellite markers is available, with a known location on the linkage, cytogenetic or radiation hybrid map. Following a two dimensional PCR screening, BACs are identified that contain these specific markers. The contigs created by the fingerprinting can be positioned relative to each other by incorporating this new information. The 5' and 3' ends of the contigs will be sequenced and new markers will be constructed, followed by additional 2D PCR screening. This will allow for the anchoring of additional contigs on the existing maps of the chicken genome.

**A029**
**Characterization of 10,000 canine ESTs**

H. MURUA ESCOBAR[1], L. BORRMANN[1], R. NIMZYK[1], J. BULLERDIEK[1],
I. NOLTE[2]
*[1] Center for Human Genetics, University of Bremen, Bremen, Germany; [2]Clinic for Small Animals, Veterinary School, Hanover, Germany*

During the past years the dog has become a more and more interesting model organism for human diseases including cancer. Nevertheless, molecular genetic tools allowing a more advanced knowledge of canine molecular genetics are far behind of what is known for humans.
The representation of canine expressed sequence tags (ESTs) at the NCBI database reflects the current situation. The NCBI EST database contains currently (March 2002) roughly 11,000,000 entries covering all organisms. Compared to this number, the dog stays clearly underrepresented with just 7301 entries.
Herein we present first results of our program aimed at large scale sequencing of canine cDNAs. So far 10,000 cDNA clones have been sequenced thus almost doubling the number of available canine ESTs. Furthermore, the results reveal that based on the gene level the homology between men and dogs is much higher than estimated so far.

**A030**
**Comparative mapping and sequencing between chicken chromosome 28 and human chromosome 19p**

RICHARD CROOIJMANS[1], LAURIE GORDON[2], SANDRA CORNELISSEN[1], TARIK RABIE[1], JAN VAN DER POEL[1], MIREILLE MORISSON[3], ALAIN VIGNAL[3], LISA STUBBS[2], MARTIEN GROENEN[1]
*[1]Wageningen University, Animal Breeding and Genetics Group, Wageningen, The Netherlands; [2]D.O.E. Joint Genome Institute, Walnut Creek and Genomics Division, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California, USA; [3]Institut National de la Recherche Agronomique, Laboratoire de Génétique Cellulaire INRA, Castanet-Tolosan, France*

Extensive conservation of synteny is observed between the chicken and human genomes, which is particularly apparent for many of the small micro-chromosomes in chicken such as GGA28. Mapping of over 40 genes to chicken chromosome 28 indicates that this chicken chromosome over almost its entire length is syntenic to human chromosome 19p.
Thirteen BAC contigs covering over 7 Mbp of this chromosome have been constructed and aligned with the linkage and radiation hybrid maps. BAC contig construction was initiated from markers known to be located on chromosome 28 and extended by chromosome walking. Additional BACs and BAC contigs were identified using chicken EST sequences homologous to genes located on human chromosome 19p and by fingerprinting all BACs from the Wageningen chicken BAC library (see abstract of Aerts et al.). Verification of the chromosomal location of these additional BACs was done by two-colour FISH or by radiation hybrid mapping. This enabled a detailed alignment of GGA28 with human chromosome 19p and resulted in the identification of multiple rearrangements on this chromosome relative to human and mouse. Representative BACs that constitute a minimal tiling path have been selected for sequencing by the Joint Genome Institute in Walnut Creek, California, USA.

**A031**
**A BAC map covering 90% of chicken chromosome 10**

RICHARD CROOIJMANS[1], ROSILDE DIJKHOF[1], TINEKE VEENENDAAL[1], MIREILLE MORISSON[2], JAN VAN DER POEL[1], ALAIN VIGNAL[2]; MARTIEN GROENEN[1]
*[1]Wageningen University, Animal Breeding and Genetics Group, Wageningen, The Netherlands; [2]INRA, Laboratoire de génétique Cellulaire, Castanet-Tolosan, France*

Chicken chromosome 10 (GGA10), one of the

larger microchromosomes and approximately 30 Mb in size, is one of the best studied chromosomes in chicken. The genetic markers known to be located to this chromosome were used for BAC contig mapping. Additional BACs were identified by chromosome walking and using STS markers derived from chicken EST sequences. The chicken EST sequences were selected based on known GGA10-HSA15 conserved synteny. Map locations of these BACs were confirmed either by FISH, SNP typing in a reference population or by mapping on the chicken RH-panel. This resulted in the development of over 450 STS markers and the isolation of more than 900 BAC clones. To further increase the BAC coverage of this chromosome, BAC fingerprint information from Image and FPC of the Wageningen BAC library was used (see abstract Aerts et al.). These combinations of approaches resulted in the identification of BACs for GGA10 that cover 25 Mb of sequences, which is close to 90% of this chromosome.

**A033**

**Construction of a framework map of the Shirakawa/University of Nevada Reno bovine Radiation Hybrid (SUN-bRH7) panel**

TOMOHITO ITOH[1], TOSHIO WATANABE[1], NAOYA IHARA[1], CRAIG W. BAETTIE[2]; YOSHIKAZU SUGIMOTO[1]

*[1]Shirakawa Inst. Animal Genet., Fukushima, Japan; [2]University of Nevada, Reno, USA*

Whole-genome radiation hybrid (WG-RH) panels have widely been used for localizing genes and anchoring physical maps. In order to map bovine genes and ESTs, we have prepared a 7000 rad bovine-hamster WG-RH panel (Mariani et al, 27th ISAG). Here we report the characterization of the Shirakawa/University of Nevada Reno bovine Radiation Hybrid (SUN-bRH7) panel and provide an initial RH map of the bovine genome.

Retention frequencies of bovine chromosomes were estimated by Genescan analysis of microsatellite loci with an ABI 3700 DNA sequencer. The framework map was created using the RH2PT, RHMAXLIK program of RHMAP 3.0 with a LOD threshold of four. Finally, this order was used the create placement map option of RHMAPPER.

To construct an RH map providing comprehensive coverage of the bovine genome, we selected more than 1400 microsatellites from the USDA bovine linkage map and used to amplify the SUN-bRH7 panel comprising 92 hybrid clones. An average retention frequency of 19% was calculated with 1000 scorable markers. The RH map programs established linkage groups covering BTA1-29, X and Y at a LOD > 4.0. Assignment for each linkage group was based on the USDA bovine linkage map. Our initial map forms a frame for mapping bovine genes and ESTs.

**A034**

**A cost effective method for the production of small gene number canine macroarrays**

A. RICHTER[1], H. MURUA ESCOBAR[1], B. MEYER[1], K. BECKER[1], I. NOLTE[2], J. BULLERDIEK[1]

*[1]Center for Human Genetics, University of Bremen, Bremen, Germany; [2]Clinic for Small Animals, Veterinary School, Hanover, Germany*

DNA hybridisation arrays are one of the major trends in molecular biology. Covering a wide range of topics including cancer research, arrays are being used in gene expression profiling and genotyping. While the domestic dog (*Canis familiaris*) is supposed to join the company of model organisms for human disease, canine DNA arrays are still less common, often making the in-house production of custom arrays necessary. High-density DNA arrays allow the investigation of a large number of genes per experiment, but are relatively costly due to the technical equipment needed for their production and analysis of experimental data, thus limiting their availability for many laboratories. In this study, we evaluated the production of low cost, small gene number canine nylon arrays that can be made and analysed with common laboratory equipment. DNA of randomly chosen clones from a canine testis tissue cDNA library was amplified using different approaches, the resulting products (being whole plasmids, inserts isolated from plasmids and PCR products) hand-spotted on nylon membranes. Hybridisation conditions were optimised using $^{32}$P-labelled cDNA from a canine mammary tumour cell line. Array analysis was conducted using standard laboratory techniques and PC imaging software. While whole plasmids were prone to unspecific hybridisation, inserts and PCR products showed specific signals. PCR products amplified from bacterial clones appeared most suited due to reduced production steps and cost.

**A035**

**Genetic and functional characterization of a candidate mutation for callipyge in sheep**

MARIA A. SMIT[(1)], CAROLE CHARLIER[(2)], KARIN SEGERS[(2)], FRANCESCA BARALDI[(2)], TRACY L. SHAY[(1)], FRÉDÉRIQUE CERQUEIRA[(3)], ROBERT FEIL[(3)], GARY D. SNOWDER[(4)], CHRISTOPHER A. BIDWELL[(5)],

MICHEL GEORGES[(2)] & NOELLE E. COKKETT[(1)]
*[(1)]Utah State University, Department of Animal, Dairy, and Veterinary Sciences, Logan, UT, USA, [(2)]University of Liège, Department of Genetics, Liège, Belgium, [(3)]Institut de Génétique Moléculaire, Montpellier, France, [(4)]USDA/ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA and [(5)]Purdue University, Department of Animal and Poultry Sciences, West Lafayette, IN, USA*

In an attempt to identify the causative callipyge (*CLPG*) mutation, we and others have recently sequenced 180 kb spanning an imprinted cluster of genes whose expression pattern is altered by the *CLPG* mutation. The only *CLPG*-specific mutation identified was a single nucleotide polymorphism (SNP) that lies within a conserved intergenic region of the imprinted domain. In the present study, we tested the reported callipyge founder ram ("Solid Gold") for the presence of the SNP. Interestingly, Solid Gold was found to be heterozygous at the SNP site, but with a greater proportion of the wild-type allele than the *CLPG*-specific mutation. Solid Gold was then genotyped for microsatellite markers known to be in complete linkage disequilibrium with the *CLPG* allele, and he carried the marker haplotype corresponding to that of his callipyge descendants. These results lead us to suggest that the mutation event creating the SNP occurred in Solid Gold during early embryonic development, resulting in germline and somatic tissue mosaicism at the SNP position. This finding further supports the hypothesis that the newly identified SNP is the causative *CLPG* mutation. We predicted that if the SNP is causative and functions as part of a regulatory element, this would result in a perturbation of its epigenetic conformation. Preliminary results from bisulfite sequencing and chromatin immunoprecipitation experiments indicate that there is an allele-specific epigenetic mark at the SNP site.

**A036**
**Chicken and Pig Normalised Multi-Tissue cDNA Libraries**

A. BONNET[1], F. HÉRAULT[2], G. TOSSER-KLOPP[1], V. LE MEUTH-METZINGER[2], F. BENNE[1], C. DÉSERT[2], C. CABAU[1], S. VILLEGER[1], M.B. SOARES[3], F. BONALDO[3], M. DOUAIRE[2],
F. HATEY[1]
*[1]INRA Laboratoire de Génétique Cellulaire Castanet-Tolosan France; [2]INRA-ENSA Rennes France; [3]The University of Iowa Iowa City USA*

INRA is carrying on a genomic programme - AGENA - devoted to farm animals (cattle, pig, trout and chicken) dealing with genome structure, functional genomics and bio-informatics. We present here the construction of multi-tissue cDNA libraries for chickens and pigs as a first step in functional genomics. Total RNA from a large set of tissues (up to 42) from animals at different stages of development or in different physiological conditions were collected. Standard multi-tissue libraries were made from 5 to 9 different tissues. These libraries were mixed and the pool was normalized: 46 x $10^6$ clones (average insert length 900 bp, empty clones < 2%) were obtained for chickens and 6.4 x$10^6$ clones (average insert length 1000 pb, 2% empty clones) for pigs. First data obtained by hybridization or specific amplification of either actin or exogenous sequences indicated that the normalization process was successful. Sequencing (3' and 5' ends) of the libraries is now underway. In order to reduce the residual redundancy, the sequencing strategy will be iterative: sequencing of a set of 5 to 10,000 clones will be followed by subtraction of these clones from the starting library. 200,000 ESTs are planned. The clones will be spotted onto membranes and microarrays for gene expression studies. All EST sequence data management, clustering, submission to public databases and annotation will be achieved using the specially in-house designed bioinformatics software package SIGENA.

**A037**
**Generation of a 12,000 rads radiation hybrid panel for fine mapping in pig. First comparisons between IMpRH (7,000 rads) and IMNpRH2 (12,000 rads)**

MARTINE YERLE, PHILIPPE PINTON, CHANTAL DELCROS, NADÈGE ARNAL, DENIS MILAN, ANNIE ROBIE
*Institut National de la Recherche Agronomique, Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France*

We have constructed a 12,000 rads porcine whole-genome radiation hybrid panel to complement thefirst generation 7,000 rads panel (IMpRH) and allow higher resolution mapping studies both in specific areas of interest and on the whole genome. We have analyzed 243 hybrid clones produced, on the basis of their genome retention frequency to constitute a final panel of 90 hybrid clones with an average retention frequency of 35.4%. The resolution of this 12,000 rads panel (IMNpRH2) was compared to the resolution of 7,000 rads panel (IMpRH) by constructing framework maps in the 2.4 Mb region of porcine chromosome 15 containing the

acid meat RN gene. In this region, two-point analysis was used to estimate RH distances and demonstrated their reliability with the estimation of physical distances. This study demonstrates that the 12,000 rads panel constitutes a powerful tool to construct high-resolution maps. IMNpRH2 (12 to 14 kb/$cR_{12,000}$) is two to three times more resolvent than IMpRH (35 to 37 kb/$cR_{7,000}$). As expected, the increase in the radiation dose allows an increase of the mapping resolution in terms of kb/cR compared to IMpRH, with the same suppleness of use for mapping experiments. In addition the RH map constructed in the region investigated proved to be more homogeneous on IMNpRH2 than on IMpRH.

**A040**
**Comparative map between GGA15 and HSA12 and HSA22**

DANYEL JENNEN, BRAM KAMPS, JAN VAN DER POEL, RICHARD CROOIJMANS, MARTIEN GROENEN
*Wageningen University, Animal Breeding and Genetics Group, Wageningen, The Netherlands*
To improve the physical and comparative map of chicken chromosome 15 (GGA15, former linkage group E18W15) BAC contigs were constructed around loci previously mapped on this chromosome by linkage analysis. The BAC clones were used for both sample sequencing and BAC end sequencing. STS markers derived from the BAC end sequences were used for chromosome walking. In total 224 BAC clones were isolated, covering almost 30 % of GGA15, and 114 STS were developed (102 STS derived from BAC end sequences and 12 STS derived from sequences within genes). The partial sequences of the chikken BAC clones were compared to sequences present in the EMBL/GenBank databases, and revealed matches to genes, ESTs and genomic clones located on human chromosome 12q24 and 22q11-q12. Furthermore 12 chicken orthologues of human genes located on HSA22q11-q12 were directly mapped within BAC contigs of GGA15. In addition 3 more genes (in total 15 genes) from HSA 22 and 8 genes from HSA 12 were mapped to GGA15. These results provide a better alignment of GGA15 with the corresponding regions in human and mouse and identify several intrachromosomal rearrangements between chicken and mammals.

**A041**
**Tumour and tissue bank for dogs and cats - base for various genetical and molecular-biological studies**

S. WINKLER[2], H. MURUA ESCOBAR[1], J.T. SOLLER[1], K.CULMSEE[2],
J. BULLERDIEK[1], I. NOLTE[2]
[1]*Center for Human Genetics, University of Bremen, Bremen, Germany;* [2]*Clinic for Small Animals, Veterinary School, Hanover, Germany*
The limiting factor for various histological and genetic studies is the absence of the adequate tissues. The establishment of a "Canine/Feline Tissue Bank" covering different races and tissue types builds the base for various future studies. Samples were taken from normal and neoplastic tissues of dogs (~87.5 %) and cats (~12.4 %). In the past few years the dog has become an increasingly important model for genetic diseases affecting both species dogs and humans, e.g. haemophilia, narcolepsy, and various types of cancer. For these diseases genetic factors are assumed to be involved in both species. Factors like age, breed, sex, and hormonal reasons show involvement in different tumours. For example Rottweilers show elevated incidence of osteosarcomas compared to Dachshunds showing reduced appearance. Other tumours rely on sex and age such as canine tumours of the mammary gland. In female dogs showing adenocarcinomas of the mammary gland the age of 5 – 6 years is affected with 3.1% compared to the age of 11–12 years with 29.7%. The tissue bank currently consists of more than 10,000 samples taken from different tissues of 452 patients with several tumours for molecular examination and of tissues from 453 "healthy" patients for reference. To draw up our own empirical data, in addition to information about type and localisation of the tumour, information about breed, age and sex were recorded for every dog/cat that was treated because of a neoplastic diseases.

**A042**
**Development of an integrated large scale sample collection and DNA preparation system and its application to paternity in sheep**

UWE LUKSCH[1], MONIKA GUTSCHER[1], JÖRDIS SEMMER[2], GOTTFRIED BREM[1], JOHANNES BUITKAMP[2]
[1]*Agrobiogen GmbH Larezhausen, Germany;* [2]*Bayerische Landesanstalt für Tierzucht, Abt. Bio- und Gentechnik, Poing, Germany*
With the development of techniques for the genotyping of individuals, there exists an increased demand for the collection of large numbers of samples to be processed in the course of scientific projects such as QTL association studies and practical applications, marker assisted selection using bottom-up designs, or genetic diagnosis of inherited diseases. Currently the samples are

mainly collected as blood, milk, sperm, or tissue. Each of these methods has its drawbacks, and they are usually to cost and time consuming. To overcome these drawbacks, a system has been developed that allows the sampling of a small tissue probe using established ear-tag technology. The TypiFix® system provides a simple, inexpensive and reliable method (failure rate for the most precious samples is under 0,1 %) for the collection and long-term storage of samples at room temperature. In combination with a new „One Step" DNA isolation process (the passage of the DNA through a column of newly applied absorption materials which hold back the undesired side components such as protein and low molecular substances and provides an average of 20 µg DNA at a quality level equivalent to existing systems), the preparation of a large number of samples can be archieved in a short time.
Approximately 600 DNA samples have successfully been used for the amplification of a microsatellite set for paternity control among lambs at the test station of the Bavarian Institute of Animal Production.

**A043**
**Monitoring the physical integrity of the callipyge locus by molecular combing**

Fanny Di Silvestro[(1)], Carole Charlier[(1)], Sandrine Caburet[(2)], Aaron Bensimon[(2)], Noelle Cockett[(3)] & Michel Georges[(1)]

*[(1)]Department of Genetics, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, [(2)]Laboratoire de Biophysique de l'ADN, Institut Pasteur, Paris, France and [(3)]Department of Animal, Dairy and Veterinary Sciences, College of Agriculture, Utah State University, Logan,USA*

The ovine callipyge phenotype is an inherited muscular hypertrophy subject to an unusual parent-of-origin effect referred to as "polar overdominance". The CLPG locus has been mapped to a 400 Kb interval on distal OAR18q. This interval has been shown to contain an imprinted domain comprising at least four genes: the paternally expressed DLK1 and PEG11, and the maternally expressed GTL2 and MEG8 genes. We have previously shown that the CLPG mutation enhances the expression of these four genes in cis without affecting their imprinting status. By resequencing 180 Kb within this interval we and others have identified an SNP that is perfectly associated with the callipyge phenotype. It is not yet known however whether this SNP is the causal CLPG mutation.
To exclude possible chromosomal rearrangements (insertions/deletions or inversions) that might be associated with the CLPG allele and might have gone unnoticed by direct sequencing, we are studying the physical integrity of the CLPG locus by using dynamic molecular combing associated with FISH. In this process, DNA molecules, attached at one end, are combed on a silanized surface by a receding air-water interface. Combed DNA is then hybridized to DNA probes that are labelled with biotine or digoxigenin, and revealed with fluorescent antibodies (respectively Texas red or FITC).
In our study, we have prepared combed DNA from homozygous +/+ and CLPG/CLPG individuals. Six BACs corresponding to a minimum tiling path spanning the 400 Kb interval known to contain the CLPG locus were used as probes. Detailed results will be presented.

# Section B: MHC and Immunogenetics

**B001**

**Sequence analysis of a segment between the two swine leukocyte antigen (SLA) class I gene clusters in the SLA class I region**

ASAKO ANDO[1], ATSUKE SHIGENARI[1], TAKASHI SHIINA[1,] CLAIRE ROGEL-GAILLARD[2], PATRICK CHARDON[2], HIROSHI YASUE[3], HIDETOSHI INOKO[1]

*[1]Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan; [2]Laboratoire de Radiobiologie et d'Etude du Génome, INRA-CEA, Jouy-en-Josas, France; [3]Genome Research Department, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan*

A contig map of the swine leukocyte antigen (SLA) class I region spanning about 1 Mb was constructed by alignment of YAC and BAC clones. In this contig, 158 kb and 307 kb segments corresponding to the non-classical and classical SLA class I genes clusters have been recently sequenced, respectively. To obtain the entire sequence of the SLA complex and compare the genomic structure between the pig and human MHCs, we determined genomic sequences of four BAC clones carrying the segment between the non-classical and classical SLA class I gene clusters by the shotgun method. These clones included a total of 433 kb genome segments from the HCR gene (most centromere-side) to the FB19 gene (most telomere-side) in the SLA class I region. The genomic sequences thus obtained in swine were compared to those of the corresponding human class I segments. Nine homologues with respect to the nine human genes, *HCR*, *SPR1*, *SEEK1*, *S*, *STG*, *TFIIH*, *DDR, FLOTLLIN,* and *FB19* were identified in this segment. We also identified a pig homologue of the new human gene represented by the cDNA clone (KIAA1885) which is expressed in brain. Furthermore, the length of this segment in the SLA class I region was only 433 kb, as contrasted with as long as 582kb in the HLA class I region. This remarkable difference in size between them could be explained by a higher density of repetitive sequences in the human MHC.

**B002**

**Reference Strand Mediated Conformation Analysis (RSCA) of Equine MHC Class II Loci**

BROWN, J.J., OLLIER, W.E.R., THOMSON, W., CARTER, S.D., MATTHEWS, J.B., CLEGG, P.D.

*Mammalian Immunogenetics Research Group, Veterinary Science Faculty, University of Liverpool, Crown Street, Liverpool L69 7ZJ, United Kingdom*

Normal immune surveillance depends on the ability of Major Histocompatibility Complex class II molecules (MHC II) to bind peptide antigens and carry them to the cell surface for presentation to T-cells. Polymorphisms in DNA coding for the MHC class II antigen binding sites can significantly affect antigen binding and hence disease susceptibility. The MHC class II DRA locus is distinct from most other class II loci, for little or no sequence variation is seen in most species. This, however, is not the case with domestic equidae. To date, five equine DRA alleles have been detected by single strand conformation polymorphism (SSCP) studies of DRA exon 2 in all equid species. Here, we report the discovery of new DRA alleles after screening a random panel of equids using sequenced based typing (SBT) and RSCA. The future objective of our research is to characterize Equine MHC class II genes with respect to the extent of polymorphism, haplotypic association between alleles of different loci establishing linkage disequilibrium, determining the distribution of alleles & haplotypes in different breeds/equids, and to determine the functional consequences and clinical relevance of ELA class II polymorphisms in relation to disease susceptibility/resistance (Sarcoids, Sweet itch, Strangles, Grass sickness, Colic).

**B003**

**Structure and polymorphism of the upstream regulatory region of bovine and equine *DRB* genes**

SILVIANA DÍAZ[1], MARÍA V. RIPOLI[2], PILAR PERAL-GARCÍA, GUILLERMO GIOVAMBATTISTA

Universidad Nacional de La Plata, Facultad de Ciencias Veterinarias, Centro de Investigaciones en Genética Básica y Aplicada). La Plata, Argentina ggiovam@fcv.medvet.unlp.edu.ar

[1]Fellow from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

[2]Fellow from Universidad Nacional de La Plata (UNLP)

In the present work we communicate the sequence and polimorphism of the proximal upstream regulatory region (URR) of bovine and equine *DRB* genes, since up to now there are not sequence information about this region of MHC class II genes in cattle and horses. The genomic DNA was extracted from whole blood samples corresponding to 49 bovine from 11 cattle breeds

and 73 horses from 4 equine breeds. The URR was amplified by using the reverse primer designed from the consensus among *DRB* sequences from different species, and as a forward primer we used the oligonucleotide proposed by Turco *et al*., 1990. Six and four URR variants were detected in bovine and equine respectively through SSCP method. The PCR products were cloned and sequenced. The obtained DNA sequences are composed of highly conserved sequence motifs that include from 5′ to 3′ direction the W, X, Y, CCAAT and TATA-like boxes, showing the same organisation of the conserved regulatory elements than previously reported *DRB* genes. In addition, we observed the following: (i) the polymorphic sites were found within and between conserved sequence motifs, (ii) each DNA sequence correspond to a different SSCP variant, and (iii) our nucleotide sequences exhibit more identity with *HLA-DRB* sequences than *HLA-DQB* sequences. These evidences suggest that the sequence would correspond to the bovine and equine *DRB* promoter. However, we are still not able to assign this sequence to a specific *DRB* gene neither in horses nor in cattle.

**B004**

**Analysis of expressed sequence tags (ESTs) in the thyroid glands of chickens suffering from spontaneous autoimmune thyroiditis, an animal model of human Hashimoto thyroiditis**

DUSAN VASICEK[1], PETE KAISER[2] KATARINA VASICKOVA[1], MICHAL KOPECNY[1]; K. HALA[1]

[1]*Institute for Pathophysiology, University of Innsbruck, Innsbruck, Austria;* [2]*Institute for Animal Health, Compton, Berkshire RG20 7NN, UK*

The obese strain (OS) of chickens suffer from spontaneous autoimmune thyroiditis (SAT) and provide an animal model for human Hashimoto's thyroiditis. SAT is regulated by approximately three autosomal genes, one of which, regulating susceptibility of the thyroid gland to autoimmune attack, is recessive. So far, markers for this characteristic have not been identified. The mechanism of initiation of SAT is not yet known. It could be due either to the action of specific gene/s products, or to differential gene/s expression in the thyroid of disease-prone individuals. The aim of this study was to understand the molecular regulation of these processes. Healthy CB inbred strain birds were used as controls. We used suppression subtractive hybridisation (SSH) to obtain clones of genes that are different or differentially expressed between affected (OS) and healthy (CB) chickens. For SSH, RNA was prepared from thyroid lobes of 3-day-old OS and CB chickens, respectively. A subtracted cDNA library from OS and CB thyroids was generated and used for differential screening. DIG labeled subtracted mixtures from OS and CB thyroids, respectively, were used as hybridisation probes. All clones showing negative signals after differential screening probably represent differentially expressed (2-5 times) transcripts between OS (SAT affected) and CB thyroids. More than two hundred clones were selected after this screening, sequenced and database-searched using BLAST. Forty OS origin clones and 21 CB origin clones have no match in the public DNA databases. No cDNA of OS origin was found in CB origin clones. We have identified several clones which are expressed only in the OS strain, and not in the healthy control CB strain. Some clones showed quantitative differential expression between the two strains. Several OS clones matched with transcripts expressed in macrophages (A1/OS, B15/OS), activated T lymphocytes (B2/OS), or during the inflammatory process.

This work was supported by FWF grant Nr.: P12732-MED, and the BBSRC.

**B005**

**Characterisation of Major Histocompatibility Complex (MHC) FLA-DRB genes in the domestic cat**

LORNA J. KENNEDY[1], RUTH RYVAR[1], ROSALIND M. GASKELL[1], DIANE D. ADDIE[2], KIM WILLOUGHBY[1], STUARD D. CARTER[1], WENDY THOMSON[1], WILLIAM E.R. OLLIER[1], ALAN D. RADFORD[1]

[1]*Mammalian Immunogenetics Research Group, Veterinary Science, University of Liverpool, UK;* [2] *Department of Veterinary Pathology, Bearsden Road, Bearsden, Glasgow, UK*

To date, 71 feline leukocyte antigen (FLA) DRB alleles have been identified in studies of cats in the USA and Japan. The number of DRB genes present in the cat is not yet clear. PCR amplification and DNA cloning were used to identify the FLA-DRB alleles in a group of 33 British domestic cats of varying breeds from three locations within the UK. Some cat family pedigrees were also analysed. Applying the strict criteria for assigning new alleles as used by established mammalian MHC nomenclature committees, we identified 13 FLA-DRB alleles, including four previously unreported alleles. We found many sequences that were 1-2 base pairs different from these alleles, and have shown that these are probably artifacts of PCR amplification. When the same criteria for allele acceptance were applied to other previously reported sequences, 11 further alleles were confirmed. Thus there is good evi-

dence for 24 FLA-DRB alleles fulfilling nomenclature criteria. Preliminary results of Reference Strand Conformational Analysis on the same cat samples suggest individual cats have between one and three DRB loci. Within families, haplotypes carrying different numbers of DRB alleles can be identified. This study suggests that there may be a different distribution of DRB alleles in the UK compared to the USA, and that different FLA haplotypes may carry different numbers of DRB genes, as is the situation in many other mammals including man, apes and cattle.

**B006**

**Sequence-based genotyping at eight major histocompatibility complex (SLA) loci in Westran pigs**

JUN-HEON LEE[1], STACY WALTERS[2], TINA PATEL[2], DENBIGH SIMOND[1], RICHARD ALLEN[2], WAYNE HAWTHORNE[2], PHIL O'CONNELL[2], JEREMY CHAPMAN[2], DOUGLAS SMITH[3], CHRIS MORAN[1]

*[1]Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2006, Australia; [2]National Pancreas Transplantation Unit, Westmead Hospital, NSW 2146, Australia; [3]Transplant Immunology Laboratory, Baylor University Medical Center, Dallas, TX 75246, USA*

Since pigs may be donors for xenotransplantation and SLA molecules elicit a xenoreactive response, we examined the *SLA1*, *SLA6*, *SLA7*, *SLA14*, *DQA*, *DQB*, *DRA* and *DRB* loci in inbred Westran (Westmead Hospital transplantation) pigs, bred for transplantation research. Three Westran generation-six inbred animals and a Large White control were used to assess SLA variants. Three clones from each locus in each animal were sequenced to assess within line diversity and similarity to known SLA sequences. Westran pigs have novel alleles at the *SLA7*, *SLA14* and *DQA* loci. Only *SLA1* appeared to be segregating, consistent with low microsatellite marker heterozygosity from recent deliberate inbreeding but also their derivation from a feral stock from Kangaroo Island, South Australia, established by the release of a single pair in 1803. The SLA genotypes will be useful for attempting modulation of immune responses in xenotransplantation.

**B007**

**Molecular genetic dissection of the major histocompatibility *B* complex in the chicken**

RONALD M. GOTO[1], W. ElWOOD BRILES[2], MARCIA M. MILLER[1]

*[1]Beckman Research Institute, City of Hope National Medical Center, Duarte, California USA; [2]Northern Illinois University, DeKalb, Illinois, USA*

Major histocompatibility complex (*Mhc*) *B* haplotypes in the chicken differentially control immune responses to infectious disease. Particular *B* haplotypes are known to confer genetic resistance to diseases caused by infection with Marek's virus, Rous sarcoma virus, and *Eimeria tenella*. The differential responses to infectious disease in the chicken may be related to the compact nature of the *B* class I (*B-F*) and class II (*B-L*) gene regions and to the levels of expression of the loci within these two regions. As yet however the exact basis of *B* system conferred disease resistance remains unknown. *B* recombinant haplotypes are powerful means for more closely defining *Mhc* genes conferring disease resistance. Recombinant haplotypes have already narrowed the *B* gene region controlling Marek's disease responses. If available, animals bearing additional, more finely defined recombinant haplotypes might be tested along side parental haplotypes to further map disease responses to particular *B* subregions or loci. Identification of *B* recombinant haplotypes has been hampered by lack of means by which to easily identify alleles at the *B-F* and *B-L* loci. We have developed methods for *B-G, B-F,* and *B-L* typing to rapidly characterize *B* haplotypes. We demonstrate the capacity of these methods to define genotypic differences among a series of recombinant haplotypes developed in a common lineage and to reveal potential recombinant haplotypes within closed breeding populations.

**B008**

**Structure of the Bovine MHC *DRA* and *DRB3* genes**

GEORGE C. RUSSELL[(1)], JUDITH A. SMITH[(2)], JOHN L. WILLIAMS[(1)] & ROBERT A. OLIVER[(1)]

*[(1)] Roslin Institute, Roslin, Midlothian UK and [(2)] Department of Veterinary Parasitology, University of Glasgow, Glasgow UK.*

The cattle MHC class II *DR* gene product is a heterodimer encoded by the *BoLA-DRA* and -*DRB3* genes. Several groups have isolated cDNA and genomic clones for these genes, but the genomic organization around the first exon of both genes has yet to be described due to the length of the first intron. We have used a combination of long-range PCR, cloning and sequencing to define the organization of the *DR* genes on existing genomic clones and in genomic DNA. We estimate the size of the coding region of the *DRA* gene to be 4.7 kilo-base pairs (kbp), while that of the *DRB3* gene is about 11.4 kbp. Sequencing of these

full-length genomic PCR products confirmed that they carried the complete *DRA* and *DRB3* genes respectively and allowed the design of probes and primers to isolate and characterise the class II promoter regions and the *DRB3* 3'-end.
Fragments carrying the 5'-end of the *DRB3* gene and its promoter were identified in both the lambda clone A1 and on a BAC clone carrying the *BoLA-DR* genes. Interestingly, hybridization of a *DRB3* exon 1 probe to BamHI digests of these clones showed a 10 kbp promoter fragment on the BAC clone and a 5 kbp fragment on clone A1. The larger fragment was sub-cloned and a 1.7kbp fragment including exon1 and the promoter was sequenced. A 3 kbp fragment encoding exons 4-6 and the entire 3'-untranslated region of the *DRB3* gene was sub-cloned from A1 and sequenced. This now provides us with improved characterization of the existing lambda clones carrying the *DRA*0101* and *DRB3*0101* alleles, and also cloned 5'- and 3'- flanking fragments for generating *DRB3* allele-specific constructs.
This work not only improves our understanding of bovine class II gene structure, but also provides the material to allow functional *DR* gene pairs encoding selected alleles to be assembled for analysis by transfection or transgenesis.

**B009**
**Evolution of the SLA class I region**

CHRISTINE RENARD, MARCEL VAIMAN, PATRICK CHARDON
*Laboratoire de Radiobiologie et d'Etude du Génome INRA CEA Jouy en Josas, France*
Chromosomal distribution of the MHC class Ia, Ib and MIC sequences vary considerably in mammals. In the pig, class I genes are distributed in 2 clusters denoted β and κ whereas in humans a third cluster α exists. Close to TNF, the β cluster contains MIC genes in both species and either class Ib in the pig or class Ia in humans. Further along the chromosome, the κ cluster encompasses the whole set of SLA class Ia loci but no functional class Ia gene in HLA. Lastly at the telomeric end of HLA, the α cluster presents a large set of sequences from the 3 series. We analyse the sequences available in pigs and humans. We identified an elementary duplication unit in the SLA κ cluster comprising a mosaic of repeats ending with a specific motif. Dot matrix analysis underlined similarity between human and pig orthologous κ fragments and unexpectedly between fragments from the pig κ cluster and fragments from human α cluster. Presumably, SLA11 and SLA4 were the oldest loci and the remaining class Ia loci arose from 5 additional subsequent rounds of gene duplication. By contrast intergenic sequences from the SLA β cluster displayed limited similarity between them and no relationship with motifs of the SLA κ cluster. Remarkably, only significant alignments exist between the pig MIC2 containing segment and several human MIC segments from the α and β HLA clusters. Thus the history of SLA and HLA gene duplication differs and, within each species, the β and κ clusters were subject to independent evolutionary pattern.

**B010**
**Characterisation of duck (Anas Sp.) MHC region**

SILVANA ROTUNDO[1], GIULIANA SALTINI[2], LAURA SIRONI[3], SILVIA CEROLINI[3], PAOLA MARIANI[1]
*[1]FPTP-CERSA, Segrate (MI), Italy; [2]CNR-ITB, Segrate (MI), Italy; [3]University of Milan, VSA Department, Milan, Italy*
In contrast to the well studied human and murine MHCs, very little is known about the genomic organization of non-mammalian MHCs. Although some information is already available for a number of avian MHC systems, chicken MHC, also referred to as B complex, is the most studied and better known. Within the B locus there are two loci containing the classical class I (B-F) and class II (B-L) β genes homologous to human. Chicken MHC also contains the B-G (class IV) gene, so far identified only in this species. Within the B locus there is only one gene homologous to the human class III, the complement component C4. We started characterising the duck MHC region by isolating MHC gene specific sequences. We derived primers specific for MHC classical genes from chicken, pheasant, and quail sequences deposited in GenBank. When possible, conserved regions were determined by aligning the sequences. We amplified duck specific MHC gene sequences using genomic DNA as template. The PCR products were sequenced and database searches (EMBL, Genbank) were carried out using BLAST to ascertain the homology of the duck sequences with MHC gene sequences in other avian species. Thus, duck specific primers were designed, further rounds of PCR were run and the products sequenced. Polymorphisms within the MHC region were investigated by sequence and SSCP analyses.

**B012**
**Bovine MHC class II DRB3 genes diversity in Japanese black, Japanese shorthorn, Jersey and Holstein cattle**

YOKO AIDA[1], MITSUO MORITA[2], SHIN-

NOSUKE TAKESHIMA[1]
[1]*RIKEN, Retrovirus Research Unit, Wako, Saitama;* [2]*Livestock improvement association of Japan Inc., Maebashi, Gunma, Japan*

We sequenced exon 2 of the bovine MHC (BoLA) class II DRB3 gene from 200 Japanese black, 102 Holstein, 100 Japanese Shorthorn and 69 Jersey cattle using new PCR-Sequence-based typing method. The 35 different previously published alleles and the three novel alleles were identified. In 38 alleles, 19 breed specific alleles were identified. These alleles were 80.0% to 100% identical at the nucleotide level to *BoLA-DRB3* cDNA clone NR1. These specific alleles did not have a specific cluster in Neighbor-joining tree, indicating that these breeds have a common variation in point of the value of the average of nucleotide, amino acid, synonymous and non-synonymous substitution. To differentiate the allelic variations between four distinct breeds, we determined the gene frequencies of the *BoLA-DRB3* alleles in each breed and compared with those of other populations. All breeds examined showed extremely high *DRB3* diversities, with heterozygosity rates of between 90.5% and 94.1%, which were near the heterozygosity rates expected of between 88.7% to 91.4%. The *DRB3*1101* was detected as a most frequent allele in Holstein (16.7%), the *DRB3*4501* was in Jersey (18.1%), *DRB3*1201* was in Japanese Shorthorn (16.0%) and *DRB3*1001* was in Japanese Black (17.5%). Furthermore, we constructed the UPGMA tree based on distances of allele frequency in each breeds. Holstein and Japanese Black were the closest to each other, and Jersey was farther from these both breeds than Japanese shorthorn.

**B013**
**Comparing of antigen binding groove of MHC class II DR molecule of cattle and human**

SHIN-NOSUKE TAKESHIMA, YOKO AIDA
*RIKEN, Retrovirus Research Unit, Wako, Saitama, Japan*

The major histocompatibility complex (MHC) polymorphism occurs predominantly at residues involved in peptide binding, and there is compelling evidence that the polymorphism is maintained by some form of balancing selection. The essential role of the MHC molecules for immunological recognition of foreign peptide antigens implies that the cause for this selection is related to the influence of MHC polymorphism on host defense against pathogens. To study the function of bovine MHC (BoLA)-DR molecule, we analyzed the selective force of bovine and human DR 1 chains. First, we compared the average number of nonsynonymous substitution per site with that of synonymous substitutions per site in pairwise of 103 *BoLA-DRB3* and 236 human MHC *(HLA)-DRB1* alleles. The rate of nonsynomymous substitutions in *BoLA* alleles was higher than that of *HLA* alleles. Next, we detected the selective force at single amino acid site in bovine and human DR 1 domains by adaptsite program package which reported by Suzuki and Gojobori (1999). Of the 22 ARSs, 6 were inferred as positively selected but none were inferred as negatively selected in BoLA. By contrast, 8 were inferred as positively selected and 3 were inferred as negatively selected in HLA. Collectively, these result shows that, several amino acids might have different biological functions between human and cattle, and, in addition, excess number of nonsynomymous was might be the result of small negatively selected amino acid sites in cattle.

**B014**
**Molecular cloning of cDNA encoding porcine interleukin-16**

TOMOKO EGUCHI[1], TOMOHIKO EBATA[2], YOHTAROH TAKAGAKI[3],
JUNICHIRO FUJIMOTO[4], HIROSHI YASUE [1]
[1]*National Institute of Agrobiological Sciences, 2 Ikenodai, kukizaki, Inashiki, Ibaraki 305-0901, Japan,* [2]*Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan,* [3]*Kitasato University school of medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan,* [4]*National Research Institute for Child Health and Development, 3-35-31, Taishido, Setagaya, Tokyo 154-8509, Japan*

Interleukin-16 (IL-16) is a chemotactic cytokine that binds to the CD4 receptor, and considered to be involved in T cell functions. Analysis of swine IL-16 would contribute to understanding of T cell-related immune-response system which is relevant to animal health as well as xenotransplantation. In the present study, in order to characterize swine IL-16, cDNA clones coding IL-16 were isolated from “full-length” cDNA library of swine “gamma/delta” T cell populations (described elsewhere). The cDNA inserts of the clones were sequenced to demonstrate that swine IL-16 mRNA was 2,296 base pairs (bp). The hypothetical ORF for IL-16 cDNA is 1,905 bp, which encodes a 635 amino-acids sequence (pro-IL-16) (MW=67,177). When the amino-acid sequence was compared with those of human, crab-eating monkey and mouse, the homologies were calculated to be 94.3%, 94.2% and 92.8%, respectively.

The comparison also predicted that the swine pro-IL-16 is excised to yield a matured IL-16 consisting of 121 amino-acids (MW=12,523).

**B016**

**Analysis of genomic structure and repertoire of swine T cell receptor α chain gene**

HIROHIDE UENISHI[1], RYUJI YAMAMOTO[1,2], TOMOKO EGUCHI[1], HIROSHI YASUE[1], YOHTAROH TAKAGAKI[2], TAKASHI AWATA[1]

*[1]National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan; [2]Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan*

T cells express heterodimeric receptors (TCR), recognize antigens using these receptors and exhibit various functions such as cytotoxicity or immune regulation. TCR genes consist of four molecules, α, β, γ and δ. T cells are classified into two types by their expressing receptor pairs, αβ and γδ. γδ T cells account for only several percents of all T cells in peripheral blood of human and mouse, however, amount of γδ T cells of swine are almost half of the whole T cells in periphery. Swine γδ T cells are notable according to their unique character, such as MHC class II expression and antigen-presenting ability to αβ T cells. TCR α and δ chain genes (TRA/TRD) are located on the same locus, and the locus spans about 1Mbp of the germline genomic DNA. We focus TRA/TRD locus because its analysis may be a clue to elucidation of a unique fashion of lineage of αβ/γδ T cells in artiodactyls. We revealed genomic structure of the region including the TRA constant (TRAC) region and joining (TRAJ) segments. We newly identified one pseudo-TRAJ segment in the germline sequence, however, whole structure of this locus is strikingly conserved among swine, human and mouse. We also identified 91 clones of swine TRA cDNA and confirmed that 44 TRAJ segments identified in the swine germline sequence are expressed in peripheral blood. Length of CDR3 of swine TCR α chain molecules is longer than those of human and mouse, and this suggests a different manner for recognition of antigens in swine.

Protection of recombinant mouse single chain antibodies against orthopox virus envelope proteins correlates with antigen affinity

**B014**

**Protection of recombinant mouse single chain antibodies against orthopox virus envelope proteins correlates with antigen affinity**

Michaela Alex[1], Katharina Hoelzle[2], Claus-Peter Czerny[1],

*[1]University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany; [2]University of Zurich, Institute of Veterinary Bacteriology, Zurich, Switzerland*

The 14 kDa fusion protein (ORF A27L), the 32 kDa adsorption protein (ORF D8L) and a 35 kDa membrane protein (ORF H3L) localized in the envelope of intracellular mature orthopox virus particles are key determinants in early and late virus/host interactions and mainly responsible for the induction of B- and T-cell specific immune responses. High-affinity neutralizing antibodies may be of therapeutic value in human and veterinary medicine for alleviating the symptoms in infected individuals and for prophylaxis to infection. We have engineered a panel of neutralizing single-chain variable fragments (scFvs) from BALB/c-mouse hybridoma cell clones producing monoclonal antibodies (MAb) that bind to sequential and conformation-dependent protective antigenic sites of the fusion protein (aa 32-39), adsorption protein (aa 290-304), and 35 kDa protein (aa 210-324). The DNA of the constructs of about 750 bp was sequenced and analyzed comparatively. Framework (FRW) and complementary determing regions (CDR) of the recombinant antibodies were defined on the deduced amino acid sequences. After cloning into the pQE30 vector (Qiagen, Hilden) the scFvs were expressed in E. coli and purified by an $N^{2+}$-terminal 6xHis-Tag. Binding to their epitopes was determined in standardized Michaelis-Menten kinetic studies together with the corresponding full-size MAbs. The kinetic studies indicated, that binding capacities of the scFvs were 11-27-times lower than binding capacities of the corresponding MAbs. Neutralizing potencies of purified scFvs against vaccinia virus were tested in vitro in plaque reduction assays and in vivo in a mouse challenge model with the neurovirulent vaccinia virus strain Munich 1. Protection correlated with antigen affinity.

# Section C: Functional Genomics

## C001
### Diet and gene expression - Bridging the gap with small intestine specific expressed sequence tags (ESTs) and cDNA microarrays in the chicken

CHRIS M. ASHWELL[1], HYUN S. LILLEHOJ[2], ROSELINA ANGEL[3]
[1]*Growth Biology Laboratory, USDA-ARS;* [2]*Parasite Biology, Epidemiology, and Systematics Laboratory-USDA-ARS, Beltsville, MD;* [3]*Department of Animal and Avian Sciences, UMD, College Park, MD*

The predominant factor influencing growth in broiler chickens is nutrient absorption across the small intestine. Animal agriculture expends its greatest resources on animal feeds and must develop novel strategies to maximize nutrient absorption and retention, due to new environmental regulations regarding animal wastes. Additionally, the advent and use of novel feed additives and transgenic crops in animal agriculture has increased the value of ascertaining the exact impact, if any, dietary components have on animals at the molecular level. These studies were undertaken to both expand the knowledge of expressed genes for chicken, as well as to identify specific genes involved in small intestinal function. A normalized cDNA library was constructed from small intestines of both normal broiler chickens and those infected with *Eimeria acervulina*, a pathogen known to limit nutrient absorption. Of the total number of unique ESTs sequenced, approximately 4,000 were randomly selected, representing both novel genes and GenBank homologs, to be amplified for spotting onto glass slides for subsequent hybridization studies. With a goal of reducing the phosphorus content of manures, the first experiments using these microarrays are to determine the effects of limiting dietary phosphorus on gene expression, specifically identifying those genes involved in phosphorus absorption and retention. Utilizing these resources, these and future studies will identify the effects of dietary manipulation on the expression of genes in the small intestine. Monitoring intestinal gene expression may also be a valuable tool for evaluating the impacts of novel feed additives and transgenic crops as dietary components in animal agriculture.

## C002
### Analysis of tissue-specific gene expression patterns using a 7653 gene cattle microarray

MARK R. BAND[1], JONATHAN U. PELED[2], ROBIN E. EVERTS[2], ZONGLIN L. LIU[2], HARRIS A. LEWIN[1,2]
[1]*The W.M. Keck Center for Comparative and Functional Genomics;* [2]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

cDNA microarrays have been shown to be useful for monitoring global gene expression patterns in normal and diseases states and in response to various environmental stimuli. We have constructed cattle cDNA microarrays containing 7653 elements, of which ~80% have putative human orthologs. The cattle cDNA clones used for creation of the array were selected from more than 17,000 expressed sequence tags (ESTs) derived primarily from a normalized and subtracted placenta cDNA library. Clones were annotated by sequential BLASTN and TBLASTX searches against multiple public domain databases (see accompanying abstract by Everts et al) including 2917 genes that have one or more *Gene Ontology* functional annotations. Fifteen different tissues were analyzed in order to identify gene expression patterns associated with specific organs and tissue types and to establish a functional baseline for future studies. Hybridizations were performed with Cy3 and Cy5-labelled cDNA derived from RNA collected from the tissue samples. All samples were compared to a universal control sample comprised of a mix of cattle RNAs. Three exogenous plant genes were used as spiking controls for data normalization. Results of various clustering methods to correlate gene expression profiles and define sequences lacking annotation will be presented. This research illustrates the potential of microarrays for understanding tissue-specific gene regulation in response to different environmental stressors.

## C003
### Affects of the brown locus (*TYRP1*) on coat color in cattle

TOM G. BERRYERE[1], SHEILA M. SCHMUTZ[1], C. MICHAEL COWAN[2], JOHN

POTTER[3]
[1]*University of Saskatchewan, Department of Animal and Poultry Science, Saskatoon, Canada;* [2]*Genetic Visions, Inc., Middleton, Wisconsin, USA;* [3]*John Potter, Spruce Grove Farm, Galien, MI, USA*

Skin biopsies from several cattle of different coat colors were used to prepare mRNA. The entire coding sequence of cattle tyrosinase related protein 1 (*TYRP1)* was obtained using cDNA prepared from mRNA. The cattle amino acid sequence was found to be 97-86% similar to goat, horse, human and mouse sequence. A microsatellite detected in intron 5 of *TYRP1* from genomic DNA was used to linkage map this gene to cattle chromosome 8 between microsatellites *BL1080* and *BM4006.* Although 2 nonconservative amino acid changes were detected, no association was found with diluted shades of black or red in Simmental nor Charolais cattle. Nor was an association found with the dun coat color, inherited as a dominant trait, which occurs in Galloway cattle. Therefore this gene does not appear to be one of the dominant or co-dominant diluter genes. However, in Dexter cattle dun coat color is inherited as an autosomal recessive trait. An amino acid change was found in the homozygous state in all 26 dun Dexter cattle examined, irrelevant of shade of dun which ranges from a pale golden to dark brown. We suggest the dun Dexter is more correctly called a "brown cow". Black Dexters had either one (28) or no (16) copy of this allele. We examined over 88 cattle of other breeds including Brown Swiss, Canadienne, Flamande, Guernsey, Jersey, Angus, Charolais, Hereford, Limousin, and Simmental and did not find this *TYRP1* mutation in any of them.

**C004**
**Expression of *PEG11* transcripts in the muscles of normal and callipyge lambs (Ovis aries)**

CHRISTOPHER A. BIDWELL[1], TRACY L. SHAY[2], CAROLE CHARLIER[3], KARIN SEGERS[3], MICHAEL GEORGES[3], NOELLE E. COCKETT[2]
[1]*Purdue University, Department of Animal Sciences, West Lafayette, Indiana USA;* [2]*Utah State University, Department of Animal, Dairy and Veterinary Sciences, Logan, Utah USA;* [3]*University of Liège, Department of Genetics, Liège Belgium*

The region of ovine chromosome 18 that includes the callipyge mutation contains an imprinted gene cluster of at least six transcripts. Paternal expressed gene 11 (*PEG11*) has a putative protein-coding sequence that produces a paternally derived sense strand transcript and maternally derived antisense strand transcripts (*antiPEG11*). Expression of *PEG11* and *antiPEG11* transcripts was analyzed using strand specific probes and northern blot analysis. Expression of a 6.5 kb *PEG11* transcript was detected in muscles that become hypertrophied including the longissimus dorsi (LD), semimembranosus (SM) and gluteus medius (GM) in 14-day, 56-day, and 84-day-old paternal heterozygous callipyge lambs ($+^{mat}/C^{pat}$). The *PEG11* transcript was not detected in the LD, SM or GM from the other three possible genotypes (+/+, $C^{mat}/+^{pat}$ or CC). The *PEG11* transcript also was not detected in the supraspinatus (SP), which does not undergo hypertrophy, regardless of the lambs' genotype. Two *antiPEG11* transcripts (1.7 kb and 0.8 kb) were readily detected in the LD, SM, GM and SP muscles of 14-day, 56-day and 84-day-old $C^{mat}/+^{pat}$ and CC lambs but were weakly detected in $+^{mat}/C^{pat}$ callipyge lambs and not detected in +/+ lambs. The callipyge mutation has altered the expression of both *PEG11* and *antiPEG11* transcripts. Expression of the *PEG11* transcript only in the LD and pelvic limb muscles of the $+^{mat}/C^{pat}$ genotype was consistent with polar overdominant inheritance of muscle hypertrophy in callipyge lambs.

**C005**
**Physical mapping of genes expressed in the *Corpus luteum* of Cattle**

TINA B. BØNSDORFF[1], ANDRÉ EGGEN[2], MATHIEU GAUTIER[2], FRODE LINGAAS[1], HANS-CHRISTIAN AASHEIM[3], KNUT RØNNINGEN[1], INGRID OLSAKER[1]
[1]*Department of Morphology, Genetics and Aquatic Biology, The Norwegian School of Veterinary Science, Oslo, Norway;* [2]*Laboratoire de genetique biochimique et de cytogenetique, Institut national de la recherche agronomique, Jouy-en-Josas, France;* [3]*Department of Immunology, The Norwegian Radium Hospital, Oslo, Norway*

Fertility, measured as % non-return, is highly ranked in the merit index by the Norwegian cattle-breeding organisation because of the considerable economical importance of this

trait. The estimates of heritability for fertility traits are small (0.01-0.05). However, there are indications that the genetic variation is significant for these traits, which means that there is a potential to make long-term improvements in fertility. A deeper understanding of the molecular mechanisms controlling fertility, i.e. the regulation of ovarian function, will contribute to the improvement of methods and strategies to increase fertility and reproductive performance in cattle as well as other mammals including man. The *corpus luteum* of the ovary secretes progesterone and plays a central role in the regulation of cyclicity and maintaince of pregnancy. In order to identify bovine genes expressed in the *corpus luteum* at the most active state, i.e. 10 days after ovulation, a representational differential analysis (RDA) was performed, with skeletal muscle as the subtractive agent. Eleven of eighty initially analysed clones were selected for further studies based on their sequence and expression profiles. A *corpus luteum* cDNA library was constructed and cDNA clones for the RDA fragments were identified, sequenced, and mapped by using a bovine radiation hybrid cell panel. The mapping results as well as expression analysis of the clones will be presented.

**C006**
**Porcine *IGF2* promoter usage and isoforms in fetal and adult porcine tissues**

MARTIN H. BRAUNSCHWEIG[1], MINH NGUYEN[2], MICHEL GEORGES[2], LEIF ANDERSSON[1]
*[1] University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala, Sweden; [2]University of Liege, Department of Genetics, Liege, Belgium*

*IGF2* is a candidate gene for a paternally expressed QTL on pig chromosome 2p affecting skeletal and cardiac muscle mass. The aim of this study was to characterize the forms of *IGF2* transcripts expressed in different tissues and developmental stages. The porcine *IGF2* gene encompasses 10 exons of which exons 1, 2, 3, 4, 4b, 5 and 6 represent 5' UTRs. The exons 7, 8, and part of exon 9 encode the precursor protein of the 67-amino-acid *IGF2* peptide. The *IGF2* gene is imprinted and primarily expressed from the paternal allele. The four promoters show tissue- and development-specific activity. Promotor 1 (P1) is mainly used in adult liver. Four isoforms have been found with the transcript including exons 1, 2, 3, 7, 8 and 9 being predominant. This transcript can further be subdivided in two isoforms due to alternative poly(A) site selection. Promoter P1 usage was also observed in fetal liver and fetal ham. In these tissues there is an additional variant with exons 1, 2, 3, 7, 8 and 9 due to alternative splicing of exon 2. Promoter 2 (P2) usage results in transcripts encompassing exon 4, 7, 8 and 9 and exon 4, 4b, 7, 8 and 9, respectively and is found in all tissues. Promotor 3 (P3) and 4 (P4) are predominantly used in various fetal tissues, in adult kidney, adult muscle and to a low extent in adult liver. The transcript isoforms include exons 5, 7, 8 and 9 and 6, 7, 8 and 9 for promoter P3 and P4 usage, respectively. For both transcripts isoforms resulting from different polyA signal usage have been observed.

**C007**
**The hairless (*hr*) gene in the ovine species**

RAFFAELLA FINOCCHIARO[1], ANNA CAROLI[2], ELENA BUDELLI[3], BALDASSARE PORTOLANO[1], PATRIZIA BOLLA[3], PIETRO GIACCONE[1], GIUSEPPE DAMIANI[4]
*[1]University of Palermo, Department S.En.Fi.Mi.Zo., Palermo, Italy; [2]University of Bari, Department of Animal Health and Welfare, Valenzano, Italy; [3]University of Milan, Department VSA, Milano, Italy; [4]IBBA-CNR, Milano, Italy*

The hairless (*hr*) gene is often responsible for congenital hypotrichosis in mammalian species. The protein codified by this gene is a transcriptional corepressor for thyroid hormone receptors. So far no molecular study has been carried out on *hr* gene in the ovine species. By using the primers designed on the conserved human, rat, and mouse sequences and the long PCR technique, the entire ovine *hr* gene was successfully amplified. Sequencing allowed the continuous alignment of 62% the ovine *hr* coding sequences, from exon 2 to 9. The highest homology (80% identity) was found with human *hr* gene. The homology scores (ranging from 76% identity of ovine-mouse comparison to 95% of rat-mouse comparison) indicate that *hr* gene is strongly conserved. The phylogenetic tree agrees with the well known evolutionary pathway of mammals, indicating that the divergences of *hr* gene in Ruminants, Rodents, and Primates stemmed from a common ancestor. Functional constraints may be re-

sponsible for the low mutation rate, and for the reduced phylogenetic divergence suggesting an important physiological role of *hr* gene. This hypothesis agrees with recent experimental evidences indicating that the gene product plays a wider role than previously suspected in development. Several pleiotropic effects were detected in different mouse tissues. Therefore the investigation of *hr* gene in domestic animals could reveal interesting influences on productive traits in addition to the well known effect on hair growth.

**C008**

**Sequencing and mapping of the porcine *Pyruvate Carboxylase* (*PC*) gene**

GUILLERMO DÁVALOS[1], MARCEL AMILLS[1], JOSÉ LUÍS NOGUERA[2], ARMAND SÁNCHEZ[1]

*[1]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Àrea de Producció Animal, Centre UdL-IRTA, Lleida 25198, Spain*

The pyruvate carboxylase (PC) gene is a member of the biotin-dependent enzyme family and catalyzes the ATP-dependent carboxylation of pyruvate to oxalacetate. In mammals, PC plays an important role in gluconeogenesis and lipogenesis. We have amplified three different regions (exon 1 to 10, exon 9 to exon 15 and exon 15 to the 3'UTR) of the pyruvate carboxylase cDNA in six Iberian, Pietrain and Large White pigs. Amplified products were sequenced forward and reverse and a silent mutation C $\rightarrow$ T was identified at exon 6. This polymorphism can be typed by primer-extension analysis. Furthermore, physical mapping of the pig PC gene was performed by using the INRA-Minnesota porcine radiation hybrid panel (IMpRH). The porcine PC gene mapped to the 2p14-17 interval, near the SW2623 microsatellite (LOD score = 13.91). Overall sequence identity between pig PC and its human and murine counterparts ranged from 86-91 %.

**C009**

**Extensive *DLK1* expression profiling substantiates the callipyge polar overdominance model in sheep implicating a *trans* effect of coregulated imprinted genes in producing the callipyge phenotype**

ERICA E. DAVIS[1], CHARLOTTE HARKEN-JENSEN[2], CAROLE CHARLIER[1], ANETTE KLEIM[2], TRACY L. SHAY[3], BØRGE TEISNER[2], NOELLE E. COCKETT[3], MICHEL GEORGES[1]

*[1]University of Liège, Department of Genetics, Liège, Belgiu;, [2]University of Southern Denmark, Department of Immunology and Microbiology, Odense, Denmark; [3]Utah State University, Department of Animal, Dairy, and Veterinary Sciences, Logan, UT, USA*

*Delta-like* (*DLK1*) is a paternally expressed gene located in the imprinted domain encompassing the ovine callipyge (*clpg*) locus. Comparative analysis of *DLK1* across the four possible genotypes has revealed that the callipyge mutation induces overexpression of mRNA in both $CLPG^{M}/CLPG^{P}$ and $+^{M}/CLPG^{P}$ animals, while at the same time, only the latter individuals exhibit the callipyge phenotype. Addressing the need for more extensive characterization of *DLK1* relative to callipyge, a multi-faceted expression study is underway in which *DLK1* expression is being examined on a panel of tissues collected from animals representative of the four callipyge genotypes spanning three stages of development. Despite the overexpression of *DLK1* previously demonstrated on the mRNA level in two distinct genotypes, preliminary results reveal that only callipyge $+^{M}/CLPG^{P}$ individuals display *DLK1* protein in muscle fibers. These results provide evidence justifying the proposed model for callipyge polar overdominance implicating a *trans* effect of coregulated imprinted genes in producing the callipyge phenotype. Based on the perfect association of *DLK1* protein with the callipyge phenotype combined with the known role of *DLK1* in cell proliferation and differentiation, an *in vivo* approach is being used to elucidate the direct involvement of *DLK1* in producing the muscular hypertrophy. A transgenic construct consisting of the ovine *DLK1* ORF placed under the control of the Myosin Light Chain-3F promoter has been introduced into the mouse by pronuclear microinjection. Seven transgenic lines are currently being established and will be subjected to analysis of both *DLK1* transgenic expression and phenotype.

**C010**

**Prion protein (*PRNP*) genotype frequencies in German breeding sheep suggest feasibility of breeding for scrapie resistance**

CORD DRÖGEMÜLLER, TOSSO LEEB, OTTMAR DISTL
*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany*

Genetic susceptibility to scrapie is associated with polymorphisms in three different codons of the ovine prion protein (*PRNP*) gene (136, 154, 171). In a number of countries, studies of *PRNP* genotypes linked to scrapie revealed the resistance of homozygous $PRNP^{ARR}$ / $PRNP^{ARR}$ animals and the high risk of $PRNP^{VRQ}$ /$PRNP^{VRQ}$ and $PRNP^{VRQ}$ /$PRNP^{ARQ}$ animals in scrapie affected flocks. Therefore, the selection of $PRNP^{ARR}$ /$PRNP^{ARR}$ genotypes may be a strategy for controlling clinical scrapie at the population level. We genotyped 1674 German breeding sheep from 15 different breeds in Germany. Apart from the wildtype allele $PRNP^{ARQ}$ at least four mutually exclusive allelic variants were found. The greatest variability within the *PRNP* gene was encountered in Texel sheep, where 14 different *PRNP* genotypes were found. In the important meat breeds Suffolk, German Whiteheaded, and Blackheaded Mutton the $PRNP^{ARR}$ allele was predominant. In these breeds simulation studies showed that four generations are necessary to breed scrapie resistant pedigree flocks. For the Texel sheep, the German Merino, the German milk and land sheep breeds examined here the frequency of the $PRNP^{ARR}$ allele was much lower and for several breeds no homozygous rams were available for breeding purposes. The breeding strategy in those breeds depends on the number of heterozygous animals. Nevertheless, resistant pedigree flocks can be achieved in nine generations at the most without loosing genetic heterogeneity of the breed.

## C011
## Segregation analyses of deafness in a large pedigree of Dalmatians

CLAUDE GAILLARD[1], VILMA YUZBASIYAN-GURKAN[2], EMILY ROBINSON NOLAN[2], TAKESHI MIYAKE[1], ANDRÉ JAGGY[3], GAUDENZ DOLF[1]
[1]*University of Bern, Institute of Animal Genetics, Bern, Switzerland;* [2]*Michigan State University, Small Animal Clinical Sciences, East Lansing, MI, USA;* [3]*University of Bern, Dept. of Clinical Veterinary Medicine, Bern, Switzerland*

Sensorineurale deafness is a common congenital disorder in Dalmatians with a genetic background for which different modes of inheritance have been proposed. The objective of our study was to investigate the mode of inheritance of this disease by segregation analyses using maximum likelihood procedures. These analyses were performed under four models, namely a major gene, a mixed inheritance, a polygenic and an environmental model. The data of a large population from the USA and a smaller one from Switzerland were pooled as the diagnoses were established based on the same protocol (brainstem auditory evoked responses) and the incidences were quite similar. A total of 1697 dogs with 1441 known phenotypes entered the study. The prevalence of deafness was 19.2% (14.3% unilaterally deaf, 4.9% bilaterally deaf). Females showed significantly ($p=0.030$) more affected animals (21.6%) than males (16.3%). The data were analyzed in two sets, the first including normal, uni- and bilateral deaf dogs, and the second normal and deaf dogs. With the first set (3 liability classes) the differences between the genetic models was not significant. With the second set (2 liability classes) the mixed inheritance model was significantly better than all other models. This model showed the presence of a major gene with a completely recessive deleterious allele ($q=0.05$) and important polygenic effect ($h^2=0.62$).

## C012
## Characterization of bovine ectodysplasin 1 (*ED1*) mutations in cattle with hypotrichosis and oligodontia

CORD DRÖGEMÜLLER, OTTMAR DISTL, TOSSO LEEB
*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany*

The ectodysplasin 1 gene (*ED1*) encodes a signalling molecule of the tumor necrosis factor (TNF) family that is involved in fetal development of ectodermal appendages. Mutations in the *ED1* gene are responsible for X-linked anhidrotic ectodermal dysplasia (ED1) characterized by impaired development of hair, teeth, and eccrine sweat glands in human and mice. We report the construction of a 480 kb BAC contig harboring the complete bovine *ED1* gene on BTA Xq22-q24. A large genomic

region including exon 3 of the *ED1* gene is deleted in cattle with hypotrichosis and oligodontia in a family of black and white German Holstein cattle with four affected maternal half sibs. An RT-PCR assay demonstrated the X-linked recessive segregation of the *ED1* deletion and established the grand-maternal origin of the mutation. A second case of congential X-chromosomal hypotrichosis and oligodontia in a family of red and white German Holstein cattle with three affected males is caused by a point mutation within the 5' splice site following *ED1* exon 8b. Interestingly, cDNA sequencing analyses revealed that this mutation alters the splicing of both alternative *ED1* transcripts thus indicating the presence of an important regulatory element for the correct splicing of the *ED1* transcripts at the exon 8 / intron 8 junction. As the clinical, pathological and genetic findings in human ED1 show similarities to the described phenotype in cattle, this bovine disorder may serve as an animal model for human ED1.

**C013**

**Transcript profiling of bovine leukemia virus (BLV) infected and uninfected cell lines using a cDNA microarray containing 7653 cattle genes**

ROBIN E. EVERTS[(1)], MARK R. BAND[(2)], ZONGLIN L. LIU[(1)], JONATHAN U. PELED[(1)], CHARU G. KUMAR[(1)], AL BARI[(2)], LEI LIU[(2)], & HARRIS A. LEWIN[(1,2)]
*[(1)]Department of Animal Sciences and [(2)]The W.M. Keck Center for Comparative and Functional Genomics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

A 7653-element cDNA microarray was used to investigate the effects of bovine leukemia virus (BLV) infection on host gene expression. Transcription profiling was performed on the uninfected B-lymphocyte cell line $BL3^0$ and its infected, genetically identical daughter cell line $BL3^*$. A standard RNA hybridization control was developed that consists of RNA extracted from 3 cell lines and brain tissue from 3 heifers. The reference RNA hybridizes to ~90% of the spots on the array. The sequences on the array were derived primarily from a normalized and subtracted cattle placenta cDNA library. Annotation was based on similarity searches using BLASTN and TBLASTX against the human UniGene database. Of the 7653 sequences, 6039 had an expectation (e) value better than $e^{-5}$ with at least one sequence in a UniGene cluster. GeneOntology (GO) terms were parsed from LocusLink and putative functions were assigned to a significant fraction of the genes on the array. Approximately 170 genes were upregulated and 100 genes were downregulated in $BL3^*$ as compared to $BL3^0$. BLV infection was associated with decreased transcript levels of 23/46 genes encoding ribosomal proteins suggesting a negative effect on protein synthesis. By contrast, 10 transcription factors and many genes associated with cell cycle and cell proliferation were upregulated in $BL3^*$. These results demonstrate the power of microarray analysis for studying the effects of retrovirus infection on host gene expression.

**C014**

**A second generation ordered comparative map of the cattle and human genomes**

ANNELIE EVERTS-VAN DER WIND[1], SRINIVAS KATA[3], SHREEDHAR NATARAJAN[2], MARK R. BAND[2], MARK REBEIZ[1], LEI LIU[2], TOM GOLDAMMER[3], CHERYL A. GREEN[1], STEPHANIE McKAY[3], ROBIN E. EVERTS[1], JIM E. WOMACK[3], HARRIS A. LEWIN[1,2]
*[1]Department of Animal Sciences; [2]W. M. Keck Center for Comparative and Functional Genomics, University of Illinois at Urbana-Champaign, Urbana, IL*
*[3]Department of Vete-rinary Pathobiology, Texas A & M University, College Station, TX*

A second generation ordered comparative map of the cattle and human genomes was produced using a 5000 rad whole genome radiation hybrid panel and *in silico* predictive mapping tools. A total of 720 new markers were added to the published map, bringing the total number of mapped markers to 2034 with LOD >9. Of these, 1423 are cattle genes or ESTs with significant ($E<e^{-5}$) hits in human UniGene of which 1179 have GB4 mapping data, 349 are framework microsatellites and 262 are cattle ESTs with no hits against human UniGene. Most of the newly mapped genes were chosen using the COMPASS software, allowing specific targeting of gaps in the comparative map as well as sparsely populated map intervals. The new version, COMPASS II, uses the new update of UniGene as a target for BLASTN search and annotation, and the first generation whole-genome comparative map as the basis

for prediction of cattle chromosome and RH bin location. Comparative coverage is 94%, with average marker spacing on the human GB4 map of 9.2 cR. The large number of genes and ESTs mapped in this study allows for a comprehensive comparison of gene order between the cattle and human genomes. The number of conserved segments and genomic rearrangements is considerably large, but still smaller than between human and mouse. This new comparative map will provide a resource for phylogenomic analysis of mammalian chromosomes and will greatly facilitate the identification of candidate genes for economically important traits.

**C015**

**Study of candidate genes for glycolytic potential in pig muscle**

LUCA FONTANESI, ROBERTA DAVOLI, LEONARDO NANNI COSTA, EMILIO SCOTTI, VINCENZO RUSSO
*DIPROVAL - Sezione di Allevamenti Zootecnici, Faculty of Agriculture, University of Bologna, Reggio Emilia, Italy*

Glycolytic potential (GP) of porcine skeletal muscles has been shown to be a predictive measure of meat quality. A high value of GP may be caused by alterations of the glucose metabolism in this tissue. Thus, for this study, we selected as candidate genes those that code for enzymes that play an essential role in the glycolysis and glycogen metabolism of the skeletal muscle. Physical mapping was obtained for *PYGM* (Sscr 2), *ALDOA* (Sscr 3), *AGL* (Sscr 4), *PFKM* (Sscr 5), *GYS1* (Sscr 6), *PKM2* (Sscr 7), *ENO3* (Sscr 12), *GAA* (Sscr 12), *PRKAB1* (Sscr 14) and *PGAM2* (Sscr 18). SNPs were identified for *PYGM*, *LDHA*, *PKM2*, *GAA*, *PRKAB1* and *PGAM2* and linkage mapping was obtained for the latter four genes. Allele frequencies for these loci were studied in seven pig breeds (Large White, Landrace, Duroc, Belgian Landrace, Piétrain, Hampshire and Meishan). GP of *m. biceps femoris* at 1 and 24 hrs *post mortem* and other meat quality traits were measured in 507 commercial pigs. Among these animals, 60 pigs were selected according to the GP value and typed to exclude the presence of the *RYR1 n* allele and of the *PRKAG3 RN*$^-$ allele. Moreover these animals were analysed for the markers identified and for a polymorphism already presented in literature for *GPI* and association analyses were performed. The results indicate significant associations of *PKM2* with GP and glycogen content and of *GPI* with lactate value at 1 hr *post mortem* ($P < 0.05$).

**C016**

**Characterization of the equine *AEG1* gene and its role in stallion fertility**

ALEXANDER GIESE[1], RONY JUDE[1], HEIDI KUIPER[1], FRANCOIS PIUMI[2], ALEXANDRA SCHAMBONY[3], HARALD SIEME[4], GÉRARD GUÉRIN[2], OTTMAR DISTL[1], EDDA TÖPFER-PETERSEN[3], TOSSO LEEB[1]
*[1]Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany; [2]INRA, Laboratoire de Génétique biochimique et de Cytogénétique, Jouy-en-Josas, France; [3]Institute of Reproductive Medicine, School of Veterinary Medicine Hannover, Hannover, Germany;*
*[4]Hanoverian State Stud Celle, Celle, Germany*

Acidic epididymal glycoprotein 1 gene (AEG1), also known as cysteine-rich secretory protein 1 (CRISP1), is a member of the CRISP protein family. The CRISP proteins are characterized by 16 conserved cysteine residues at the C-terminus and expressed in the male genital tract. It has recently been shown that the amount of CRISPs that are tightly bound on sperm surfaces correlates with the fertility of stallions. Therefore, their genes are of interest as candidate genes for inherited male fertility dysfunctions and as putative quantitative trait loci for male fertility traits. Here, we report the cloning and DNA sequence of 90 kb of horse genomic DNA from equine chromosome 20q22 containing the complete equine *AEG1* gene, which consists of eight exons spanning 31 kb. The transcription start site was mapped by the RLM-RACE technique and three closely spaced transcription start sites were annotated. Further analysis of equine *AEG1* transcripts did not reveal any evidence for alternative splicing. RT-PCR analysis demonstrated that *AEG1* is expressed in different parts of the epididymis, whereas it is hardly detectable in the testis. The naturally occurring diversity of the equine *AEG1* gene in different horse breeds was investigated and several polymorphisms are reported, including one that affects the amino acid sequence.

**C017**

**Identification and chromosome assignment of genes potentially different expressed in meat and dairy cattle**

TOM GOLDAMMER[1], UTE DORROCH[1], RONALD M. BRUNNER[1], SRINIVAS R. KATA[2], JAMES E. WOMACK[2], MANFRED SCHWERIN[1]

*[1]Research Unit for Molecular Biology, Research Institute for the Biology of Farm Animals, Dummerstorf, Germany; [2]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA*

We investigate in the identification and localization of genes potentially different expressed in liver and intestine of lactating German Holstein and Charolais cows to analyze active metabolic pathways and chromosome regions that could cause the different phentotypes of these milk and meat type cows. The homology search for twenty-four bovine expressed sequence tags (ESTs) in human genome data bases resulted in hits with high sequence similary with 18 genes and 6 unknown coding sequences in genomic DNA clones. The human DNA sequences were comparative assigned in the bovine genome using a cattle-hamster somatic hybrid cell panel, a cattle-hamster 5000 rad whole genome radiation hybrid panel, and fluorescence *in situ* hybridization (FISH). Synteny could be declard for 20 ESTs. Twenty-three ESTs were RH-mapped and assigned in the established cattle $WGRH_{5000}$ map. The software RHMAPPER was used for calculation of RH-mapping data. Two genes were assigned by FISH on cattle chromosomes. The identified genes represent potentially candidates for the description of metabolic differences in meat and dairy cows at the gene transcription level and generally for economical important traits. The new loci contribute to the completion of the bovine gene map and the comparative assignments will increase our knowledge about genome evolution between cattle and human.

**C018**

**Functional analysis of bovine MC1R and ASP using *in vitro* cell systems**

DARIA A. GRAPHODATSKAYA, HANNES W. JOERG, GERALD F. STRANZINGER

*Swiss Federal Institute of Technology, Institute of Animal Sciences, ETH-Zentrum, Zurich, Switzerland*

Coat colour in mammals is determined by the relative amounts of eumelanin (black) and pheomelanin (red) produced in melanocytes. The mealanocortin 1 receptor (MC1R) is activated by α-melanocyte stimulating hormone (α-MSH) which increases the amount of cAMP in the cell. This activates tyrosinase resulting in eumelanin synthesis. The Agouti signalling protein (ASP) acts as an antagonist to MC1R by binding to the receptor and preventing the MC1R-MSH interaction. The cAMP concentrations were measured in cells lines expressing 5 MC1R alleles and stimulated with α-MSH. The recessive red allele **e** and the dominant black allele $\mathbf{E^D}$ were unresponsive to a wide range of αMSH concentrations. Two alleles from brown cattle $\mathbf{E^{d1}}$, $\mathbf{E^{d2}}$ and one allele found in red cattle $\mathbf{e^f}$ responded to an α-MSH stimulation in a dose-dependent manner. Cells transfected with the $\mathbf{e^f}$ allele reached the same cAMP concentrations as those with the $\mathbf{E^{d1}}$ and $\mathbf{E^{d2}}$ alleles at 10 times a higher concentration of α-MSH. These results indicate that the **e** MC1R allele is a non-functional receptor, $\mathbf{E^D}$ is a constitutively activated receptor, and $\mathbf{E^{d1}}$ and $\mathbf{E^{d2}}$ are hormone activated receptors. The delay in the $\mathbf{e^f}$ allele response may explain the similarity of **e** and $\mathbf{e^f}$ phenotype. No differences were found in the coding sequences of ASP between red, black and brown cattle. Incubation of the cells transfected with ASP together with the cells transfected with $\mathbf{E^{d1}}$ and $\mathbf{E^{d2}}$ resulted in decreased cAMP production.

**C019**

**The bovine gastrointestinal tract: A gene expression profile**

CHRISTIANE HANSEN[1], ANNA FU[1], YAN MENG[1], CHANGXI LI[1], ERASMUS OKINE[1], CHRISTOPH W. SENSEN[2], PAUL GORDON[2], STEPHEN S. MOORE[1]

*[1]Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB Canada; [2]Dept. of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB Canada*

Understanding the genetic mechanisms at work in the bovine gastrointestinal (GI) tract would aid considerably in our understanding of ruminant digestion. However, while the gross functions of most regions of the bovine GI tract are known, a lack of information concer-

ning the specific processes occurring in each region exists. In addition, little is known about these regions from a genetic point of view. We have used gene expression profiling of the bovine rumen, reticulum, omasum, abomasum, duodenum, jejunum and ileum to examine differences between them. Directionally cloned cDNA libraries of each of the tract's regions were constructed using the Stratagene ZAP cDNA synthesis kit and approximately 2000 clones were sequenced for each region. Sequences were submitted to the MAGPIE program, a system for the automated analysis of biological sequences, functional assignments were made for the various ESTs, gene ontology assignments were then made and sequences were assembled into contigs where possible. It was found that significant differences in gene expression exist as one moves from one compartment to the next. While lysozymes are very abundant in the abomasum, for example, they are virtually absent from the other regions. Ribosomal sequences too showed differences in expression between regions. Finally, a number of the GI regions did not appear to express a particular type of sequence preferentially. A comprehensive overview of the differences found will be presented.

**C020**

**Molecular cloning and characterization of mRNA for *Equus caballus* tenomodulin gene (*TNMD*)**

TELHIAS HASEGAWA[1], MUTSUMI MATSUTA[1], FUMIO SATO[1], ATSUTOSHI KUWANO[2]

*[1]Japan Racing Association, Equine Research Institute, Laboratory of Molecular and Cellular Biology, Utsunomiya, Japan; [2]Japan Racing Association, Equine Research Institute, Clinical Sciences and Pathobiology Division, Utsunomiya, Japan*

Although the superficial digital flexor tendon (SDFT) is frequently injured in equine athletes and presenting a significant problem in equine sports medicine, there are not enough information regard to mechanisms on development and regeneration of tendon tissues. Because tendon cells, so called 'tenocytes', should contribute to both processes, characterization of tenocytes is essential in these studies. Thus, we attempted to clone and characterize an mRNA encodes equine tenomodulin (synonyms: tendin, chondromodulin-I like protein, and ChM-IL), a recently discovered member of proteins produced in tendon tissue. Tenomodulin has been mined from human and murine EST databases by similarity with chondromodulin-I (ChM-I) mRNA, an anti-angiogenic extracellular matrix (ECM) protein secreted by cartilage cells. The reconstructed 1214 bases nucleotide sequence of equine tenomodulin encodes 371 amino acid sequence, and showed high similarity and common motifs with human and murine tenomodulin sequences. The expression of this molecule in the regenerating part of injured SDFT tissue had been visualized by *in situ* hybridization. Therefore, the role and function of this molecule are subjected to study as well as its possibility as a molecular marker of functional tenocytes.

**C022**

**African Swine Fever virus (ASFV): microrarray analysis of differential gene expression in porcine macrophages**

PAUL A. HOPWOOD[(1)], FUQUAN ZHANG[(2)], CHARLES ABRAMS[(2)], LINDA DIXON[(2)], ALAN ARCHIBALD[(3)], RICHARD TALBOT[(3)], DAVE BURT[(3)] &. STEWART LOWDEN[(1)]

*[(1)] Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK. [(2)] Institute of Animal Health, Pirbright, Surrey, UK [(3)] UK Centre for Functional Genomics in Farm Animals, Roslin Institute, Edinburgh, Scotland, UK*

A porcine cDNA microarray has been constructed to examine gene expression profiles in macrophages infected with African swine fever virus (ASFV). ASFV is a serious threat to the pork industry worldwide, causing rapidly fatal haemorrhagic fever in domestic pigs. However, although the virus infects African species (e.g warthog and bushpig), they are resistant to disease through unknown mechanisms. Using a microarray to investigate global gene expression in infected cells has provided insights into the virus-host interaction & immune-evasion strategy and to improved understanding of viral pathogenesis. The parallels with other haemorrhagic fevers such as Ebola and Marburg suggest ASFV as a valuable model for these diseases.

The microarray consists of 2500 cDNAs comprising selectively cloned target transcripts including cytokines, cell surface

markers and signal transduction molecules, and clones derived from subtracted macrophage libraries. To establish the differential gene expression pattern associated with ASFV infection, the microarray has been interrogated with probes from *Sus scrofa* macrophages; both uninfected and infected *in vitro* with a virulent strain of ASFV. We will present this data and also describe ongoing experiments which aim to investigate gene expression in macrophages from ASFV-resistant species and cells infected with mutant virus. These studies will further elucidate the mechanisms of viral pathogenesis and host-resistance.

**C023**
**Polymorphic markers for three genes expressed in the horse macrophage**

PETR KRÁLÍK, LUDMILA LUKESZOVÁ, JÁN MATIAŠOVIC, PETR HOŘIN
*University of Veterinary and Pharmaceutical Sciences, Institute of Animal Breeding and Genetics, Brno, Czech Republic*
Detection of polymorphisms of genes expressed in macrophages is a prerequisite for application of functional genomics in studying mechanisms of innate immunity and of genetic resistance to intracellular pathogens. Here, polymorphisms of three horse genes crucial for macrophage function were studied. A part of the horse interferon-gamma gene from 5' UTR down to exon 4 was amplified and screened using a set of restriction endonucleases. Two polymorphic sites were found by AfaI, one localized within intron 1 and the second within intron 3. Homozygotes as well as heterozygotes in both polymorphic sites were found in Old Kladruber horses. Thoroughbreds were found to be polymorphic only in the intron 3 polymorphic site. A PCR-RFLP method for detecting previously reported allelic variants of the interleukin-12 p40 gene was developed. A 1587 bp long PCR product containing polymorphic nucleotide position 714 in exon 6 was cleaved with the Aci I restriction endonuclease detecting the A/G substitution involved. All three possible genotypes were identified in Old Kladruber horses. A *GT* microsatellite was found within the 3´UTR of the TNF-alpha receptor 1 gene. Preliminary results suggest existence of at least three alleles whose length varied from 42 to 58 GT repeats.

**C024**
**Characterisation of Gene Expression in *RN*-Pigs Carrying a Mutation in *PRKAG3***

PER HORN[1], JAKOB HEDEGAARD[2], RENE LAMETCH[2], EMØKE BENDIXEN[2], CHRISTIAN BENDIXEN[1]
*Departments of [1]Animal Breeding and Genetics; [2]Animal Product Quality; Danish Institute of Agricultural Sciences, P.O. Box 50, DK-8830 Tjele, Denmark*
The porcine *RN*-genotype has large effects on meat quality and processing yields. This genotype possess a dominant mutation resulting in a ~ 70 % increase in muscle glycogen content in glycolytic muscle cells, which is converted into lactate postmortem resulting in a lowered ultimate pH. Furthermore, a number of other physiological changes are observed, including enlarged sarcoplasmatic compartments and higher density and altered morphologically of mitochondria. The *RN*$^-$ gene has been mapped to chromosome 15 and was recently identified as the *PRKAG3* gene encoding a muscle specific isoform of the regulatory $\gamma 3$ subunit of adenosine monophosphate activated protein kinase (AMPK). The *RN*-genotype is a single nucleotide substitution in *PRKAG3* causing a change from arginine to glutamine.
We have examined the gene expression patterns in the *RN*-pigs by restriction fragment differential display (RFDD), 2D-gels and Northern blot analysis. RFDD showed the majority of differentially expressed genes to be of mitochondrial origin. Further analysis showed a general increase in mitochondrial gene expression probably accounting for the higher respiration seen in *RN*-pigs and this phenomenon is likely to be explained by the higher density of mitochondria. The 2D-gels showed an elevated expression of UDP-glucose pyrophosphorylase 2 (*UDPG2*). This observation was confirmed by Northern blotting and can, at least in part, explain the higher glycogen content seen in the *RN*-pigs.

**C025**
**Characterization of the porcine peroxisome proliferator activated receptor gamma coactivator 1 *(PPARGC1*)**

Kathleen Jacobs[1], Gary Rohrer[2], Heinz Barthenschlager[3], Antonin Stratil[4], Mario Van Poucke[1], François Piumi[5], Martine Yerle[6],

Marc Mattheeuws[1], Alex Van Zeveren[1] & Luc Peelman[1]
[1]*Department of Animal Nutrition, Genetics, Breeding and Ethology, Ghent University, Merelbeke, Belgium,* [2]*USDA ARS US Meat Animal Research Center, Clay Center, NE, USA,* [3]*Institute of Animal Husbandry and Breeding, Department of Animal Breeding and Biotechnology, University of Hohenheim, Stuttgart, Germany,* [4]*Institute of Animal Physiology and Genetics, Academy of Sciences of Czech Rpublic, 277 21 Libechov, Czech Republic,* [5]*Laboratoire de Radiobiologie et d'étude du génome, INRA CEA jouy-en-Josas, France &* [6]*Laboratoire de Génétique Cellulaire, INRA, Castanet-Tolosan Cedex, France.*
Peroxisome proliferative activated receptor gamma coactivator 1 (PPARGC1) is a coactivator of nuclear receptors with an important function in adaptive thermogenesis. It influences genes involved in regulation of body weight and composition. Therefore, *PPARGC1* can be considered as a candidate gene for carcass and meat quality traits. A BAC clone, isolated using a *PPARGC1* PCR fragment has been mapped by FISH to Sscr8p21. A $(CA)_n$-microsattelite (*SGU0001*) isolated from the BAC has been mapped to porcine chromosome 8 by RH mapping. The most significantly linked marker (2pt analysis) is *SWR1101* (57cR; LOD=5.47). *SGU001* was also mapped at the same position as marker KS195 (32.5 cM) by linkage mapping on the MARC reference family. *PPARGC1* was located between the most proximal marker *SW905* and the following *SW933* with the respective supports 8.03 and 11.92 in sex specific linkage analysis on the Hohenheim reference family by using an AseI polymorphism located in exon 8 of the gene. The coding exons of the porcine gene were sequenced and compared to human, mouse and rat sequences. The coding regions and surrounding sequences harbouring known splice sites of the gene were scanned for polymorphisms. Five intronic and four exonic SNP's were found. No SNP's were found in known regulatory sequences. Some exonic SNP's have effect on the translation product of the gene. Transcription of the gene was detected by RT-PCR in porcine adipose, muscle, kidney, liver, brain and heart tissues and in lower amounts in duodenum and adrenal tissues.

**C026**
**Partial cloning and chromosomal mapping of the porcine *ADRP* gene**

TAE-HUN KIM[1], NAM-SUN KIM[1], BONG-HWAN CHOI[1], JUN-HEON LEE[2], CHRIS MORAN[2], IL-CHEONG CHEONG[1]
[1]*Animal Genomics & Bioinformatics Division, National Livestock Research Institute, RDA, Suwon 441-350, Republic of Korea;* [2]*Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2006, Australia*
Adipose differentiation related protein (*ADRP*) expression increases rapidly from a very low level as undifferentiated adipocytes mature. We report here the molecular cloning, characterization, and chromosomal localization of the porcine *ADRP* gene. Partial cDNA was amplified from total RNA of porcine adipose tissues by RT-PCR using consensus primers designed from human and cattle sequences. PCR products of 700 bp and 580 bp were cloned and sequenced. 1213 bp of porcine sequence was compared with human, cattle, and mouse showing amino acid sequence identities of 89%, 89% and 82%. Analysis of the amplification pattern in the 27 clones of the French porcine-rodent somatic cell hybrid panel allowed regional assignment to SSC1q2.3–q2.7. The human location at HSA9p21.3 and porcine location are consistent with the known syntenic relationship between SSC1 and HSA9. The characterization of this gene provides a useful starting point for evaluation of a positional candidate gene for frequently reported fatness QTL in the long arm of SSC1.

**C027**
**Candidate genes for progressive external ophthalmoplegia (PEO) in cattle**

HEIDI KUIPER[1], GABI HAUKE[1], CORD DRÖGEMÜLLER[1], TOSSO LEEB[1], JOHN L. WILLIAMS[2], OTTMAR DISTL[1]
[1]*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany;* [2]*Roslin Institute (Edinburgh), Rosling, Midlothian, Scotland*
Bilaterial convergent strabismus with exophthalmus (BCSE) is a widely spread hereditary disease in many cattle populations. It is known that autosomal dominant progressive external ophthalmoplegia with various mitochondrial DNA deletions in man can be caused

by mutations in the genes *C10orf2* (chromosome 10 open reading frame 2), *POLG,* (polymerase (DNA directed) gamma) and *SLC25A4* (solute carrier family 25, member 4). In our study we mapped the three genes *C10orf2, POLG,* and *SLC25A4* by flourescences *in situ* hybridization on bovine metaphase spreads. Using a comparative approach human cDNA clones from these genes were obtained to screen the bovine RPCI-42 BAC library. Positive bovine BAC clones were labelled with digoxigenin and then used for FISH on GTG banded chromosomes. The cattle ortholog of the gene *POLG* was mapped to BTA21q17-q22 and the orthologs of *C10orf2* and *SLC25A* were mapped to BTA26q13-q21 and BTA27q14-q15, respectively. The results of our FISH experiments were corroborated by radiation hybrid mapping of STS markers derived from the BAC end sequences using the 3,000 rad Roslin/Cambridge bovine whole-genome radiation hybrid panel. RH mapping results confirm the known synteny conservation between parts of HSA15 with BTA21, parts of HSA10 with BTA26, and HSA 4q with BTA27. The development of microsatellites of the candidate gene containing BAC clones provides the basis for future linkage studies that might clarify the role of these genes for BCSE in German Brown cattle.

**C028**

**A non-sense mutation in the *FMO3* gene causes fishy off-flavour in cow's milk**

STEFAN MARKLUND, LEIF ANDERSSON, VICTORIA GUSTAFSSON, ANNE LUNDÉN

*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden*

Fishy off-flavour sometimes occurs in milk from cows of the Swedish Red and White breed (SRB). It was recently shown that the off-flavour comes from high levels of trimethylamine (TMA) in the milk. 'Fish-odour syndrome' is a similar human phenotype characterized by high levels of TMA in the body fluids caused by recessive loss-of-function mutations in the *FMO3* gene encoding flavin-containing monooxygenase 3. This liver enzyme converts TMA to the odourless TMA oxide. We used cross-species PCR on genomic DNA to amplify and determine ~50% of the coding *FMO3* sequence from two cows producing milk with a fishy off-flavour and normal milk, respectively. The affected cow was homozygous for a R238X nonsense mutation that prevents >50% of the peptide synthesis. A pyrosequencing test showed a strong association between this mutation and off-flavour milk in a case/control material. The R238X frequency among 100 SRB individuals was 0.155 whereas we did not find the mutation in samples from a few other breeds. Sequence analysis of RT-PCR products from cattle liver mRNA revealed only barely detectable levels of the mutant *FMO3* transcript in two heterozygous carriers of R238X. This could probably be explained by nonsense-mediated mRNA decay (NMD).

**C029**

**Characterization of perixome profilator-activated receptor gamma (PPARγ) isoforms in the pig**

TOSHINORI OMI[1], ŠPELA ŠPILAR[1], BERTRAM BRENIG[2], GERALD STRANZINGER[1], STEFAN NEUENSCHWANDER[1]

[1]*Swiss Federal Institute of Techology, Institute of Animal Sciences, Zürich, Switzlerland;* [2]*University of Göttingen, Insitute of Veterinary Medicine, Göttingen, Germany*

Peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor which controls genes that are involved in the regulation of energy metabolism, cell differentiation, apoptosis and inflammation. So far, two PPARγ isoforms (γ-1b, γ-1c and γ-1d) which are isolated from a porcine fat tissue cDNA library or by RT-PCR using total RNA from fat tissue. The cDNA structure and genomic organization of the PPARγ showed that free exons are associated with the γ-1 cDNAs (A1, A', A2) and one exon (B) with the γ-2 cDNA. PPARγ-1b is a splice variant of the known γ-1a starting with exon (B) with the γ-2 cDNA. PPARγ is a splice variant of the knwon γ-1a starting with exon A1, whereas PPARγ-1c and γ-1d start from a seperate leader exon (A') which is located approximetaly 1kb downstream of exon A1. Based on sequence comparisons we found 6 SNPs in the PPARγ and γ-2 sequence. The relevance of the allelic variants will be investigated. Furthermore, expression fat γ-2 and γ-1 showed the same expression level whereas in the subcutaneous fat more γ-1 expressed than γ-2. Among PPARγ-1 transcripts the γ-1a isoform is most abundant

and the transcripts which do not contain exon A2 are much less expressed.

**C030**
**Analysis of nucleosome – Stat5 relationship in βLG transgene expression**

RAMONA N. PENA, BRUCE WHITELAW
*Division of Gene Expression and Development, Roslin Institute, Roslin, Scotland, UK*

The proximal promoter of sheep *β-lactoglobulin* (βLG) gene is well characterised and includes three Stat5 binding sites, 190-bp away, which promote prolactin induction of this gene. Using the monomer extension technique we have previously identified a strong sequence dependent nucleosome positioning over the proximal promoter of this gene which coincides with the region spanning the three Stat5 sites. In order to further analyse the role of this nucleosome-directing DNA sequence we generated six constructs where the region comprising the three Stat5 sites was inserted (both in forward and reverse orientations) at several distances from the original site. The insertion of these short DNA sequence enhanced gene expression in stably transfected HC11 cells in all forward-inserted constructs. In a second set of constructs a LacZ-based reporter gene replaced the βLG transcription unit. For each construct, the total level of LacZ expression was correlated to the percentage of expressing cells as assessed by a FACS/Scan protocol. We are currently analysing the *in vivo* effect of this sequence repetition in transgenic mice. Expression studies and nucleosome-position analysis is currently underway in these animals.

**C031**
**Mapping of candidate genes for nonsyndromic sensorineural deafness in dogs**

SIMONE G. RAK[1], CORD DRÖGEMÜLLER[1], HEIDI KUIPER[1], TOSSO LEEB[1], PASCALE QUIGNON[2], CATHERINE ANDRÉ[2], OTTMAR DISTL[1]
[1]*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany;* [2]*UMR 6061 CNRS, Génétique et Développement, Faculté de Médecine, 35043 Rennes Cedex, France*

Congenital nonsyndromic sensorineural deafness has been reported in several livestock species and, particularly, in more than 60 different breeds of dogs. The incidence of this inherited congenital anomaly is highest in Dalmatian dogs with 20-30%. Mutations in various genes are causative for congenital nonsyndromic hearing impairment in humans or mouse and 26 of these genes were chosen as candidates for mapping in dogs. In an effort to identify these canine genes, human $^{32}$P–labeled inserts of IMAGE cDNA clones were used to screen the high density filters of the RPCI-81 canine BAC library. Positive BAC clones were isolated and the BAC end sequence data were used to design canine-specific primers for mapping on the canine RHDF5000 radiation hybrid panel. The BAC clones were labeled by nick translation and used for fluorescence in situ hybridization (FISH) on GTG-banded canine metaphase chromosomes. Identification of the chromosomes was done according to the established GTG- and DAPI-banded karyotype of the domestic dog. So far, 17 candidate genes have been mapped on the canine genome. The obtained results confirm the established synteny of the latest integrated canine RH map. The development of microsatellite markers of the candidate gene containing BAC clones provides the basis for future linkage studies that might clarify the role of these genes for congenital nonsyndromic sensorineural deafness in Dalmatian dogs.

**C032**
**Statistical Analysis of Gene Expression Data from Hypertrophying and Normal Muscle Tissue**

JAMES M. REECY[1], DAN NETTLON[2]
[1]*Department of Animal Science;* [2]*Department of Statistics, Iowa State University, Ames, IA 50011*

Knowledge of the molecular mechanisms underlying skeletal muscle growth could lead to new methods for enhancing lean tissue deposition in livestock. Affymetrix GeneChip technology was used to measure the relative levels messenger RNA transcripts in the soleus muscle isolated from gastrocnemius ablated or sham operated rats. We used this data on transcript abundance to screen for evidence of increased or decreased gene expression in hypertrophying tissue relative to control tissue. An ANOVA approach, coupled with bootstrapping of residuals, was used to determine a p-value for each gene, where small p-values are indicative of differential expression be-

tween treatment and control tissues. Resampling-based multiple testing techniques were used to adjust p-values to control the overall type I error rate and provide a list of genes that may play a role in promoting skeletal muscle growth. We found 19 genes with step-down adjusted p-values less than 0.05. The small number of animals used in this study (three rats per treatment) is a concern. To address this concern we computed adjusted p-values designed to control the false discovery rate. These FDR-adjusted p-values are designed to produce a list of differentially expressed genes of which a certain percentage (5%) is believed to be false positives. We found 125 genes with FDR-adjusted p-values less than 0.05. The use of FDR analysis dramatically increases the number of differentially expressed genes.

**C033**

**Relationship between expression patterns of *C/EBP family* and *PPARgamma* genes and marbling scores in the muscle of Japanese Black Cattle**

REI SAKOYAMA, YUKIO TANIGUCHI, TAKAHISA YAMADA, YOSHIYUKI SASAKI
*Graduate School of Agriculture, Kyoto University, Kyoto, Japan*

Intramuscular fat accumulation is an economically important trait in beef cattle and an unique but yet unidentified molecular mechanism is thought to be involved in the expression of this trait. As a first step to elucidate this mechanism, we investigated expression patterns of the transcription factors which play important roles in adipocyte development, i.e., C/EBPalpha, beta and delta and PPARgamma, in the muscle in accordance with marbling scores. Competitive-PCR analysis revealed that expression levels of C/EBPalpha and PPARgamma but not C/EBPbeta and delta, increased in proportion to an increase of marbling scores. In immunohistochemical study, we detected C/EBPalpha signals mainly in intramuscular fat cell nuclei, C/EBPbeta and delta signals mainly in cell nuclei in connective tissue and muscle bundle and rarely in intramuscular fat cell nuclei, and PPARgamma signals mainly in cell nuclei in connective tissue and in some degree in intramuscular fat cell nuclei. The result of association of C/EBPalpha and PPARgamma expression levels with marbling scores is consistent with immunohistochemical detection of preferential signals for C/EBPalpha and PPARgamma in intramuscular fat cell nuclei over the muscle tissue. Thus, C/EBPalpha and PPARgamma may be useful as indicators for marbling scores in finished cattle. At present, we are going to investigate age-dependent expression patterns of these factors in *Musculus longissimus* of Japanese Black and Holstein breeds and to elucidate the molecular mechanism underlying intramuscular fat development during bovine fattening.

**C034**

***TYRP1* mutations in dogs affect coat and nose color**

SHEILA M. SCHMUTZ, TOM G. BERRYERE
*University of Saskatchewan, Department of Animal and Poultry Science, Saskatoon, Canada*

Skin biopsies of several dogs of various coat colors were used to prepare mRNA and cDNA which allowed us to obtain the first 1536 base pairs of the 1612 bp of tyrosinase related protein 1 (*TYRP1*). Three polymorphisms were found to be associated with brown coat color in dogs which had normal melanocortin 1 receptor (*MC1R)* sequence: a premature stop codon in exon 5, a proline deletion in exon 5, and a cysteine to serine change in exon 2. These mutations caused brown nose and pad color in dogs which had at least one copy of wild type *MC1R*. Dogs which were homozyogous for a premature stop codon in *MC1R*, had yellow to red coat color but the pigmentation of their nose and pads was brown instead of black when they contained 2 copies of these *TYRP1* mutations. All 100 dogs from 29 breeds fit the coat and nose colors predicted by these mutations. No differences in shade of brown were noted amongst homozygotes of the 3 different *TYRP1* mutations. All 3 mutations are proposed to be relatively old since they all occur in longhaired, shorthaired, and wirehaired breeds. This study documented gene interactions at the molecular level between *MC1R* and *TYRP1* which were proposed by Little (1957) and Winge (1950), but not been previously proven.

**C035**

**Interacting phenotypic effects of co-existing variants within a single gene - cellular stress response is significantly affected by interactions between promoter and 3´-UTR variants of the porcine *hsp70.2* gene**

MANFRED SCHWERIN[1], STEFFEN MAAK[2], RAINER FUERBASS[1]
[1]*Department of Molecular Biology, Research Institute for the Biology of Farm Animals, Dummerstorf, Germany,* [2]*Institute of Animal Breeding and Husbandry with Veterinary Clinic, Martin-Luther-University, D-06108 Halle, Germany*

Cellular tolerance to stress is mediated by a family of proteins termed heat-shock proteins (HSP). As shown in this study of porcine primary fibroblasts *hsp70.2* is induced to produce abundant amounts of transcript upon heat shock treatment. However, transcript levels and heat shock response was found to be different in various individuals. While previously described functional promoter variants of this gene can partly account for the high variability of heat-induced transcript levels, they are unrelated to the observed highly variable absolute amounts of *hsp70.2* transcripts. Comparative sequence analysis revealed an alteration of the 3´-UTR sequences in these samples. The variant 3´-UTR allele proved to extend the half life of the *hsp70.2* mRNA. It is suggested that the cellular stress response is significantly affected by the action and interaction of both, promoter and 3´-UTR variants of the *hsp70.2* gene occurring naturally in different pig breeds. The mRNA stabilising 3´-UTR variant was significantly more frequently associated with the promoter-"down" variant in two pig populations analysed. This implies that the mutant 3´-UTR may be advantageous for the carrier in the context of an impaired promoter. Our results demonstrate that polymorphisms with opposite effects on gene expression occur even within a single gene. This makes evident the limits of candidate gene experiments where one polymorphism in a gene is investigated on its relationship to phenotypes.

**C036**

**Candidate genes for embryonic survival in the Pig**

ANDREAS SPÖTTER[1], CORD DRÖGEMÜLLER[1], HEIDI KUIPER[1], BERTRAM BRENIG[2], TOSSO LEEB[1], OTTMAR DISTL[1]
[1]*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany;* [2]*Institute of Veterinary Medicine, University of Göttingen, Göttingen, Germany*

Candidate genes being involved in the embryo implantation in the pig between day 10-20 were chosen in consideration of expression patterns of endometrial proteins during early pregnancy. Additional selection criteria were the knowledge about the function of homologous proteins in other species and QTL studies. For the genes *CTSL*, *ITIH4*, *EGF*, *LIF* polymorphic DNA markers (microsatellites or SNPs) were developed and they were physically assigned by FISH and RH mapping. Furthermore, *LIF* and *CTSL* were molecularly characterized. CTSL (cathepsin L) is a lysosomal cysteine protease. The complete DNA sequence of this gene which spans about 5.6 kb and consists of 8 exons was determined. *CTSL* was assigned to SSC10q11→q12. The *LIF* (leukemia inhibitory factor) gene which encodes a pleiotropic cytokine, spans about 6.3 kb and consists of 5 exons including three alternative first exons (1D, 1M, 1T) spliced onto common second and third exons. *LIF* was mapped on SSC14q21→q22. ITIH4 belongs to the inter-α-trypsin inhibitor family of serine protease inhibitors. This gene was localized on SSC13q21→q22. The polypeptide EGF (epidermal growth factor) stimulates growth and proliferation of skin epithelia in the embryo. In the uterus it may induce endometrial growth and differentiation. *EGF* was mapped on SSC8q23→q24. The developed markers will be used for upcoming association studies to show significant additive and dominant gene effects on the embryonic survival and number of piglets born alive.

**C037**

**Differences in Stearoyl-CoA Desaturase mRNA Levels in Muscle between Japanese Black and Holstein cattle**

MASAAKI TANIGUCHI[1], HIDEYUKI MANNEN[2], YASUHIKO SHIMAKURA[3], AKIO OKA[4], HIROKO WANABE[5], MASANORI KOMATSU[5], GREGORY S. HARPER[6]; SOICHI TSUJI[2]
[1]*Kobe University, Graduate school of Science and Technology, Kobe, Japan,* [2]*Kobe Univer-*

*sity, Faculty of Agriculture, Kobe, Japan, [3]Animal Inspection Office, Gifu, Japan, [4]Animal Science Division, Hyogo Central Institute of Agriculture, Kasai, Japan, [5]Department of Livestock and Grassland Science, National Agricultural Research Center for Western Region, Oda, Japan, [6]CSIRO Livestock Industries, Brisbane, Australia*

Japanese Black cattle have a fatty acid profile that differs from that of other breeds in terms of unsaturated fatty acids. The enzyme stearoyl-CoA desaturase (SCD, also known as Δ9-desaturase) has been implicated in creating this breed difference. In this study, we compare the level of the SCD gene expression in muscle between two cattle breeds, Holstein and Japanese Black. First of all, we cloned SCD cDNA and sequenced it so that the RT-PCR is applicable. In so doing, we identified five nucleotide substitutions in the cDNA sequence. As those substitutions are linked each other, two haplotypes, A and G, were observed. The gene frequency of haplotype A is 55% in Japanese Black and 39% in Holstein. The nucleotide substitution at 878bp causes the substitution of amino acid alanine for valine in the protein. There were significant differences of SCD mRNA content and unsaturated fatty acids composition between two breeds; Japanese Black showed higher SCD mRNA (3.4-fold) in muscle and higher unsaturated fatty acids (1.3-fold) in fat tissue than Holstein. However, there were no differences of SCD mRNA content and unsaturated fatty acid composition between two haplotypes, and no correlation between SCD mRNA content and unsaturated fatty acid content in fat tissue within a breed.

**C038**

**Sequencing and physical mapping of the porcine *dopamine β-hydroxylase* gene**

ANNA TOMÁS, MARCEL AMILLS, ARMAND SÁNCHEZ

*Universitat Autònoma de Barcelona. Departament de Ciència Animal i dels Aliments, Barcelona, Spain*

*Dopamine β-hydroxylase* (*DBH*) is the enzyme that catalyzes the conversion of dopamine to norepinephrine. The transfer of catecholamines across the placenta is essential for embryo survival. Female mice with targeted disruption of the *DBH* gene showed an impaired maternal behavior caracterized by a lack of pup retrieval. We have amplified one fragment of the porcine *DBH* cDNA encompassing exon 4 and 12 in eight pigs belonging to four different breeds (Iberian, Landrace, Large White and Pietrain). Direct sequencing of the PCR product revealed 87%, 82% and 88% nucleotide identity with the orthologous human, mouse and bovine sequences, respectively. The pig *dopamine β-hydroxylase* mRNA was expressed in the uterus, ovary, testicle and hypophysis. Moreover, we have performed the physical mapping of the *DBH* locus by using the INRA-University of Minnesota somatic cell radiation hybrid panel (IMpRH). The *DBH* gene was significantly linked to the EST *SSC10D08* and the microsatellite marker SW1301 on 1q2.13 with LOD scores of 12,99 and 6.09, respectively. Comparative mapping between pig and human revealed that this result is consistent with the chromosomal location of the human *DBH* gene at Hsa9q34.

**C039**

**Improving the human-pig comparative map: sequencing of 16,000 ests and mapping 175 pig genes with human orthologs**

CHRISTOPHER K. TUGGLE[1], JON A. GREEN[2], CARLOYN FITZSIMMONS[1], RAMI WOODS[2], RANDALL S. PRATHER[2], SERGEI MALCHENKO[3], M. BENTO SOARES[3], CHAD A.ROBERTS[4], KEVIN PEDRETTI[4], THOMAS CASAVANT[4], DANIEL POMP[5], J. BRAD EDEAL[5], YUANDAN ZHANG[1], MAX F. ROTHSCHILD[1], KEVIN GARWOOD[6]; WILLIAM BEAVIS[6]

*[1]Department of Animal Science, Iowa State University, Ames, IA USA; [2]Department of Animal Science, University of Missouri-Columbia, Columbia, MO USA; [3]Department of Pediatrics, University of Iowa, Iowa City, IA USA; [4]Department of Electrical and Computer Engineering, University of Iowa, Iowa City, IA USA; [5]Department of Animal Science, University of Nebraska, Lincoln, NE USA; [6]National Center for Genomic Resources, Sante Fe, NM USA*

We are developing extensive sequence and mapping data for cDNAs expressed in female reproductive tissues. We have produced a total of 22 libraries from different stages of estrus or gestation for embryo, anterior pituitary, hypothalamus, ovary, uterus, and term placenta. More than 16,000 sequences from random

clones have been produced and 90% submitted to Genbank. The average read length across this dataset is >400 base pairs. As assessed by clustering analysis, these data represent nearly 10,000 different genes. A BLAST analysis using 9,336 clusters, as of February 2002, indicates that 4,099 of these clusters are unique relative to porcine Genbank genes/ESTs (BLAST score <50). To facilitate selection of genes for comparative mapping, we have developed software to predict the cytogenetic location of pig ESTs. We identified human loci with a BLAST score >200 to our EST dataset, and then predicted the pig location of high-scoring ESTs based on human cytogenetic and RH mapping data, along with human:pig chromosome painting information. Pig EST matches to human loci with consistent cytogenetic and RH mapping locations total 1,486. Within the human genome, there is an average distance of 9.4±5.8 cR between loci with a pig EST match. To date, 175 loci have been mapped using both the SCHP and the RH panel, with nearly complete agreement to prediction. A website (http://pigest.genome.iastate.edu) has been established for access to these sequences and the analysis data.

**C040**

**Identification of one single nucleotide polymorphism at exon 7 of the porcine *cytosolic malate dehydrogenase (MDH1)* gene**

ORIOL VIDAL[1], ARMAND SÁNCHEZ[1], LUÍS VARONA[2], MARCEL AMILLS[1]

*[1]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Àrea de Producció Animal, Centre UdL-IRTA, Lleida 25198, Spain*

Cytosolic malate dehydrogenase (MDH1) is an enzyme that catalyses the conversion of oxalacetate to malate, that afterwards is converted to pyruvate by the malic enzyme. These two biochemical reactions are coupled and release most of the NADPH required for fatty acid synthesis. We have sequenced the pig *MDH1* gene in six Landrace (LD), Large White (LW) and Pietrain (PI) pigs. Total RNA from liver was reverse transcribed and amplified yielding one fragment of 1 Kb (from exon 2 to 9). This fragment was sequenced forward and reverse. We found one silent C→ T mutation at exon 7. We have designed a primer-extension analysis protocol in order to type this polymorphism and search for associations with fat deposition and fatty acid composition.

**C041**

**Targeted gene identification using exon trapping on bovine chromosome 6**

ROSEMARIE WEIKARD, CHRISTA KÜHN, TOM GOLDAMMER, MANFRED SCHWERIN

*Research Institute for the Biology of Farm Animals, Research Unit Molecular Biology, Dummerstorf, Germany*

Linkage analyses strongly suggest several QTL for production, health and conformation traits in the middle region of bovine chromosome 6 (BTA6). For identification of genes located in this region we focused on both comparative mapping of genes from orthologous human chromosome 4 onto BTA6 by high-resolution radiation hybrid mapping and targeted identification of coding sequences in subchromosomal regions of BTA6 poorly covered with information from the human gene map. A bovine BAC library was screened with 60 BTA6 markers comprising the region of BTA6 flanked by markers *BMS2508-ILSTS87*. As an initial step towards systematic transcript analysis in the critical BTA6 region we performed exon trapping on four selected region-specific BACs. Twenty one unique putative exon traps were detected. The chromosomal location of 19 putative exons was confirmed by remapping them onto their parent BACs by PCR. The trapped sequences were used as templates to screen public gene databases for *in silico* gene identification. However, there was no identity to known genes and ESTs, although 6 exon traps revealed substantial similarity to bovine repetitive sequences. To this point, 14 of the trapped sequences are novel and could be predicted to originate from unknown genes. The present exon sequence information provides probes for screening cDNA libraries to isolate full-length cDNA and contributes to establish a detailed transcription map for the specific BTA6 region for positional cloning efforts.

**C042**

**Comparison of Pedigrees of BSE infected Holstein-cows with the normal Holstein population in Germany**

MAIK WITTEMEIER[1], WALTER SCHULZ-SCHÄFFER[2], BERTRAM BRENIG[1], HER-

MANN H. SWALVE[3], EKKEHARD SCHÜTZ[4], WILHELM WEMHEUER[1]
*[1]University of Göttingen, Institute of Veterinary Medicine, Göttingen, Germany; [2]University Clinics of Göttingen, Göttingen, Germany; [3]University of Halle-Wittenberg, Institute of Animal Breeding and Animal Husbandry, Halle/S., Germany; [(4)] Chronix Biomedical, Benicia, CA, USA*

The question whether there is a genetic disposition for susceptibility of BSE infection is still under debate. Furthermore it is unclear yet whether the risk of BSE differs between or within the cattle breeds. There is a large and convincing body of evidence that genetic differences of PrP in both, Scrapie in sheep as well as CJD in Humans having a major impact on the disease risk. Therefore it seemed likely to find such disease favouring variants in *the Bos Taurus PrP gene* as well. But in contrast, in cattle no variant of the PrP gene could been found to be associated with an increased risk of acquiring BSE. However, that does not exclude still undiscovered genetic dispositions for this disease at other gene loci. The objective of the study was therefore, to generate pedigrees of BSE-positive cows in comparison to a control group, with consideration of race, age and region, to prove whether there are significant differences. We tried to achieve a 5-generation pedigree of all black and white and red and white German Holstein-cows, which were tested BSE-positive. The inspection of 90 cows found in 2000, 2001 and early 2002 resulted in 40 complete pedigrees. A randomly selected age, breed and region matched group of disease-free animals (n=xx) served as control. We found no differences between the BSE and the control groups of pedigrees. In Germany, even the distribution of the breeds of all infected cows is similar to the typical regional cattle population. The result of this study suggests that there is no distinct genetic disposition for a BSE- infection in the German Holstein population. In addition we could show that there is no strain difference in susceptibility of BSE between the German cattle breeds.

**C043**
**A comparative RH map of the polled gene region in cattle**

ANNE WÖHLKE, CORD DRÖGEMÜLLER, ANKE BADER, HEIDI KUIPER, TOSSO LEEB, OTTMAR DISTL
*Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover, Germany*

In cattle the polled phenotype shows tight linkage with genetic markers of the centromeric region of BTA1, but several studies have not been able to order the polled locus relative to the markers used. Without knowledge of true marker and gene order on proximal BTA1 maps, the selection of candidate genes using comparative mapping data may lead to failure. Current comparative maps between HSA21 and the proximal part of BTA1 are insufficient to define chromosomal rearrangements due to the low density of mapped genes in the bovine genome. Recently, the availability of the complete sequence and gene catalogue of the long arm of HSA21 has provided valuable tools for a more detailed comparative analysis of corresponding segments on BTA1. In this study we constructed a comprehensive RH map of the proximal part of BTA1 on the 3,000 rad Roslin/Cambridge bovine whole-genome radiation hybrid panel. A set of known bovine markers, i.e. 10 microsatellites, 5 genes and 2 cattle ESTs, was used to construct a RH map. Furthermore, we mapped eight new STS markers derived from bovine RPCI-42 BAC clones. These BAC clones contain bovine orthologs of HSA21 genes, i.e. *GRIK1*, *CLDN8*, *TIAM1*, *HUNK*, *SYNJ1*, *OLIG2*, *IL10RB*, and *KCNE2*, and were physically assigned by FISH to BTA1q12.1-q12.2. Concluding, the RH-mapping of 25 loci greatly improved the map resolution of the proximal part of BTA1 and revealed previously unknown details of the cattle-human comparative map.

**C044**
**Characteristics of gene expression levels in somatic nuclear transfer-derived cloned bovine placenta based on array analysis of 1,353 genes**

HIROSHI GOHMA[1], YUKIO TANIGUCHI[1], HIROSHI YASUE[2], KAZUYOSHI HASHIZUME[2], TAKAHISA YAMADA[1], YOSHIYUKI SASAKI[1]
*[1]Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]National Institute of Agrobiological Sciences, Ibaraki, Japan*

Somatic nuclear transfer-derived cloned bovine fetus and placenta are known to be susceptible to a developmental abnormality associated with abortion or stillbirth, as compared to em-

bryo transfer-derived ones. This susceptibility may be partly attributable to an abnormal expression of genes at early developmental stage in the former cloned bovine fetus and placenta. Our present study was thus designed to characterize gene expression levels in the somatic nuclear transfer-derived cloned bovine placenta. We have firstly made a catalog of 1,521 clones isolated randomly from directionally cloned cDNA library of 60 embryonic day-old placenta, yielding clone collection of 795 species. We have next made a macroarray comprising of clones of 1,353 species obtained from placenta cDNA library as well as from 60-day-old whole fetus cDNA library. Using the macroarray, we have examined expression levels of 1,353 genes in 60 embryonic day-old abnormal placentas derived from the cloned cattle, and in age-matched normal placentas from the cloned and artificially inseminated cattle. There was more marked difference in gene expression level between abnormal and normal placentas than between normal placentas. This difference almost corresponded to an increase rather than a decrease in abnormal against normal placentas. Three genes, *HBA1*, *HBA2* and *SPTB*, and one gene, *IGF2*, respectively, expression levels of which showed decrease and increase in the abnormal placenta, have been suggested to be possible candidates for responsible genes for susceptibility to developmental abnormality in the cloned cattle.

**C045**

**A novel polymorphism in exon 5 of the Bovine Growth Hormone gene and its relationship to backfat thickness in Hanwoo cattle**

DU-HAK YOON[1], HONGHE CAO[2], YONG-MIN CHO[1], OLIVER HANOTTE[3], IL-CHEONG CHEONG[1]

[1]*Division of Animal Genomics and Bioinformatics, National Livestock Research Institute, RDA, Suwon 441-350, Korea,* [2]*nstitute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, China;* [3]*International Livestock Research Institute (ILRI), PO Box 30709 Nairobi, Kenya*

The Growth Hormone (*GH*) is a member of the somatotropin/prolactin family of hormones. Its role in growth control has been extensively studied in human, mice and livestock. We report here a novel PCR-RFLP polymorphism within the exon 5 of the bovine Growth Hormone (*bGH*) gene and its relationship to backfat thickness in Hanwoo cattle (Korean cattle). A 522 bp fragment from eight unrelated Hanwoo cattle was amplified by PCR and subsequently cloned and sequenced. An *Msp*I RFLP corresponding to a C to T transition was observed at position 2258. It is predicted to be a missense mutation (Arg to Trp) at codon 166. Codominant Mendelian segregation of the two alleles was confirmed in two full-sib F2 families ($n$ = 32, African taurine *B. taurus* □ African zebu *B. indicus*). *Msp*I allele frequencies were calculated in 13 different breeds of cattle (European and Asian taurines). An analysis of variance (ANOVA) was conducted to investigate the effects of the three genotypes on live weight, backfat thickness and carcass traits in 64 Hanwoo bulls. A significant association ($P < 0.05$) was found with backfat thickness. This result indicates that the *bGH* RFLP newly identified might be used as a marker for selection against backfat thickness in Hanwoo cattle.

**C046**

**Cloning and mapping of cattle caspases**

Y.Q.YUAN[1,2], L. PEELMAN[1], A. DE KRUIF[2], A. VAN ZEVEREN[1], A. VAN SOOM[2]

[1]*Department of Animal Nutrition, Genetics, Breeding and Ethology, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium;* [2]*Department of Reproduction, Obstetrics and Herd Health, Ghent University, Salisburylaan 133, B-9820, Belgium*

Sequential activation of cysteine-aspartic acid proteases (caspases) plays a central role in the execution of cell apoptosis. Studies in caspase inhibiting or inactivating could be helpful in developing drugs. Currently 13 caspase family members have been characterized in mouse while 11 caspases were discovered in human. However, only bovine caspase 13 has so far been described. The aim of this study was to search for other bovine caspases and map them. Fragments of the caspases were amplified by RT-PCR followed by cloning and sequencing. Mapping of genes was done by use of radiation panel. Caspase 1, 4, 5, 6, 7 and 8 have been successfully detected, cloned and mapped at this moment.

**C047**

**Mapping of the canine *HMGA1* gene - a gene frequently involved in human mesenchymal tumors**

K. BECKER[1], H. MURUA ESCOBAR[1], A. RICHTER[1], B. MEYER[1], I. NOLTE[2], J. BULLERDIEK[1]

*[1]Center for Human Genetics, University of Bremen, Bremen, Germany; [2]Clinic for Small Animals, Veterinary School, Hanover, Germany*

The dog has become a more and more interesting model organism for human diseases including cancer. Parallels between human and canine tumors have often been described. Accordingly, the results of canine gene mapping studies will be of considerable significance. Rearrangements of its human counterpart on 6p21 involving the *HMGA1* gene have been described in a variety of mesenchymal tumors as e.g. pulmonary chondroid hamartomas, uterine leiomyomas and lipomas. So far, it is not clear yet if comparable translocations occur in the corresponding canine tumor as well. To further elucidate that question, we have mapped the canine *HMGA1* gene. By fluorescence in situ hybridization (FISH), we have mapped the canine *HMGA1* to CFA 23. The assignment of the canine *HMGA1* gene to CFA 23 clearly shows that the chromosomal region where the canine *HMGA1* gene has been mapped to is not a hotspot of chromosomal breakpoints seen in canine tumors. Therefore in contrast to humans the activation of that gene as a result of chromosomal translocations does not seem to play a considerable role in canine tumors. This may be due to the fact that the corresponding changes are not able to induce benign tumors in the dog or to stimulate their growth. Alternatively, there may be factors favouring the occurrence of the structural changes in humans which are lacking in dogs.

**C048**

**Functional analysis of the bovine kappa casein promoter**

MARUŠA DEBELJAK[1], WOLFGANG DOPPLER[2], ANTONELLE BALDI[3], PETER DOVC[1]

*[1]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domzale, Slovenia; [2]University of Innsbruck, Department of Medical Chemistry and Biochemistry, Innsbruck, Austria; [3]University of Milano, Veterinary faculty, Milan, Italy*

The 2140 bp fragment of the bovine kappa casein (K-CN) promoter region has been sequenced. DNA sequence analysis revealed a number of potential binding sites for transcription factors as YY1, Stat5, NF1, AP2, CREBP, CREB and GRE. The consensus TATA box sequence was found proximal to the transcription start site. Due to their important role in the regulation of lactoprotein gene expression we focused on the functionality of the Stat5 binding sites in the bovine K-CN promoter. DNA fragments containing Stat5 sequences present in the bovine K-CN promoter were used for electro mobility shift assay (EMSA) and DNase1 footprinting. The cell lysate from transfected COS7 cells, over-expressing the C terminally deleted forms of Stat5a and Stat5b proteins was used as a source of enriched transcription factor. EMSA and DNase1 footprinting revealed specific binding of Stat5 to the target sequences. In order to test the promoter activity *in vitro* we transfected bovine mammary gland derived cells (BME UV1/2) with the luciferase reporter gene construct containing the short (925 bp) and the long (2064 bp) version of the K-CN promoter. In the luciferase assay almost no expression was found after transfection with the shorter promoter variant, however, the longer promoter fragment induced weak but specific expression, suggesting that 2000 bp of the K-CN promoter are sufficient for induction of expression under *in vitro* conditions.

**C049**

**Characterization of a candidate gene for performance in racehorses**

N.A. ELLIS, I. TAMMEN, F.W. NICHOLAS, H.W. RAADSMA

*ReproGen, Centre for Advanced Technologies in Animal Genetics and Reproduction, The University of Sydney, Camden and Sydney, Australia.*

The control of arterial blood pressure is largely regulated by angiotensin-converting enzyme *(ACE)*, a component of the renin-angiotensin system. In humans, a polymorphism in the *ACE* gene is associated with elite endurance performance. This gene is therefore one of interest when investigating the effect of genetics on racing performance in horses. The aim of this study is to fully characterise the equine

*ACE* gene. A BAC clone containing this gene has been obtained through the International Equine Gene Mapping Workshop (IEGMW). Using comparative information, approximately half of the gene has been sequenced from the BAC. Collaboration with the IEGMW has also led to the mapping of the gene on the somatic cell and radiation hybrid panels, with the results confirmed by FISH mapping. *ACE* was found to be on ECA 11p13, which agreed with predictions from comparative maps. Two pools of horse DNA are being created for screening for polymorphisms based on sequencing. Following the detection of polymorphisms, association studies investigating possible links between identified *ACE* haplotypes and racing ability will be performed. Phenotypic data is being collected in collaboration with the Sydney University Equine Performance Laboratory, which has facilities for the measurement of physiological parameters of racing performance. Other performance data such as racing records will also be included in the analysis. Future work will include more in-depth analysis of associations with performance, with the aim of identifying DNA markers for athletic ability in race horses.

**C050**

**Molecular genetic characterisation of the gene causing chondrodysplasia in Dexter cattle**

JULIE A.L. CAVANAGH[1], IMKE TAMMEN[1], PETER A. WINDSOR[1], FRANK W. NICHOLS[2], HERMAN W. RAADSMA[1]

*[1]Reprogen, Centre for Advanced Technologies in Animal Genetics and Reproduction, The University of Sydney, Camden, Australia and [2]Reprogen, The University of Sydney, Sydney, Australia*

Dexter cattle are a small breed of cattle originating in Ireland that have been bred in Australia for many decades. There have been reports of mutant, aborted foetuses in this breed of cattle, described as chondrodysplastic foetuses (or "bulldog" calves) occuring worldwide. The affected foetuses display disproportionate dwarfism, a short vertebral column, marked micromelia, a relatively large head with a retruded muzzle, cleft palate, protruding tongue and a large abdominal hernia. Dexter chondrodysplasia is inherited in an incompletely dominant manner. As part of an approach to controlling the disease in Australia, Dexter Cattle Australia (DCA) chose to initiate and support research to develop a DNA-test to identify carrier animals, prevent carrier x carrier matings, and hence reduce the incidence of chondrodysplastic foetuses. Potential candidate genes were identified, and homozygosity mapping in regions predicted to contain these genes identified a region of interest. A candidate gene predicted to be in this region was mapped on the bovine linkage and physical maps, confirming location in the region of interest. One disease causing mutation was identified in the candidate gene, a 4 bp insertion causing a frame shift which leads to a premature termination codon. The resulting protein is predicted to contain less than one third of the original amino acid sequence. A DNA test has been developed to identify animals heterozygous for the mutation.

**C052**

**PorDictor – predictors of pork quality derived from gene expression profiles of prenatal muscle tissue**

BARBARA HARLIZIUS[1], MARINUS F.W. TE PAS[2], KIN-CHOW CHANG[3], ROBERTA DAVOLI[4], JAN W.M. MERKS[1], ROLAND WÖRNER[5], HUBERT EPING[6], EDUARD MURANI[7], SIRILICK PONSUKSILI[7], KARL SCHELLANDER[7], JAN M. PRIEM[2], NUNO DA COSTA[3], MASSIMO CAGNAZZO[4], LUCA FONTANESI[4], BARBARA LAMA[4], HUBERT HENNE[5], KLAUS WIMMERS[7]

*[1]Institute for Pig Genetics BV, 6641 SZ Beuningen, The Netherlands; [2]ID-Lelystad, Institute for Animal Science and Health, 8200 AB Lelystad, The Netherlands; [3]University of Glasgow Veterinary School, Department of Veterinary Pathology, Glasgow G61 1QH, UK; [4]University of Bologna, Dipartimento di Protezione e valorizzazione Agroalimentare (DIPROVAL) Sezione di Allevamenti Zootecnici, 42100 Reggio Emilia, Italy; [5]Züchtungszentrale Deutsches Hybridschwein GmbH, 21337 Lüneburg, Germany; [6]Landesverband Rheinischer Schweinezüchter, 53115 Bonn, Germany; [7]University of Bonn, Institute of Animal Breeding Science, 53115 Bonn, Germany*

Meat quality traits are of major economic importance in livestock production. The genetic background of meat quality is to a large extend determined by factors active during prenatal muscle development. Recently

an EU funded project, PorDictor (QLK5-2000-01363), has been started aiming at the identification of new predictors for pork quality derived from gene expression profiles of embryonic/fetal skeletal muscle. The project comprises (1) identification of candidate genes for meat quality traits by comparing breeds that are extremes in meat quality and muscularity and (2) confirmation of gene effects on meat quality in commercial crossbreeds. In total about 2000 embryos/fetuses of seven key developmental stages of the breeds Duroc and Pietrain have been collected for analysis of differential gene regulation in (presumptive) M. longissimus dorsi. Several techniques of expression profiling, i.e. cDNA-microarrays, differential display-RT-PCR, in situ hybridisation, subtractive hybridisation and quantitative real time RT-PCR are applied to detect breed-specific, stage-specific and/or phenotype-associated transcripts. These will be screened for polymorphism and their association with meat quality traits will be evaluated in performance tested porkers of the commercial crosses Pietrain x Landrace and Duroc x Large White. The project will provide new insights in muscle development and largely contribute to improve meat production and to overcome the existing antagonisms between leanness and meat quality traits.

**C053**

**Variation at the porcine *KIT* locus influence peripheral blood cell measures**

A. JOHANSSON[1], G. PIELBERG[2], I. VAARA[3], L. ANDERSSON[2], I. EDFORS-LILJA[1]

[1]*Department of Biosciences and Process Technology, Växjö University, Växjö, Sweden;* [2]*Department of Animal Breeding and Genetics, SLU, Uppsala, Sweden and* [3]*Central Hospital, Växjö, Sweden*

The *KIT* gene encodes the mast/stem cell growth factor receptor essential for normal development of melanocytes, hematopoietic stem cells and germ cells. Dominant white coat colour in pig is due to a gene duplication and a single-nucleotide splice mutation, whereas the Belt phenotype ($I^{Be}/I^{Be}$) in the Hampshire (H) breed is assumed to be caused by a regulatory *KIT* mutation. A reduced number of peripheral blood leukocytes in pigs homozygous for the dominant white allele have earlier been observed in the $F_2$-generation of an intercross between Wild Boar and Yorkshire pigs. In the present study a total of 16 litters, crosses between H and Yorkshire/Landrace (Y/L) or pure Y, were analysed regarding *KIT* genotypes and various blood cells measures. Blood samples, 3 to 7 per piglet, were collected from the age of 2 days and until 2 months after birth. Several blood parameters were recorded, including numbers of erythrocytes and leukocytes, haemoglobin and hematocrit levels. The *Kit* genotypes were determined using pyrosequencing. Several genotypes were found amongst the Y/L sows confirming a high allelic diversity at the *KIT* locus. A good agreement was found between coat colour and genotypes, as all piglets carrying the $I^{Be}$ allele had black spots, except one. A segregation distortion was indicated in the inheritance of the $I^{Be}$ allele and a preliminary analysis indicated that pigs carrying the $I^{Be}$ allele had higher numbers of leukocytes and hematocrit levels.

**C054**

**Detection of genes differentially expressed in porcine leukocytes due to transport stress by using cDNA–AFLP and Differential Display**

PATCHARIN KRUTMUANG, SIRILUCK PONSUKSILI, KARL SCHELLANDER, KLAUS WIMMERS

*University of Bonn, Institute of Animal Breeding Science, 53115 Bonn, Germany*

Transport stress is one of the problems in pig production. The process of pig management often includes the transport of piglets from several producers to fattening units and then to slaughterhouses. Stress is the reaction of the body to stimulus, which disturbs the normal homeostasis, physiology and biochemical activities of an animal. Stress response likely leads to adverse effects on growth, behavior, reproductive performance, meat quality and disease resistance. The objective of this investigation is to identify candidate genes which are differentially expressed under transport stress. cDNA-AFLPs were used to compare the expression pattern of leukocytes before and after transport stress. Differential display analysis was conducted for expression profiling of leukocytes of animals with high and low cortisol response after transport stress. Five and six potential candidate genes/ESTs were detected by cDNA-AFLP and differential display, respectively. The chromosomal localiza-

tion of these ESTs was done using the IMpRH-panel. Quantitative real-time PCR was used to confirm differential expression of the ESTs. Two ESTs detected by cDNA-AFLP were found to be differentially expressed ($P<0.05$) before and after transport. One EST from differential display was differentially expressed ($P<0.001$) between the high and low cortisol group. These ESTs represent candidate genes for understanding and for dissecting genes which may have major effect on stress resistance.

**C055**

**Mapping of some maternal transcript ESTs in cattle pre-implantation embryos**

SOLOMON G. MAMO[1], KLAUS WIMMERS[1], MARCUS GILLES[1], KATA SRINIVAS[2], KARL SCHELLANDER[1], SIRILUCK PONSUKSILI[1]

[1]*University of Bonn, Institute of Animal Breeding Science, 53115 Bonn, Germany;* [2]*Texas A&M University, Department of Veterinary Pathology, Texas, USA*

Gene mapping is an important tool towards identification of candidate genes, applications in comparative genomics and evolutionary studies. The objectives were to identify and allocate genes expressed in cattle embryo pre-implantation stage. Nine ESTs (Expressed Sequence Tags) derived from matured oocytes and 4- cell embryo cDNA libraries were mapped using radiation hybrid panel. A primer pair was designed per EST to amplify products and their identity was confirmed by sequencing. RH mapping revealed average retention frequencies, calculated as the proportion of clones retained a given marker, of 16.5 %. Six markers (ESTBb003, ESTBb007, ESTBb008, ESTBb011, ESTBb4c1 & ESTBb4c6) have strong similarity with genes; *Sterol C5 desaturase, Normal keratinocyte mRNA, KIAA0197 protein, Host cell homolog, Catenin associated delta 1 & Capping protein.* They are assigned to chromosomes 15, 14, 15, 10, 15 and 3, respectively. These localizations fit the current human bovine comparative map as shown by using the COMPASS software. Some of the functions of these genes include cholesterol biosynthesis, calcium binding, intracellular protein transport, cell-cell adhesion, protein complex assembly and role in cell motility. This study ordered some of the developmentally important genes that are expressed at maternal transcript level. Further study to evaluate their contribution to embryo quality and developmental competence will complement this study.

**C056**

**Expression analysis of *HMGA1* in normal and neoplastic canine mammary cell lines using macroarrays**

B. MEYER[1], H. MURURA ESCOBAR[1], A. RICHTER[1], K. BECKER[1], I. NOLTE[2], J. BULLERDIEK[1]

[1]*Center for Human Genetics, University of Bremen, Bremen, Germany;* [2]*Clinic for Small Animals, Veterinary School, Hannover, FRG*

Mammary neoplasms are twice as frequent in dogs (*Canis familiaris*) as in humans and account for more than 50% of the tumours observed in the female dog. Due to their similar histological and biological characteristics canine mammary carcinomas are considered to be valid models for studying the molecular mechanisms underlying tumour development in humans. A gene frequently involved in neoplastic transformation and metastatic tumour progression is *HMGA1*, an architectural transcription factor, which is expressed in high levels during embryogenesis and in dividing cells, whereas it is only detectable at very low levels or even absent in non-dividing fully differentiated cells. Overexpression of *HMGA1* recently has been observed in primary human breast cancer. Increased expression was also correlated to increased metastatic potential of both mouse and human mammary epithelial cancer. In order to investigate mRNA expression levels of *HMGA1* in canine mammary tumours, macroarray and northern blot analyses of normal, benign, and malignant mammary cell lines were carried out. For the benign mammary tumour cell line these experiments revealed a strong expression of *HMGA1* as well as of the tumour-relevant genes *c-MYC* and *HER2*. Furthermore the northern blot analysis showed signals for all three examined cell lines, indicating expression of the *HMGA1* mRNA not only in the benign but also in the normal and malignant mammary cell lines verifying the results obtained by the arrays.

**C057**
**Coat color and mutations of MC1-R locus infour sheep breeds**

LUIS V. MONTEAGUDO[1], M. TERESA TEJEDOR[1], ISIDRO SIERRA[2], ALICIA POSTIGLIONI[3], ROSA GAGLIARDI[3], MIGUEL D'ANGELO[3], RAUL BARRAO[1], RICARDO PONZ[1], M. VICTORIA ARRUGA[1]
*[1]University of Zaragoza, Laboratory of Cytogenetics and Molecular Genetics, Zaragoza , Spain; [2]University of Zaragoza, Department of Animal Production and FoodScience, Zaragoza, Spain; [3]Universidad de laRepública, Laboratory of Cytogenetics , Montevideo, Uruguay*
MC1-R locus was investigated by PCR-RFLP methodology inMerino (black variety, an ancient breed), and in several black individualsfrom Merino Corriedale, Rasa Aragonesa and Salz sheep breeds. M73K andD121N mutations ofMC1-R were found in the black Merino and Corriedale individuals. In fact,both mutations cosegregated with dominant black color coat in the Blackvariety of Merino and in Merino Corriedale. However, in Rasa Aragonesa andSalz breeds, black color appeared as a recessive character and was not associated to these mutations.Genotype coincidence was always found for both mutations; in every studiedbreed, individuals were homozygous dominant, heterozygous or homozygousrecessive for both M73Kand D121N mutations. These findings suggest the existence of only two alleles, one of themcarrying simultaneously both mutations and the other one free of them.Dominant mutations associated to black color should be considered as anancestral character, with a commun and remote origin in Black Merino and Merino Corriedale sheep breeds.

**C058**
**SNP detection and linkage mapping of the porcine *TGFB1* and *RLN* genes as candidates for reproductive traits**

SIRIWADEE CHOMDEJ, SIRILUCK PONSUKSILI, KARL SCHELLANDER, KLAUS WIMMERS
*University of Bonn, Institute of Animal Breeding Science, 53115 Bonn, Germany*
Reproductive performance comprises of genesis of conceptuses, their prenatal development and growth as well as postnatal rearing of sucklings. We are aiming to address candidate genes for these processes of reproduction. There is experimental evidence that transforming growth factor beta 1 and relaxin affect the prenatal development and the rearing ability, especially the mammary gland phenotype. In order to identify SNP within the *TGFB1* and *RLN* gene porcine cDNA-amplicons were comparatively sequenced in animals of five pig breeds. A transition (A>G) was detected in exon 5 (position 797 of cds) of *TGFB1*. A SSCP procedure was established for genotyping. Two SNPs were identified in the *RLN* gene, a transversion from T to G in intron 1 at position 9, and a transversion from A to C in exon 1 at position 22. RFLP and SSCP are used to genotyping these SNPs. The SNP within exon 1 at position 22 of *RLN* leads to an amino acid exchange (isoleucine to leucine). The segregation of all alleles was observed in 21 families of DUMI F2 resource population and Mendelian inheritance of the alleles could be demonstrated. Linkage mapping of the genes is in agreement with previous physical mapping data. The SNPs identified here are valuable tools for ongoing linkage and association analyses with traits related to reproduction.

**C059**
**Mapping and identification of the gene causing hereditary zinc deficiency in Angus cattle**

IMKE TAMMEN[1] , ROGER W. COOK[2], JANE GITSCHIER[3], FRANK W. NICHOLAS[1], HERMAN W. RAADSMA[1]
*[1]University of Sydney, Centre for Advanced Technologies in Animal Genetics and Reproduction – ReproGen, Camden & Sydney, Australia; [2] NSW Agriculture, Wollongbar, Australia and University of California, Howard Hughes Medical Institute and Departments of Medicine and Pediatrics,San Francisco,USA*
Bovine hereditary zinc deficiency (lethal trait A46) was first reported in Friesian cattle in 1964. It is inherited as an autosomal recessive disorder and is considered to be homologous to Acrodermatitis enteropathica (AE) in humans. In 1993 hereditary zinc deficiency was diagnosed in a herd of Angus cattle in Australia. Although the disease appeared to be confined to a single herd it was further investigated due to its potential to provide a model for gene therapy as affected animals survive with treat-

ment. We applied a breeding program to enlarge the Angus pedigree and used retrospective sampling to obtain DNA from affected Friesian cattle. Due to the limited material we initiated a homozygosity mapping approach to map the defective gene in cattle. Before completion of the genome screen, the human gene for AE was mapped to HSA 8q24.3 and recently identified. Applying comparative mapping information, we mapped hereditary zinc deficiency in Angus cattle to a region on chromosome 14, which is homologous to human HSA8q24.3. The data for Friesian cattle was inconclusive. After identification of the gene causing AE, we started sequencing the orthologous bovine gene and identified a nonsense mutation in Angus cattle, which is not present in affected Friesian cattle. Further research will be required to determine if the disease in Friesian cattle is caused by a different mutation in the same gene.

**C060**

**Fatty acyl desaturase and elongase genes of marine and freshwater teleosts**

MORRIS AGABA, NICOLA HASTINGS, DOUGLAS R. TOCHER, CATHERINE A. DICKSON, JOHN R. SARGENT, ALAN J. TEALE

*Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK*

Enzymes that lengthen and desaturate the carbon chain are required for biosynthesis of the long chain highly unsaturated fatty acids (Lc-HUFA) arachidonic and docosahexaenoic acids. The Lc-HUFA are essential for normal health and development of all vertebrates. Marine teleosts, unlike their freshwater counterparts, appear to have a dietary requirement for Lc-HUFA that may be due to defective fatty acid elongation and/or desaturation. In examining this possibility we are comparing the structures of the desaturase and elongase genes of representative marine and freshwater species, and the function of their products. The zebrafish (*Danio rerio*) elongase and desaturase genes were the first that we characterised. Interestingly, the products of both genes appear to be less functionally constrained than the desaturases and elongases of higher vertebrates. The zebrafish desaturase gene encodes a novel bifunctional enzyme having both delta 5 and delta 6 desaturase activities, while the elongase substrate specificity ranges from short chain monounsaturates to Lc-HUFA. The elongase and desaturase genes have now been cloned from an anadromous (Atlantic salmon) and several marine species. Predicted protein sequences show that the gene products have high homology among fish species. Functional characterisation is ongoing.

**C061**

**Quantification of differentially expressed transcripts in in-vitro produced cattle pre-implantation stage embryos using fluorescence monitored real time PCR technique**

DAWIT TESFAYE, KLAUS WIMMERS, MARKUS GILLES, KARL SCHELLANDER, SIRILUCK PONSUKSILI

*University of Bonn, Institute of Animal Breeding Science, 53115 Bonn, Germany*

Determining the physiological timetable of differential gene expression in pre-implantation stage embryos requires the ability of accurately quantifying stage specific mRNA transcripts. The aim of this study was quantification of differentially expressed transcripts in in-vitro produced cattle 8-cell, 16-cell, morulae and blastocyst embryos. Of 16 clones identified and sequenced, clones 2C14 and 1C9 which were identified in 8-cell stage and share high homology with human *Pleckstrin homology, Sec 7 coiled domain 2 (PSCD2)* mRNA and *Nucleosome assembly protein 1 (NAP1L1),* respectively, were quantified using ABI Prism 7000 instrument. cDNA from the four stages of embryos were subjected to real time PCR quantification utilising specific primers designed using the Primer express software and SYBR green PCR master mix (Applied Biosystems). ß-actin was used for internal normalization. The relative expression level of 1C9 clone to 8-cell stage amounted 1.05, 0.82 and 0.75 fold difference in 16-cell, morulae and blastocyst stages, respectively. Similarly, the relative expression level of clone 2C14 to 8-cell stage amounted to 0.97, 0.97, 0.96 fold difference in 16-cell, morulae and blastocyst stage respectively. These results indicated that while clone 1C9 showed a significant reduction in expression up to blastocyst stage, a weak difference was observed in the expression of clone 2C14. Further quantification of these clones in other developmental stages and different embryo qualities will supplement these findings.

**C062**

**Mapping and examination of ion channel genes as candidate genes for the hereditary disease 'Congenital progressive ataxia and spastic paresis' in swine**

A. KRATZSCH, B.KORCZAK, S. NEUENSCHWANDER, G. STRANZINGER, P. VÖGELI
*Institute of Animal Science, ETH-Zurich, Zurich, Switzerland*

The congenital progressive ataxia (CPA) and spastic paresis in pigs, recently identified in Switzerland, is a disease with unknown etiology. It manifests itself shortly after birth as a severe neuropathy. The disease seems to be controlled by a single autosomal recessive allele designated *cpa*. In a previous study we demonstrated close linkage of *cpa* to the microsatellite *Sw902* on porcine chromosome 3 (SSC3). *Sw902* is mapped in close proximity to the physically and genetically mapped *IL1* locus on SSC3q13-21. Available human-swine comparative maps predict correspondence of this region to human 2q1-2 region, where ion channel genes ($Ca^{2+}$, $Na^{+}$) and a cholinergic receptor gene are mapped. Epilepsy and ataxia in humans seem to be caused by mutations in these genes. The calcium channel beta 4 gene (*CACNB4*) was mapped to SSC3q14-q21, while the sodium channel gene (*SCN2A*) and the cholinergic receptor gene (*CHRNA1*) were located on SSC15. So far mutation screening, expression studies and drug treatment of the porcine *CACNB4* gene did not reveal any modification in affected piglets.

**C063**

**Physical mapping of a bovine chromosome region with an effect on milk fat content**

ANDREAS WINTER, ARIANE ALZINGER, RUEDI FRIES
*Technische Universität München, Lehrstuhl für Tierzucht, Freising-Weihenstephan, Germany*

A QTL affecting milk fat content on bovine chromosome (BTA) 14 has been independently confirmed by several studies. A mutation within *DGAT1* (diacylglycerol acyltransferase 1), a functional and positional candidate gene, is associated with the QTL effect. Directly proving the causality of this mutation by genetics means is not feasible. Therefore we attempt to provide indirect proof by excluding other positional genes in the QTL region through linkage disequilibrium studies. To this end, we made an effort to determine the gene content of the chromosomal region harbouring *DGAT1* (BTA 14q12). Sequences of human genes within a range of 640 kb around DGAT1 (HSA8q24.3) listed in the NCBI MapView were used to obtain bovine sequence information for the corresponding genes using BLAST analysis of the EST division of GenBank. Bovine EST information of five neighbouring genes and sequence information of five BAC ends of clones containing DGAT1 were used for generating PCR probes. Two compound probes were compiled, one consisting of gene-specific probes and one consisting of BAC-end-specific probes, and were used to screen a bovine BAC library (RPCI-42). A total of 19 overlapping clones encompassing about 500 kb was isolated. The order of BACs and the position relative to *DGAT1* of 25 genes were determined by BAC-end and gene-specific PCR amplification from the 19 BACs. Genes and BAC ends are now comparatively sequenced to find SNPs, which will be the basis of linkage disequilibrium studies.

**C065**

**Intra- and inter-subspecific variation in complete *Bos taurus* and *Bos indicus* mitochondrial genomes - a molecular basis for maternal lineage effects**

STEFAN HIENDLEDER[1], AXEL JANKE[2], ECKARD WOLF[1]
[1]*Department of Molecular Animal Breeding and Biotechnology, Gene Center, Ludwig-Maximilians-University Munich, Germany;* [2]*Division of Evolutionary Molecular Systematics, Department of Genetics, University of Lund, Sweden*

Maternal lineage effects have been reported to influence milk production traits, fertility and, more recently, nuclear transfer efficiency in cattle. However, information on the molecular basis of such effects is very limited. We have analyzed maternal lineages of 5 *B. taurus* breeds and one *B. indicus* breed for mitochondrial DNA (mtDNA) control region (CR) sequence polymorphism and used the CR database to infer phylogenetic relationships. All analyzed sequences fell into the two major clusters of *Bos primigenius* mtDNAs, *B. taurus* and *B. indicus*. One *B. taurus* mtDNA (Simmental) and the *B. indicus* mtDNA (Zwergzebu) were chosen for complete se-

quence analysis, cloned into plasmid vectors and sequenced by standard procedures. Sequence comparison of the new 16338 nt *B. taurus* sequence with the bovine reference sequence revealed 10 polymorphisms in total. Six polymorphic sites were located in coding regions, and one of four substitutions in protein coding genes was non-synonymous. Comparisons between the 16339 nt *B. indicus* and the *B. taurus* mitochondrial genomes showed 237 polymorphic sites in total. Non-synonymous substitutions were present in subunits of respiratory chain enzymes NADH-dehydrogenase, $bc_1$-complex, Cytochrome c-oxidase and ATP-synthase. We conclude that the molecular basis for mtDNA effects in *B. taurus* cattle is limited, but it is extensive in cattle populations where *B. taurus* and *B. indicus* haplotypes occur simultaneously, e.g. New Zealand and Australia.

**C066**
**Forensics and Animal Genetics: An Emerging Science**

M. KETCHUM, M. ALVAREZ, J. ALVAREZ, M. KONIECZNY, et al.
*DNA Diagnostics, Inc. d.b.a. Shelterwood Laboratories, Carthage, TX*

In the past, various methods of human DNA analysis have been employed to link suspects to crime scenes. Now, crimes are also being solved with the use of animal DNA testing. Both mitochondrial DNA as well as STRs are now commonly used in the animal industries. In recent years, these resources are also being used to solve crimes. Cases have ranged from fraud disclosed using equine mitochondrial DNA to linking a suspect to the crime scene of a homicide with canine mitochondrial DNA. Additionally, multi-species STRs yield results in just as wide a variety of forensic cases. Equine STRs coupled with human STRs were utilized to not only identify a racehorse injected with cocaine, but also to identify the human profile of the suspect that injected the horse. Canine STRs amplified from DNA extracted from saliva stains on a victim's clothing after a brutal dog attack resulted in linking a neighbor's dogs to the attack and resulting criminal negligence charges. Bovine and equine STRs have been utilized on numerous occasions on various cattle rustling cases as well as horse theft with great success. Wildlife enforcement also utilizes animal DNA analysis to successfully prosecute cases. Deer STRs can link meat to bloodstains or remains in poaching cases. Additionally, sex of the deer can be determined so that laws relating to the harvesting of antlerless deer can be enforced.
With multi-species DNA analysis capabilities, more crimes will be solved in the future. If a suspect owns an animal or works around animals, chances are that animal hair will be present on his person. Even with gloves or other protective measures that a suspect might take to prevent transfer of his or her own DNA, animal hair has been left behind at crime scenes time after time. Increased utilization of available animal DNA analysis methods will insure that more cases will not remain unsolved.

**C067**
**Comparative sequence analysis of the TH-IGF2 region for Q and q alleles at an imprinted QTL mapping to proximal SSC2**

MINH NGYUEN, CATHERINE COLLETTE, CARINE NEZER, NADINE BUYS, MICHEL GEORGES
*Department of Genetics, Faculty of Veterinary Medicine, University of Liège*

While performing a whole genome scan to identify QTL influencing growth and carcass traits in a Piétrain x Large White intercross, we identified a QTL with major effect on muscularity and fat deposition towards the centromeric end of SSC2 (Nezer et al., 2001). Comparative mapping information pointed towards HSA11 as the human orthologue, and thereby to *IGF2* and *MyoD* as potential positional candidates. Mapping these genes with respect to the porcine marker map showed that *IGF2* co-localized with the QTL. Assuming that *IGF2* was indeed responsible for the QTL effect and that the *IGF2* gene would be imprinted in the pig as it is known to be in the human and mice, we made the prediction that in our F2 population a significant substitution effect would be found between the paternally inherited Piétrain versus Large White alleles, but not between the corresponding maternally inherited QTL alleles. We demonstrated that *IGF2* is indeed imprinted in the pig, and that only the QTL alleles inherited from the boar influenced the phenotype, thereby supporting our hypothesis (Nezer et al., 2001). Similar results were simultaneously

obtained in a Wild Boar x Large White intercross by Jeon et al. (2001).
Despite the strong candidacy of *IGF2* (given its known role in myogenesis), our results did not formally prove that this gene caused the observed QTL effect. *IGF2* indeed maps to an imprinted domain containing other putative candidates. To refine the map position of the QTL, we (i) constructed a BAC contig spanning the *TSSC5-H19* interval containing the *KVLQT1* and *IGF2* imprinted domains and from it developed a high density microsatellite and SNP-based marker map of proximal SSC2, and (ii) identified six distinct "*Q*" and "*q*" chromosomes by marker assisted segregation analysis performed in 32 large (> 100 offspring) boar families. Comparison of the "Q" bearing (muscle increasing) boar chromosomes revealed a shared chromosome segment in the interval bounded by $p57^{KIP2}$ and the 3' UTR of *IGF2,* thereby mapping the QTL to this interval. As *INS* and *IGF2* are the only known paternally expressed genes in this well-characterized interval, both remained good causative candidates. We therefore resequenced 28 Kb corresponding to the *3' TH- 3' IGF2* interval for the shared "*Q*" haplotype as well as the six "*q*" chromosomes. We identified > 140 different SNPs in this segment of which all but 30 showed a perfect allelic segregation between the Q and q chromosomes. Further analysis is in progress to identify the putative causal mutation responsible for the QTL effect. Corresponding results will be presented.

**C068**
**cDNA cloning and characterization of the porcine *RH* gene**

TOSHINORI OMI[1], STEFAN NEUENSCHWANDER[1], ŠPELA ŠPILAR[1], PETER VÖGELI[1], EIJI KAJII[2], GERALD STRANZINGER[1]
[1]*Swiss Federal Institute of Technology, Institute of Animal Sciences, Zurich, Switzerland,* [2]*Jichi Medical School, Department of Legal Medicine & Human Genetics, Tochigi, Japan*
The Rhesus (RH) homologous gene family includes *RH (D /CE)*, *RHAG*, *RHBG*, and *RHCG* in the human. RHD and RHCE, originally identified in human red cells, encode for the antigens of the human RH blood group system and were of central importance in transfusion medicine. In this paper, we describe the nucleotide sequence of the pig RH cDNA, the chromosomal mapping and two allelic variants of the pig *RH* gene. The pig RH cDNA has an open reading frame (ORF) of 1365 nucleotides (GenBank; AB067771), which encodes for a protein of 423 amino acids. On the amino acid level, the pig RH shares 60.8% and 61.0% identity with the human RHD and RHCE, respectively. The pig RH protein appears as a composite encoding amino acids from the human RHD and RHCE. However, the pig RH protein contains 6 amino acid residues more than the human RH proteins. Hydrophobic analysis suggests that the pig RH protein also has a 12-transmembrane domain, which is conserved among all members of the RH family. RH transcripts could be detected by RT-PCR in the spleen and bone marrow but not in the heart, the kidney, or the lung. The pig *RH* gene was mapped to chromosome 6q22-23 and porcine *RHBG* gene to chromosome 4q21-22 by somatic cell hybrid panel. A SINE sequence and a short tandem repeat (STR) of $(CAAA)_n$ were detected in the pig *RH* (GenBank; AB039288), corresponding to the human *RH* intron 4. So far, two alleles, $(CAAA)_1$ and $(CAAA)_4$, have been found in the pig.

**C069**
**Evolution of the bovine Y-chromosomal multicopy TSPY gene family**

E.L.C. VERKAAR, J.A. LENSTRA
*Department of Infections diseases and Immunology and Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*
The non-recombining region (NRY) is important for maintaining integrity of the Y-chromosome. A typical feature of the NRY is the rapid evolution and amplification of male specific sequences in order to prevent recombination with the X-chromosome. The repetitive TSPY (testis specific protein Y-encoded) gene encodes a nucleosome binding protein and has been found in several species. TSPY genes have been shown to be dispersed over the whole bovine NRY. We have studied the organization of the TSPY loci by isolation and analysis of BAC clones. Southern blotting showed two types of restriction patterns. Sequence analysis of representative clones revealed that one pattern corresponds to a cluster of four closely linked and heterogeneous TSPY

pseudogenes. The BAC clones with the less complex patterns contain a single TSPY gene with an intact open reading frame, which is highly similar to published expressed sequence tags. Comparison of the sixth intron sequences from several BAC clones and from related bovine species indicated sequence heterogeneity as well als concerted evolution. The amplified TSPY sequence from taurine cattle resembled the sequence of zebu, gayal and gaur, while the TSPY sequences of bison, wisent and yak form a seperate cluster. Our data suggests that repetitive elements as well as repetitive genes on the NRY are comparable to centromeric satellite repeats by being subject to a dynamic concerted evolution, presumably via successive amplification events.

**C070**
**SWMolecular analysis of candidate genes for congenital deafness in dalmatians**

T. KUTZER, I. PFEIFFER, B. BRENIG
*Institute of Veterinary Medicine, University of Goettingen, Germany*

Congenital deafness has been reported for approximately 60 dog breeds. The disorder is usually associated with pigmentation patterns. A large amount of white in the hair coat increases the likelihood of deafness. Two pigmentation genes are often associated with deafness in dogs: the merle gene and the piebald gene (Dalmatian, English Setter). But not all breeds with these genes have been reported to be affected. The deafness usually develops in the first few weeks after birth. A degeneration of a part of the blood supply to the cochlea is discussed as a reason. In most cases the incidence of congenital deafness in different breeds is unknown because of the limited number of studies. In the Dalmatian breed the occurrence is up to 30%. Deafness in Dalmatians appears to be an autosomal recessive disorder, possibly caused by the presence of two different autosomal recessive deafness genes, or a syndrome with incomplete penetrance. Further studies will be required to determine the mechanisms. At present no genetic tests exist to identify affected or carrier animals. We investigated the alpha-tectorin (TECTA) and Connexin 26 (Cx26) genes as candidates for deafness in dalmatians. The study included 119 dalmatian dogs, 92 healthy dogs, 10 unilaterally and 17 bilaterally deaf dogs. Using PCR primers based on published human TECTA sequence, we amplified and sequenced products from genomic dog DNA isolated from EDTA blood. BLAST results for Exon 10 indicated an average of 88 % homology between the dog and the human sequence. Exon 10 sequences of affected dogs, normal animals and control animals from different breeds were compared and analyzed for potential mutation sites. The analysis for Connexin 26 is in progress.

**C071**
**cDNA cloning, molecular analysis and expression of the ovine uroporphyrinogen decarboxylase gene**

REZA NEZAMZADEH, ANDREA KREMPLER, BERTRAM BRENIG
*Georg-August University of Göttingen, Department of Veterinary Medicine, Göttingen, Germany*

Uroporphyrinogen decarboxylase (Uro-D) is a cytosolic enzyme which catalyzes the fifth step of the heme biosynthetic pathway, the sequential decarboxylation of uroporphyrinogen III yields coproporphyrinogen III. Defects at the Uro-D locus cause the genetic disease Porphyria cutanea tarda (PCT). It was first described in domestic animals ahead with light sensitivity and photodermatosis. In females the defect also leads to reduced fertility by inactivation of the ovaries and reduced estrus.
The genomic structure of the Uro-D gene has been characterized in man and cDNA sequences are knwon from human, mouse and rat. According to the human genomic organisation we found 10 exons spanning over 3 Kb. On screening animals with the disease we found a point mutation in the coding region of Uro-D gene resulting in a Leu → Pro substitution at amino acid position 392. The ovine Uro-D cDNA was isolated from sheep liver. The open reading frame with a length of 1104 bp encodes a 367-amino acid polypeptide with a predicted molecular mass of 45 kDa.

**C072**
**Isolation and characterization of the porcine ß-Glucuronidase gene (GUSB) as candidate for congenital hernia**

J. BECK[1], CHRISTOPH KNORR[1], E. SCHUETZ[2] & BERTRAM BRENIG[1]
[1]*Institute of Veterinary Medicine, Georg-August-University of Göttingen, Göttingen*

*(Germany)*
[2]*Chronix Biomedical, Benicia, CA, USA*
The gubernaculum is a loose connective tissue organ that plays a major role during testicular descent. In the pig, the first phase of transabdominal migration is brought about by growth of the gubernaculum through the inguinal canal into the scrotum. This dilates the inguinal canal preparing the pass of the testis through this bottleneck. Development of the gubernaculum during this phase is characterized by rapid cell proliferation and accumulation of intercellular water. This process is mainly mediated by synthesis of hyaluronic acid (HA), since this poly-anionic macromolecule is known to have a large hydrodynamic volume. Our assumption is that swelling of the gubernaculum exceeds through diminished degradation of HA and causes that the inguinal canal remains open, which predisposes male pigs for the development of hernia. As ß-Glucuronidase is involved in the biodegradation of HA, it is a candidate gene for this congenital disorder. Here we report the isolation, characterization, and localization of the porcine GUSB gene. Our first step was to screen a porcine genomic PAC-library by PCR, with primers derived from exon 6 & 7 of the feline GUSB gene. A clone of approximately 80 kbp was isolated, containing the whole GUSB gene. We assigned GUSB to porcine chromosome SSC3p14-p16 by FISH and confirmed our findings by hybrid panel analysis. Animals phenotypically affected with hernia inguinalis and non-affected animals were used to detect SNPs in the procine GUSB gene by CEL I digestion of heteroduplex DNA and comparative sequencing.

**C073**
**The origin of domestic cattle (BOS TAURUS) in Middle Europe: ancient DNA Analysis on Neolithic bones**

RUTH BOLLONGINO, JOACHIM BURGER, KURT W. ALT
*University of Mainz, Molecular Archaeology Group, Institute of Anthropology, Mainz, Germany*
We present the first results of a project on the origin of Middle European domestic cattle (Bos taurus). From archaeological and archaeozoological record it is not quite clear whether the first domestic cattle were imported from the Near East or if there was an autochthonal domestication of the wild aurochs (Bos primigenius) in Middle Europe. Ancient DNA analyses on Neolithic bones were performed in order to collect genetic data of different populations. Samples from all over Middle Europe, the Balkan and the Near East will be compared to find out where centers of domestication were and if crossbreeding with wild aurochs took place. The first results from mitochondrial markers (d-loop) are presented from both Neolithic domestic cattle and aurochs.

**C074**
**Molecular characterization of a 95kDa zona receptor kinase fragment**

LEONARD BULL, STEPHAN JANSEN, BERTRAM BRENIG
*Institute of Veterinary Medicine, Georg-August University of Goettingen, Germany*
Gamete binding involves complex recognition mechanisms which are directed by specialised molecules. The male adhesion molecules are divided in primary and secondary ligands. Primary ligands and their receptors are responsible for the first recognition of the gamete and the initiation of metabolic pathways as can be seen in the acrosome reaction, while secondary receptors and ligands become important shortly before the sperm's penetration through the zona pellucida. In our experiments,
we aim to find out whether the initial binding of the gametes can be modulated. We hypothesis that binding affinity to the complementary gamete can be modified by the state of phosphorylation in certain proteins. Under the assumption that the degree of phosphorylation in binding proteins is affected by environmental factors, lack of fertility and the inability to bind to the complementary gamete -either permanently or temporarily- would then become explainable. We have cloned a porcine fragment of the 95 kDa receptor kinase. Sequence analysis reveals that the porcine receptor fragment shows a homology of 88.5% and 74% to the murine and human receptor kinase. The fragment has been also successfully expressed in a bacterial system. This is an important prerequisite for further experiments in which the binding capacity in a phosphorylated and dephosphorylated state can be measured.

**C075**

**The porcine CALC-A/α-CGRP gene: Isolation, characterization, physical mapping, and detection of SNPs (single nucleotide polymorphisms)**

CHRISTOPH KNORR, BERTRAM BRENIG
*Georg-August-University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany*

Calcitonin and α-CGRP are products of the alternatively spliced gene CALC-A/α-CGRP. Calcitonin mRNA is predominantly found in thyrodial C cells and mRNA encoding the neuropeptide calcitonin gene-related peptide (CGRP) in neural tissues. In vivo, calcitonin reduces serum calcium and acts antagonistic to the parathyroid hormone. Primers derived from the canine mRNA were designed to screen porcine genomic libraries. A positive PAC clone has been isolated and the CALC-A/α-CGRP gene was completely sequenced. Calcitonin is translated after alternative splicing of exons 1 to 4 and α-CGRP is generated by alternative splicing of exons 1, 2, 3, 5, and 6. Ten SNPs (single nucleotide polymorphism) have been detected in the gene after comperative sequencing of animals of different origins. The porcine CALC-A/α-CGRP gene was localized to chromosome 2p11→p13 confirming synteny between the p arms of chromosomes HSA11 and SSC2.

**C076**

**PathoCHIP: Identification of genes expressed during the pathogenesis and control of *Haemophilus parasuis* infections in pigs**

SAFFRON DORNAN[1], LUCINA GALINA[1],CAROLE SARGENT[1], LUSHENG HUANG[1], ISABEL BLANCO[2], JOACHIM ULLRICH[3], VOLKER SPEHE[3], PAUL SELTZER[3], MARNIE MELLENCAMP[1], ANA CANALS[2] & GARY EVANS[1*]
www.pathochipproject.com
[1]*Sygen Laboratory, Department of Pathology, University of Cambridge, UK,* [2]*INIA-CISA, Madrid, Spain and* [3]*Intervet, Frankfurt, Germany*

*Haemophilus parasuis* is a gram-negative bacterium causing polyserositis primarily in young pigs. Current treatments include antibiotics and vaccination. Vaccination is of limited success and development of antibiotic resistance is also a concern.

PathoCHIP is an EC funded Framework 5 project, aiming to use microarrays to identify both host and pathogen genes that are differentially expressed during *Haemophilus parasuis* infection. It is hoped that identification of bacterial virulence genes will lead to development of novel treatments for the disease whilst identification of host candidate genes responsible for disease resistance may lead to the production of animals that are resistant or less susceptible to the disease via marker assisted selection.

An animal challenge model will be utilized and optimized to reproduce the disease in vivo. Two lines of pigs will be used in the challenges to increase the amount of genetic differences in host susceptibility to infection. Differences in host responses to the experimental challenge will be determined by assessment of clinical signs, bacteriology, pathology as well as PCR testing. Samples will be taken from all points of infection (lung, pericardium, meninges, joints, tonsils and lymph node) to allow preparation of both host and bacterial RNA. This material will be used to identify bacterial and host genes that are differentially expressed during the infection via the use of bacterial and host microarrays. A *Haemophilus parasuis* genomic DNA library containing 18,000 clones (> 6x genome coverage) will be used to analyse the bacterial genes expressed during the pathogenesis. A host pig normalized cDNA library from infected tissues containing about 15,000 clones and a pig immune tissue cDNA library containing about 9,000 clones will be used to construct the host pig microarrays which will be used to analyse gene expression differences. Differentially expressed genes will be sequenced to identify candidate genes associated with disease resistance or susceptibility. Host genes will also be identified using SSH cDNA libraries.

It is hoped that the PathoCHIP project may generate additional tools such as diagnostics to identify infected pigs, identification of bacterial genes that can be used to develop vaccines and creation of genetic tools and resources to study the interaction between other pathogens and the host.

* To whom correspondence should be addressed

# Section D: Marker, Polymorphism and Biodiversity

**D001**

**Mitochondrial D-loop and cytochrome B gene sequences as a tool to preserve the genetic diversity in an Iberian pig population**

ESTEFÂNIA ALVES, CRISTINA ÓVILO, CARMEN RODRÍGUEZ, LUÍS SILIÓ
*INIA, Departamento Mejora Genética Animal, Madrid, Spain*

A conservation programme of Iberian pig is based on the *Torbiscal* line, produced by blending four old strains of Iberian pigs: two Portuguese (Ervideira and Caldeira) and two Spanish (Puebla and Campanario) strains. The complete genealogy of all animals is available back to 1945, with 21 generations from the founders to the present animals. These genealogical records allow the identification of eight maternal lineages: five corresponding to Campanario strain and the others to Puebla, Caldeira and Ervideira strains, respectively. The complete sequence of the *D-loop* region (1246 bp) and *cytochrome B* (*CytB*) gene (1140 bp) of mtDNA were determined by direct sequencing of PCR-product from eight animals representing the eight quoted maternal lineages. We found the existence of six haplotypes defined by 14 single nucleotide polymorphisms, 10 at the *D-loop* region (positions 15544, 15558, 15578, 15714, 15715, 15741, 15758, 16127, 16139 and 16141) and four at the *CytB* gene (positions 14309, 14717, 14740 and 15264). One out of the six haplotypes was shared by three maternal lineages belonging to Campanario strain and the other five were specific to each one of the remaining lineages. Our results show that mitochondrial DNA sequence analysis is useful for the assessment of non-autosomal genetic variability in a conservation programme and to improve new conservation strategies.

**D002**

**Identification of two new variants of vitamin D binding protein (GC) in cattle**

HELEN ARTHUR, T. KEVIN BELL
*Australian Equine Genetics Research Centre, University of Queensland, St. Lucia, Australia*

Vitamin D binding protein (GC) polymorphism has been reported in many mammalian species. In addition, GC has been shown to be linked to albumin and α-fetoprotein and these constitute a gene family. In cattle, three GC variants have been described and marked breed differences have been observed. Utilizing 12% PAGE, pH 7.9, and Identification of two new variants of vitamin D binding protein (GC) in cattle immunoblotting with antiserum to the human GC protein, two new GC variants, designated GC M and GC D, have been found in Piedmontese and Angus cattle respectively. The Piedmontese variant migrates between the F and S variants and occurred only at low frequency (n=316; F = 0.30, S = 0.67, M = 0.03). An examination of samples from Angus cattle revealed a much slower migrating variant which was also relatively rare (n=1144; F = 0.11, S = 0.88, D = 0.01). Whilst GC F and S homozygotes are commonly found, in this study the GC M and GC D forms and also GC C occurred only as heterozygotes. Neuraminidase treatment of plasma samples followed by two-dimensional electrophoresis (IEF 4.5-5.4, 10% PAGE, pH 7.9) and immunoblotting indicated that the microheterogeneity observed in the five known GC variants was due in part to differences in sialylation.

**D003**

**Thoroughbred and Trotter Blood Group and DNA Allele Frequency in Hungary**

BEÁTA BÁN[1], ALICE GYURMAN[1], CSILLA JÓZSA[2]
*[1]National Institute for Agriculture Quality Control; [2]University of Veszprém*

Two thoroughbred and two trotter stocks were typed (N=88) with 5 biochemical systems (Tf, Al, Es, Gc, A1B), blood groupwithin the D system by 13 serums and a set of 12 microsatellites by ABI 310 automatic prism analyzer. The results indicated that the allele frequencies characteristic for the species in the basis of by blood group and polymorphical tests can be detected by the microsatellite tests as well. Those group which can be seperated with species on the basis of alleles by tradtional methods, can be seperated by microsatellite tests, too.

**D004**

**Evaluation of certain genetic and phenotypic parameters on growth performance in crosses of Hampshire x Indigenous Pigs in Assam**

TEJENDRA BARDOLOI, KABERI DEKA
*Assam Agricultural University, Department of Animal Genetics and Breeding,*
*College of Veterinary Science, Khanapara , Guwahati-781022,Assam ,India*

Data on growth records of 621 half-bred and 697 graded (crosses of Hampshire and Indigenous) progenies born to 122 dams mated to 70 sires were utilized to analyse the effects of various genetic and non-genetic factors, to estimate the heritability, genetic and phenotypic correlation coefficients on body weights at birth, weaning,16$^{th}$ week, maturity and at slaughter age respectively. Least squares analysis of variance revealed highly significant effect of genetic groups on body weights at birth,8$^{th}$, and 24$^{th}$ week, highly significant influence of sex on body weights at 8$^{th}$, 24$^{th}$ and 32$^{nd}$ week. Period of birth and season of birth had highly significant effects on body weights at all these stages of growth. Faster growth in half-bred and graded pigs born during winter and pre-monsoon seasons is a favourable information to the breeders to maximize profit by restricting the farrowings to these seasons. Heritability estimates by paternal half-sib correlation method indicated better performance on overall population, followed by graded and then in half-breds. The genetic correlation coefficients in graded pigs were better than overall estimates, followed by half-breds. The coefficient of determinant ($R^2$) values indicated that efficiency in prediction of body weight at 24$^{th}$ week is more, as compared to body weight at 32$^{nd}$ week, when the linear regression coefficient on body weight at 16$^{th}$ week, partial regression coefficients on body weights at 8$^{th}$ and 16$^{th}$ weeks and multiple regression coefficients on body weights at birth, 8$^{th}$ and 16$^{th}$ weeks of ages are considered to be the independent factors.

**D006**

**Restriction fragment length polymorphism analysis of a conserved region in the mitochondrial cytochrome *b* gene in the North American Bison (*Bison bison)***

DOMENICO BERNOCO, JONATHAN N. DODD, BOB G. MORRIS
*Stormont Laboratories Inc., Woodland, CA 95776, USA*

Using the restriction fragment length polymorphism test of Meyer *et al*. (1995), 447 North American Bison (*Bison bison)* were tested for variation in the mitochondrial cytochrome *b* gene. The amplicons (359 bp in length) were digested with the restriction enzyme *Inf* I and resolved by polyacrylamide gel electrophoresis. Two restriction profiles were identified: one characterized by two DNA fragments of 198 and 161 bp, generated by the presence of a single *Inf* I restriction site in the amplified region, and a second one characterized by three fragments of 198, 117 and 44 bp generated by an additional *Inf* I restriction site in the smaller fragment. The first restriction profile was expected in agreement with the published DNA sequence for bison (Hassanin, A. and Douzery, E.J.P., 1999), and was called B for bison specific. The unexpected second profile was found to be in accordance with the published DNA sequence for cattle (Anderson, S. et al., 1982), and therefore called C for cattle specific. If the presence of the C restriction profile among bison is used as an indication of past cattle-bison hybridization, then its occurrence among the tested populations was estimated at 0.028 ± 0.016 (n = 107) in commercial herds, at 0.018 ± 0.012 (n = 114) and at 0.066 ± 0.017 (n = 226) in two protected public park herds for an average of 0.045 ± 0.010. Due to the low occurrence of the C restriction profile, the differences between herds' frequencies were not statistically significant.

**D007**

**Co-amplification of ten microsatellites for identity and parentage testing in Alpaca**

MICHELE BLASI[1], ADELE LANZA[1], CINZIA VARLOTTA[1], MARYAM MOTAVALIAN[1], ANDREA ROSATI[1], GABRIELLA BOZZINI[2]
[1]*Laboratorio Gruppi Sanguigni, Italy;* [2]*Associazione Italpaca, Italy*

Alpaca breeding in Italy is in its initial stages of growth but promises to grow fairly quickly, just as it is happening in other European countries. As the alpaca breeding population increases, it inevitably requires that breeders have a means for identifying single animals and their exact maternal and parental links. For this purposes, the Laboratorio Gruppi San-

guini, a longstanding center dedicated to the genetic improvement of livestock in Italy, has defined a protocol based on the amplification of 10 microsatellites. The ten microsatellites that have been selected by our laboratory for the identification and exclusion of parentage in alpacas were extracted from published scientific literature. The microsatellites selected are the following: *LCA192, LCA23, LCA56, LCA71, LCA65, LCA5, LCA68, LCA66, LCA8, LCA77*. To demonstrate the validity of this protocol we analysed 50 non-sib Alpacas. Parentage exclusion probability has been calculated for each locus and for the all 10 loci together and is equal to 0.999. If one of the parents is sure, 999 out of 1000 mistakes in the attribution of the other parent can be identified.

Last researches based on fruit flies (*Drosophila*) (4 pairs of chromosomes) that were conducted by us, show that on the basis of traditional calculation of genotype frequency of any breed in general genotype of hybrid it is impossible to identify actual genotype of every separate animal. It is generally known that genotype of hybrids ABC, received from ABxC, is ¼ A, ¼ B and ½ C. But the analysis of possible versions of segregation shows that in practice far not all the animals have such ratio of A,B,C genotypes in themselves, and even double-breed individuals may be met (table 1).

♂**AB x C**♀

| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|
| 1A | A | B | B | B | C | C | C | C |
| | B | A | B | B | C | C | C | C |
| | B | B | A | B | C | C | C | C |
| | B | B | B | A | C | C | C | C |
| 2A | A | A | B | B | C | C | C | C |
| | B | A | A | B | C | C | C | C |
| | B | B | A | A | C | C | C | C |
| | A | B | A | B | C | C | C | C |
| | A | B | B | A | C | C | C | C |
| | B | A | B | A | C | C | C | C |
| 3A | B | A | A | A | C | C | C | C |
| | A | B | A | A | C | C | C | C |
| | A | A | B | A | C | C | C | C |
| | A | A | A | B | C | C | C | C |
| 4 | A | A | A | A | C | C | C | C |
| 4 | B | B | B | B | C | C | C | C |

Besides this, even animals that correspond to the counted parts are not the same as for genotypes. The division of chromosomes lasts in more complicated manner in population as well as in individuals during hybridization of received three-hybrids descendants with individuals of D-breed.

That's why in future it is necessary to work out new methodical approaches to evaluation and forecasting possibility of appearance of animals of given genotype.

**D009**

**Polymorphism evaluation of three bovine microsatellite markers in different Italian cattle breeds**

G. BONGIONI, A. POZZI, K. PARATI, A. GALLI

*Istituto Sperimentale Italiano "Lazzaro Spallanzani" Milano, Italy*

In Italy the official control of quality semen is regulated by law and executed by the Lazzaro Spallanzani Institute on random samples of frozen semen (at least 10% of all batches circulating in Italy). The official control includes the so called "identity test", which is done by comparing the pattern of DNA extracted from reference sample (blood or semen) versus DNA extracted from semen of each sampled batch. For this test we used at least three microsatellites belonging to the ISAG recommended set : TGLA 122, TGLA 126, TGLA 227.

The aim of this work was to compare seven Italian cattle breeds: Italian Brown (01), Italian Frisian (02), Valdostana Pezzata Rossa (03), Italian Simmenthal (04), Piedmontese (05), Rendena (10) and Grey Alpine (11) concerning the following genetic parameters: effective number of alleles (A. Ns.), Heterozygosity (He) and Polymorphism information content (PIC) obtained from these three microsatellites in 1204 sires.

The DNA extracted was amplified by PCR and alleles were visualized by 373 DNA automatic sequencer (ABI Prism–Applied Biosystems) . The following table showes the obtained results.

| | TGLA 122 | | | TGLA 126 | | | TGLA 227 | | |
|---|---|---|---|---|---|---|---|---|---|
| Breed | A. Ns. | He | PIC | A. Ns. | He | PIC | A. Ns. | He | PIC |
| 01 | 13 | 0.78 | 0.75 | 6 | 0.63 | 0.55 | 10 | 0.80 | 0.77 |
| 02 | 17 | 0.82 | 0.78 | 7 | 0.73 | 0.69 | 12 | 0.83 | 0.81 |
| 03 | 14 | 0.82 | 0.79 | 7 | 0.76 | 0.70 | 13 | 0.86 | 0.84 |
| 04 | 13 | 0.79 | 0.76 | 8 | 0.65 | 0.59 | 9 | 0.81 | 0.78 |
| 05 | 19 | 0.86 | 0.83 | 6 | 0.77 | 0.74 | 12 | 0.88 | 0.87 |
| 10 | 9 | 0.68 | 0.64 | 6 | 0.65 | 0.60 | 8 | 0.79 | 0.75 |
| 11 | 7 | 0.76 | 0.71 | 4 | 0.63 | 0.57 | 11 | 0.82 | 0.79 |

The most variable breed was represented by Piedmontese in all three locus either for Heterozygosity or for PIC.

**D010**

**Development, comparison and application of different *PRNP* typing systems**

JOHANNES BUITKAMP[1], CHRISTA KÜHN[2], MARTINA HAIDUCH[1], URSULA IRPS[3], JÖRDIS SEMMER[1], JÖRN MOSNER[3]
[1] *Bayerische Landesanstalt für Tierzucht, Abt. Bio- und Gentechnik, Poing, Germany;* [2]*Forschungs-institut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany;* [3] *GAG BioScience, Bremen, Germany*

Scrapie is a transmissible spongiform encephalopathy (TSEs) of sheep. Sheep that are experimentally infected with BSE agent via the oral route develop almost the same clinical signs as scrapie. Natural scrapie causes economic losses that could be reduced by the use of scrapie resistant sheep. It is well known that variants of the prion protein coding gene *(PRNP)* are associated with resistance or susceptibility to scrapie and several labs provide genetic tests. Depending on the application *PRNP* test systems have to meet different requirements. When used in scientific projects a precise definition of the alleles may be required including detection of novel alleles whereas mass-screening systems will need robust and cheap single-pass typing of a large number of sheep. We have implemented three different methods accomplishing these different needs. For complete analysis of the genotype we used direct PCR sequencing, for medium-throughput applications we developed a fluorescent multiplex 'amplification refractory mutation system', and for the mass-screening a fully automated MALDI-TOF based method was established. The pros and cons of these methods will be discussed.

**D011**

**Genetic Diversity of the *Falco peregrinus* populations of central Iberian Peninsula**

E. CALONGE, Y. MÍNGUEZ, S. DUNNER, J. CAÑÓN
*Dpto. Producción Animal, Facultad de Veterinaria, Madrid, Spain*

A total number of 124 *Falco peregrinus* belonging to three different ecological niches of the central Iberian Peninsula were studied with the purpose of analysing the existence of a subjacent genetic structure, and also studying the inbreeding levels associated to these niches. From the 124 birds analysed, 35 corresponded to the Mountain subpopulation, 51 to the Valley and 33 from the Regional Park of Manzanares. All birds were genotyped using 7 microsatellites (NVHfp13, NVHfp31, NVHfp79-4, NVHfp89, NVHfp92-1, UCMfp517, UCMfp347) and AFLPs (Amplified Fragment Length Polymorphisms) through the combination of TaqI and EcoRI and primers E45 and T32. Genotypes were used by scoring absence or presence of an allele with "0" or "1", and genetic distances estimated according to Dice. The graphic representation shows the grouping of Mountain and Regional Park sub-populations with the clear segregation of Valley sub-population. A low percentage (3%) of the total genetic variability, estimated using F statistics, is due to the subpopulations for which the inbreeding level was relatively high (17%). Analysis of diversity shows that if extinction of Regional Park and Mountain sub-populations is considered, 52% of the total genetic diversity would be lost.

**D012**

**Chromosomal localization, and genetic variation of the Ovine heart fatty acid-binding protein gene (H-FABP)**

JORGE H. CALVO[1], NADIRA SAÏDI-MEHTAR[2], DANIEL VAIMAN[3], JUAN J. JURADOL, MALENA SERRANOL
[1]*Departamento de Mejora genética, INIA, Madrid, Spain;* [2]*Laboratoire de Biologie Moléculaire et Génétique, Université Oran Es-Sénia, Oran, Algeria;* [3]*Laboratoire de Génétique Biochimique et de Cytogénétique, Département de Génétique Animale, INRA, Jouy-en Josas, France*

Fatty acid-binding proteins (FABPs) are small intracellular proteins involved in fatty acid transport from the plasma membrane to the sites of β oxidation and triacylglycerol or phospholipid synthesis. The heart type FABP (H-FABP) protein is present in several tissues with a high demand for fatty acids such as cardiac and skeletal muscle and lactating mammary gland. By the other hand, this protein has been associated with mammary gland development. Thus, the ovine H-FABP is a candidate gene for milk and meat quality, and for uddertraits.

Exons two, three and four and flanking intronic sequences were determined as well as a small DNA fragment of the 3' untranslated region, from an autochthonous breed specialized in

milk production (Manchega breed). Eleven SNPs were detected up to now, showing similar haplotypes. Besides, a variable CTC insertion and a variable poli A tract were also detected in intron 3. These polymorphisms have been characterized in Manchega breed.
The sheep H-FABP gene was located on chromosome 2 by sheep sequence-specific PCR on DNA from a sheep/rodent cell hybrid panel.
H-FABP pseudogene-like sequences have been isolated, showing a 7-bp deletion in putative exon 3, and various nucleotide substitutions, as well as codon stops.
In conclusion, this genetic variation will enable us to investigate the role of the H-FABP gene in milk and meat quality.

**D013**
**An IRS-PCR (Interspersed Repeated Sequence-PCR) method for the determination of sex and other traits in bovine embryos**

IRENE CAPPUCCIO, ALESSIO VALENTINI
*Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy*
Control of the sex ratio of domestic species is potentially of great commercial importance for animal production. Several methods for bovine embryos sexing have been developed. The optimal one must be accurate, non invasive, inexpensive, rapid. Methods based on PCR fit all these requirements, moreover they can allow the characterisation of bovine embryos for multiple traits and in this respect they are superior to the use of sexed sperm for the artificial insemination. The reduced amount of DNA obtained from few biopsed cells represents a major limitation to perform multiple analyses or for repeating the analysis to improve its accuracy.
Several technologies based on PCR amplification of unknown DNA sequences are available to increase the overall amount of DNA and some of them have been successfully used.
In this study an IRS-PCR (Interspersed Repeated Sequence-PCR) was performed to increase the amount of DNA present in 2-4 days bovine embryos and its amplified products were used for a successful and accurate sex typing. The possibility of multiple loci embryo characterisation is investigated.

**D014**
**Gene linkage mapping of the porcine chromosome X region harbouring QTL for fat deposition**

STANISLAV CEPICA[1], GARY A. ROHRER[2], MARTIN MASOPUST[1]
[1]*Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic;* [2]*USDA, ARS, U. S. Meat Animal Research Center, PO Box 166, Spur 18D, Clay Center, Nebraska, USA*
The QTL for backfat thickness and intramuscular fat content on SSCX is well documented in Meishan x Western breed pedigrees. The QTL has been mapped to the chromosome region between microsatellites SW2456 and SW1943. In the French pedigree with more than 1100 $F_2$ animals the QTL mapped at position 73 – 74 Kosambi cM with 95% confidence interval ranging from 65 to 85 Kosambi cM. For fine mapping and comparative positional candidate cloning, alignment of human and swine gene maps is essential. Using PCR we have partially cloned and sequenced porcine orthologs of genes from HSAX and subsequently detected SNPs (PCR-RFLPs) within seven genes. These markers have been typed on the USDA-MARC backcross pedigree. Gene location on the current USDA-MARC linkage map of chromosome X follows: AMELX - 8.7 cM, RPS4X – 74 cM, POU3F4 – 74 cM, FACL4 – 80 cM, CAPN6 – 81 cM, PAK3 – 82.5 cM, and BGN – 128,0 cM. Final order of genes mapped to the USDA-MARC 2 linkage map of the chromosome X region with 95% confidence interval of the QTL contains genes in the following order: AR – 74 cM, PGK1 – 74 cM, ZNF261 – 74 cM, RPS4X – 74 cM, PLP1 – 74 cM, POU3F4 – 74 cM, SERPINA7 – 75.5 cM, FACL4 – 80 cM, CAPN6 – 81 cM, and PAK3 – 82.5 cM. From comparison of cytogenetic and linkage maps it is apparent that the chromosome region belongs among those with a low recombination rate. Now with more than 20 genes on the porcine linkage map there is no evidence of rearrangements in gene order between porcine and human X chromosomes.

**D015**
**Milk protein variability in African *Bos Genus* breeds**

GABRIELLA CERIOTTI[1], GIULIANA

TIRELLA[1], STEFANIA CHESSA[1], ANNA CAROLI[2], CASIMIRO CRIMELLA[3]
*[1]University of Milan, Department VSA, Milano, Italy; [2]University of Bari, Department of Animal Health and Welfare, Valenzano, Italy; [3]University of Milan, Institute of Zootechnics, Milano, Italy*

The importance of milk protein loci is well known due to the relationships with productive traits. The polymorphism of $\alpha_{s1}$-casein (*CSN1S1*), β-casein (*CSN2*), k-casein (*CSN3*) and β-lactoglobulin (*LGB*) was investigated in African *Bos taurus* (Somba, Lagunaire), *Bos indicus* (Zebu Peul Soudanais) and *Bos taurus x Bos indicus* (Borgou) populations at DNA level. The employed techniques were: ASP-PCR (*CSN1S1*), PCR-RFLP (*BLG*), and PCR-SSCP (*CSN2* and *CSN3*). Milk protein *loci* were all polymorphic, except *CSN1S1* in Lagunaire. *CSN2* presented four alleles in Borgou and Zebu (*A[1]*, *A[2]*, *B*, *I*), while only *A[1]* and *A[2]* variants were found in Somba ($A^1$ = 75%) and Lagunaire ($A^1$ = 69%). The *CSN2*I* variant, recently described in European breeds, showed a rather high frequency (10%) in Zebu, and was detected at a lower frequency (1%) in Borgou. At *CSN3* locus, *B* allele was predominant in pure taurine breeds (Somba: 76%; Lagunaire: 75%), while *H* was predominant in Zebu and Borgou (35% both). *CSN*H* allele can only be detected at DNA level, being identical to *CSN*A* at protein level by electrophoretic techniques. In addition *CSN3*A[1]*, which is a silent mutation based on *CSN3*A*, was observed in Zebu (17%) and Borgou (8%). At *LGB* locus, the *B* allele was predominant, if compared to *LGB*A*, with a decreasing trend from Zebu (90%) to Borgou (81%), Somba (78%) and Lagunaire (51%). The discriminating power of milk protein genes in differentiating between indicine and taurine breeds was verified by statistical analyses.

**D016**

**Towards the construction of an integrated cytogenetic and genetic map of the rabbit**

C. CHANTRY-DARMON[1,2], D. VAIMAN[2], H. HAYES[2], H. DE ROCHAMBEAU[3], D. ALLAIN[3], C. URIEN[1], N. BOURGEAUX[1], M. BERTAUD[2], P. CHARDON[1], C. ROGEL-GAILLARD[1]
*[1]Laboratoire de Radiobiologie et Etude du Génome, UMR INRA-CEA 13.314, Jouy-en-Josas, France; [2]Laboratoire de Génétique biochimique et Cytogénétique, INRA, Jouy-en-Josas, France; [3]Station d'Amélioration Génétique des Animaux, INRA, Castanet-Tolosan, France*

Rabbit (*Oryctolagus cuniculus*) is a small but active animal sector for meat and fur in Southern Europe agriculture and is a good model for human diseases and for studies on physiology, immunology and transgenesis. However, the rabbit genome has been poorly studied until now. Since there is an increasing need of efficient mapping tools to identify genes of interest in this species, the construction of an integrated cytogenetic and genetic map has started. The aim of the work is to build a first generation microsatellite map with a resolution of 10 cM that means the isolation of about 300 polymorphic microsatellites. Microsatellite typing will be performed on informative three generation families to establish genetic distances. The availability of both a rabbit BAC library and data on reciprocal human-rabbit chromosomal painting allowed the development of an integrated approach. The BAC library has been screened by PCR or by high density filter hybridization for genes chosen for their hypothetical position on rabbit chromosomes. BAC clones for 74 distinct genes have already been isolated and 55 clones were mapped by FISH to all but the X chromosomes. Thirtytwo microsatellite sequences with more than 10 repeats were identified from 22 BAC clones and unexpectedly half of them corresponded to (TC)n repeats. PCR primers were designed for 20 microsatellites. Isolation of other microsatellites and analysis of the allelic polymorphism is in progress.

**D017**

**Radiation hybrid mapping of 22 loci containing endogenous retroviral sequences in the pig and characterization of the retroviral genomic flanking sequences**

Z. LIU[1], C. ROGEL-GAILLARD[2], N. BOURGEAUX[2], J. C. SAVE[2], M. YERLE[3], D. MILAN[3], P. CHARDON[2]
*[1]Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China; [2]Laboratoire de Radiobiologie et Etude du Génome, UMR INRA-CEA 13.314, Jouy-en-Josas, France; [3]Laboratoire de Génétique Cellulaire, INRA, Castanet-Tolosan, France*

Genomic integration of mobile elements such as transposons and retroviruses is known to

contribute to the evolution and plasticity of genomes. Sixtytwo BAC clones containing type C porcine retroviruses (PERVS) were previously isolated from a porcine BAC library and mapped by fluorescent in situ hybridization to all chromosomes except 6, 12, 15, 16 and 18, allowing us to identify 24 distinct integration loci. We report here the more precise mapping of 22 loci using the ImpRH radiation hybrid (RH) panel and sequence analysis of PERV genomic flanks. BAC end sequencing was done for all BAC clones and 104 sequence tagged sites were recovered. PCR was performed on the RH panel with 31 primer pairs designed from the STS. Two loci at position 17q12 were clearly distinguished due to the resolution of the RH mapping. Two PERV clusters had been described at positions 3p15 and 17q21 but the RH mapping results strongly suggested a unique locus at each position. Further fingerprinting analysis of the BAC clones confirmed the single locus and the existence of two alleles at each chromosomal position. A massive sub-cloning of all PERV genomic flanks was launched. One BAC clone per locus was digested by EcoR1 or HindIII and a long terminal repeat (LTR) probe specific for PERVs was used to recover the LTR flanking genomic sequences on both sides of the PERV. Analysis of the sequencing results will help to study genomic segments permissive for stable retroviral integration in the pig.

**D018**
**RFLP and SSCP polymorphism in the myogenin gene in crossbred pigs**

KRYSTYNA M. CHARON, ZUZANNA NOWAK, JÓZEF KULISIEWICZ & ALEKSANDRA HERMANOWICZ-ŚWIERCZEK
*Agricultural University, Faculty of Animal Science, Warsaw, Poland*

Polymorphism in the chosen regions of myogenin gene was analysed in two groups of crossbred fatteners, born by crossbred sows (Polish Large White x Polish Landrace) and sired by (I) Duroc boars and (II) crossbred boars (Duroc x Pietrain) from the Warsaw Agricultural University experimental farm. The total DNA was isolated from a whole blood using phenol/chloroform extraction. SSCP polymorphism was analysed in exons 1, 2 and 3, while RFLP (*Msp*I) polymorphism in non-coding region of 3' end of the myogenin gene. Primers for exons 1 to 3 were designed using computer program „Primer 3", while primers for the 3'gene end originated from literature (Ernst et al., 1993).
Exon 1 showed low polymorphism with two SSCP patterns: A (two bands) in 98% of animals from group I and 94% in group II, and E (three bands) in 2 fatteners from group I and 6 in group II. Exon 2 in all studied animals was monomorphic. Exon 3 showed high polymorphism with four patterns: A, B, C and D alleles with one to three bands. Pattern A was the most frequently observed (88% in group I, 82% in group II), the remaining patterns occurred in 2-10% of fatteners.
Sequence analysis showed substitution T→A in exon 1; deletion T in exon 3 (B allele) and substitution G→T in exon 3 (C allele). Allele D had the same sequence as allele B.
In non-coding region of 3' end of myogenin gene we found three RFLP genotypes: F - homozygote non-digested with *Msp*I (PCR product equal to 353 bp), G - homozygote digested with *Msp*I (two bands - 219 bp and 134 bp) and H - heterozygote including F and G bands. Genotype H was the most frequent in both groups of fatteners (0.85 and 0.65 in I and II group, respectively), genotype G frequency was equal to 0.13 in group I and 0.32 in group II, while genotype F was the most rare one (0.02 and 0.03, respectively).

**D019**
**Microsatellite DNA polymorphism of Jeju native horses in Korea**

GIL JAE CHO[1], YOUNG JAE LIM[1], SUNG JEAN KIM[1], BYUNG WOOK CHO[2]
[1]*Korea Racing Association, Equine Blood Typing Laboratory, Gwachon, Korea;* [2]*Miryang National University, Department of Animal Science, Miryang, Korea*

Jeju native horses (JNH) are one of the native animals that were designated as a natural monument in Korea. They have been isolated in Jeju island for a long time and some of them have been used as racing horses. Ramdom samples of 62 JNH were genotyped with 16 microsatellites markers including nine international minimum standard panel. Multiplex PCR was used for amplifying these microsatellite markers and genotyping reproducibility was observed based on size precision of the PCR fragments. Allele frequencies, heterozygosities, polymorphic information contents (PIC) and exclusion probabilities (PE) were

calculated. The number of alleles of the markers was varied between 5 and 13 with an average number of alleles of 8.38. The heterozygosities were ranged from 0.388 to 0.847 and mean expected heterozygosity was 0.721. Observed PIC was from 0.366 (HTG6) to 0.823 (ASB17) and mean PIC was 0.685. This result indicated that JNH are more polymorphic than Thoroughbred horses. The PE was observed from 0.222 (HTG6) to 0.691 (ASB17) and the total exclusionary power of all markers was 0.9999. These results can give basic information for developing parentage verification and individual identification system in JNH in Korea.

**D020**
**Genetic diversity of two rare horse breeds from Poland**

GRZEGORZ CHOLEWIŃSKI[1], BARBARA GRALAK[2], E. GUS COTHRAN[3], EWA IWAŃCZYK[1]
*[1]Agricultural University of Poznan, Poland; [2]Institute of Genetics and Animal Breeding of Polish Academy of Sciences, Jastrzebiec, Poland; [3]University of Kentucky, Lexington, KY, USA*

In Poland, as in many other countries, a program for the preservation of the genetic resources represented by different farm animals species has been developed. In this study 27 genetic markers (10 microsatellites – AHT5, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20; 7 blood group loci – A, C, D, K, P, Q, U and 10 biochemical polymorphisms – ALB, A1B, ES, GC, HBA, PGD, GPI, PGM, PI, TF) were use to assess genetic diversity of two horse breeds (the Polish Primitive Horse and Hutsul) covered by the National Farm Animal Genetic Resources Conservation Program. Blood group and biochemical diversity was relatively low in the Hutsul compared to the average for domestic horse breeds but was well above average for the Polish Primitive Horse. In contrast, the Hutsul had higher heterozygosity at microsatellite loci than did the Polish Primitive (both breeds had values near the mean of microsatellite variability for horse breeds). This difference can be accounted for by monomorphism of the HTG6 locus in the Polish Primitive Horse. Based upon genetic distance values, neither breed shows close relationship to any breed that we have tested. These breeds represent unique horse genetic resources and preservation efforts should be continued.

**D021**
**Two-point linkage mapping of the ovine calpain II regulatory gene**

H. Y. CHUNG[1], C. D. KIM[1], C. Y. CHO[1], S. H. YEON[1], H. J. JIN[1], M. E. DAVIS[2], H. C. HINES[2], A. M. CRAWFORD[3]
*[1]National Livestock Research Institute, Dept. of Performance Testing, Chenan, Korea; [2]The Ohio State University, Dept. of Animal Sciences, Columbus, USA; [3]University of Otago, Dept. of Biochemistry, Dunedin, New Zealand*

The ovine calpain II regulatory gene, which is calpain 4, was screened with primers for 8 sheep reference families containing 122 crossbred animals from New Zealand. The primer sequences were selected based on the bovine cDNA sequence (GenBank accession No. J05065), and focused on the exon regions (exons 3 and 4, 5 and 6, and 7 and 8). Genetic variants were observed using polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) analysis of the three segments, and heterozygosities for each segment were estimated to be 0.45 (CAPN4L34), 0.41 (CAPN456), and 0.48 (CAPN478). Two-point linkage analysis was performed to identify the location of the calpain 4 segments among microsatellite loci with CRI-MAP version 2.4. The results strongly suggested that the calpain regulatory gene is located on ovine chromosome 14.

**D022**
**Cytochrome B sequence variation as a tool for tracing the origin of three local Spanish pig breeds**.

ALEX CLOP[1], ANA FERNÁNDEZ[2], MARCEL AMILLS[1], JUAN CAPOTE[3], CORI RAMON[4], LEIF ANDERSSON[5], ARMAND SÁNCHEZ[1]
*[1]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Departamento de Mejora Genética y Biotecnología, INIA, Madrid 28040, Spain; [3]Unidad de Producción Animal, Pastos y Forrajes, Instituto Canario de Investigaciones Agrarias, La Laguna 60, Spain; [4]Departament de Biologia, Universitat de les Illes Balears, Palma 07071, Spain; [5]Department of Animal*

*Breeding and Genetics, Swedish University of agricultural Sciences, Uppsala S-75124, Swe.*
The pig has been domesticated both from a European and Asian subspecies of the Wild Boar. The origin of mitochondrial DNA can be traced in the maternal line by analysing *Cytochrome B* sequence polymorphism. We have typed four SNPs located at positions 15036, 15038, 15041 and 15045 of the porcine *Cytochrome B* gene in three Spanish breeds (n = 83) by direct sequencing of the PCR product. Different combinations of these SNPs yield four different haplotypes: EI (TGCG), EII (TGTG), AI (CATA) and AII (CATG). Three different Spanish breeds, Iberian (Torbiscal and Guadyerbas) (IB), Porc Negre from Mallorca (PN) and Cerdo Negro Canario (CAN), were studied. The analysis of 50 pigs from the IB breed showed that all of them had the EI european haplotype. This observation could be explained by the purity of both the Torbiscal and the Guadyerbas experimental lines. The PN pigs also had the EI haplotype (n = 22) except one individual that showed a new haplotype (TGCA). This new haplotype might have been produced by a single mutation on the first SNP of EI. Finally, CAN pigs (n = 11) displayed three different haplotypes: EI (n = 3), AI (n = 3) and AII (n = 5). This result indicates that this pig breed has a mixed European and Asian origin. Possibly, the AI and AII haplotypes were introduced by the ancient multiple settlers that colonized the Canary Islands.

**D023**

**Fishing *in silico*: searching for tilapia genes using sequences of microsatellites DNA markers**

AVNER CNAANI, MICHA RON, GIDEON HULATA, EYAL SEROUSSI
*Institute of Animal Science, Agricultural Research Organization, P.O. Box 6, Bet-Dagan 50250, Israel*
Genetic linkage maps of some edible fish species, constructed in recent years, consist of hundreds of DNA markers but only few genes. Microsatellites DNA markers are short tandem repeats, with unique flanking sequences. They are highly abundant throughout the genome and appear in coding and non-coding regions. Therefore, it is likely that the flanking sequences can be part of a gene, which can be identified by similarity searches against GenBank database. Microsatellite sequences were downloaded from GenBank and queried against the databases using Blastn and Blastx searches. Out of 312 microsatellites compared to the databases, 17 loci had a significant match to known genes. We were able to map nine of these genes in the tilapia linkage map, providing anchors for comparative mapping between tilapia and other vertebrates. The rapid *in silico* approach utilized in this study, previously used in mice and livestock, increased the number of genes in the tilapia linkage map from 14 to 23, and identified 7 more genes which match unmapped microsatellies.

**D024**

**Mapping of mammary gland-derived expressed sequence tags (ESTs) in cattle**

ERIN E. CONNOR[1], TAD S. SONSTEGARD[1], JOHN W. KEELE[2], GARY L. BENNETT[2], JOHN WILLIAMS[3], RICHARD PAPWORTH[3], MELISSA S. ASHWELL[1]
*[1]U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA; [2]U.S. Department of Agriculture, Agricultural Research Service, Clay Center, NE, USA; [3]Roslin Institute, Roslin, Midlothian, Scotland*
To improve the comparative map between the cattle and human genomes, bovine expressed sequence tags (ESTs) were assembled into tentative consensus (TC) sequences and those with an expression bias for the mammary gland (≥75% of ESTs in TC) were selected for radiation hybrid (RH) and linkage mapping. To initiate mapping, selected TC sequences were aligned with a human genome draft sequence to predict gene identity, map position, and genomic sequence structure information. Of more than 400 TC sequences analyzed, approximately 100 met criteria as candidates for mapping. These sequence alignments were used to design primer pairs for PCR that amplified a predicted intron, which limited this number to 58 primer pairs. PCR products amplified from bovine genomic DNA from sires of the USDA Meat Animal Research Center (MARC) reference population were directly sequenced to verify amplification of the targeted gene region and to identify single nucleotide polymorphisms to be used as markers for linkage mapping. Currently, 22 bovine genes are being placed on the USDA-MARC Linkage Map and/or the Roslin RH Map. Mapping of bovine ESTs improves the bovine

comparative map with the human genome sequence and assists in the integration of the bovine linkage and physical maps.

**D025**
**Genetic diversity in feral horse and burro populations**

E. GUS COTHRAN
*Department of Veterinary Science, University of Kentucky, Lexington, KY, 40546, USA*
Genetic diversity in 78 populations of feral horses has been measured using blood group, biochemical, and microsatellite polymorphisms. There is a tremendous amount of genetic diversity if feral horses, approaching that observed across domestic horse breeds. This is largely due to the diverse origins of the feral herds. Heterozygosities range from 24.6% to 43.2%. There was a general trend for variability to be correlated to population size but the relationship was not statistically significant. Eight populations have been tested multiple times over several years. In only one case was there a significant change in variation and that was a loss of variation. Twelve feral burro populations have been tested for microsatellite variation. Genetic variation levels in burros was similar to that of horses although consistently lower (*Ho*=21.7% to 55.1%). Feral populations of burrows showed lower variation than domestic Standard donkeys. There were some differences in patterns of variation within feral burro populations compared to feral horse populations. These differences may be due to differences in social structure.

**D026**
**Sequence and analysis of the myostatin promoter region in cattle**

A. CRISÀ, C. MARCHITELLI,
A. VALENTINI
*Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy*
Myostatin or GDF8 acts as a negative regulator of muscle growth; mutations in this gene are responsible for the double muscling phenotype found in several European cattle breeds. In some breeds double muscling is not associated with any disruptive mutation in the gene and a possible explanation could be attributed to the effect of transcriptional gene regulation. For this reason we have identified and characterized the upstream 5' region of the myostatin gene. From the analysis of published sequences in pig, human and cattle we have designed consensus primers to amplify a fragment of the promoter region (1270bp).The sequence analysis was carried out on two individuals each of the following breeds: Marchigiana, Romagnola, Piedmontese, Chianina, Holstein Friesan, Belgian Blue, Limousine, Pezzata Rossa, Brown and Charolaise, The promoter region contains several binding sites for transcriptional factors which may play an important role for the regulation of the protein and consequently of muscular development.
From sequence data analysis we found a T/A polymorphism at –371 (relative to ATG start codon) in Belgian Blue breed. Then we carried out a PCR-RFLP test on fifthteen to twenty individuals of each breed. The T/A variant is present both in heterozygous and homozygous condition with different allele frequencies in all breeds.

**D027**
**Polymorphism of κ-casein in Italian goat breeds: a new ACRS-PCR designed DNA test for discrimination of A and B alleles**

MARIA FELIGINI[1], VLATKA CUBRIC-CURIK[2], PIETRO PARMA[1], JASMINA LUKAC-HAVRANEK[2], GIAN FRANCO GREPPI[1], INO CURIK[3], GIUSEPPE ENNE[1]
*[1]Istituto Sperimentale Italiano "Lazzaro Spallanzani", Milan, Italy; [2]Faculty of Agriculture University of Zagreb, Dairy Science Department, Zagreb, Croatia; [3]Faculty of Agriculture University of Zagreb, Animal Science Department, Zagreb, Croatia*
Genetic polymorphism of κ-CN gene was analysed in Italian goat breeds by a newly designed ACRS-PCR DNA test for rapid characterisation of goat kappa-casein (κ-CN) A and B variants. Thus, the 167-bp PCR product surrounding the nucleotide mutation was amplified from genomic DNA and the PCR product was digested with *Mae*III. After digestion the A allele showed two fragments of 77 and 65 bp in comparison to the B allele which has given two fragments of 90 and 77 bp. The analysis of allele frequency distribution at κ-CN locus, based on 401 individual samples, revealed significant differences between three goat breeds from the north of Italy (Nera di Verzasca, Frontalasca and Alpine) with frequency of κ-CN B allele around 0.3, versus two goat breeds from the south of Italy (Mal-

tese and Sarda) with frequency of κ-CN B allele around 0.5. While two goat breeds (Maltese and Nera di Verzasca) did not show significant deviations from the Hardy-Weinberg equilibrium, a highly significant excess of heterozygote genotype (AB) was observed in Alpine, Frontalasca and Sarda goats. Here developed ACRS-PCR method for discrimination of A and B alleles as well as high frequency of κ-CN B allele give a prerequisite for simultaneous estimation of casein haplotype effects on milk production and cheese-making properties.

**D029**
**The *ink4a/arf* locus evolution in primates**

ANNE DI TOMMASO[1], CEDRIC SOLER[1], CHRISTIAN ROOS[2], ALAIN KITZIS[1], VERONIQUE LADEVEZE[1]
*[1]Laboratoire de Génétique Cellulaire et Moléculaire, UPRES EA2622, Université de Poitiers and CHU de Poitiers, France; [2]German Primate Center, Göttingen, Germany*
The human *ink4a/arf* locus encodes two cell cycle regulatory proteins, the cyclin dependent kinase inhibitor ($p16^{ink4a}$) and the p53 activator ($p14^{arf}$), through use of alternative first exons. This locus genomic organization is unique in eukaryotes, with two different proteins obtained using different reading frames. The aim of this study is to characterize the *ink4a/arf* locus origin and to determine the molecular process which allows this locus organization. The divergence between mouse or opossum $p19^{ARF}$ and human $p14^{ARF}$ is very important whereas proteins have the same nucleolar localization and function. We studied the exon 1β of $p14^{arf}$ in 12 different species of primates. We didn't find any polymorphism in studied species (monkeys, apes and humans). These sequences are very similar with only one to four amino acids substitutions as compared to the human sequence, while mouse and opossum sequences show many differences. Moreover we show three amino acid substitutions in new world monkeys and lemurs which are not present in old world monkeys, apes and humans. More surprisingly we observe a threonine at position 31 in all human sequences whereas an alanine is always present in all pre-monkey, monkey and ape sequences. Our results indicate that this substitution could play an essential role in the control of cell-cycle arrest and apoptosis. We will discuss the different partners that could be implicated in the interaction with this threonine, specially spinophilin.

**D030**
**Analysis of new variants within the ovine and bovine PrP-gene**

JULIA ECKERT, SIEGFRIED PREUSS, YI-HUA HAN, HERMANN GELDERMANN
*University of Hohenheim, Institute of Animal Husbandry and Breeding, Department of Animal Breeding and Biotechnology, Stuttgart, Germany*
Strong effects of DNA variants within the PrP (Prion Protein) gene on the incidence and pathogenesis of a number of TSE (Transmissible Spongiform Encephalopathy) syndromes have been reported. Three allelic amino acid positions in the PrP of sheep are associated with Scrapie incidence. But this association does not sufficiently explain Scrapie distribution and breed specific differences. In cattle no amino acid substitutions have been identified so far and only one silent mutation and a variable repeat motifs coding for an octapeptide are known for the bovine PrP gene. The few DNA markers available do not allow efficient genotyping. Aim of this study was to identify new polymorphic positions within the PrP gene that will allow rapid screening and may reveal possible associations with TSE-resistance. Potentially polymorphic loci were identified by "etandem" (sequence analysis package EMBOSS was kindly provided by the UK HGMP Resouce Centre) software-assisted screening of PrP sequences published in genomic databases (i.e. Genbank). Three animals for each of eleven genetically diverse sheep breeds and four animals for each of eight genetically distinct cattle breeds were submitted to PCR-based screening and analysed for polymorphisms. Divers alleles were cloned, sequenced and compared to database information. Of the 24 analysed positions in cattle nine displayed polymorphisms. The number of alleles ranged between two and seven. In eight of the 23 positions analysed in sheep polymorphisms were found with the number of alleles variing between two and five. Seven of the positions amplified in sheep have not previously been described. The allele structures displayed complex DNA differences. These results will help to refine and simplify present day genotyping of the PrP gene and to analyse the distribution

of variants among species and breeds.

**D031**
**Blood protein polymorphism in some native horse types in Turkey**

OKAN ERTUĞRUL[1], BILAL AKYÜZ[2], AHMET KOPAR[3]
[1]*Ankara University, Veterinary Faculty, Department of Genetics, Ankara, Turkey;* [2]*Erciyes University, Veterinary Faculty, Department of Genetics, Kayseri, Turkey;* [3]*Veterinary Control and Research Centre, Laboratory of Blood Groups and Genetics, Etlik, Ankara, Turkey*

Different breeds and types of horses raised in different areas in Turkey. The numbers and types of native horses are in steady decrease in time because of expanding agricultural mechanization. The bloods of 87 native Turkish horses of different types were analysed for blood protein polymorphism. The horses were from different geographical areas of Turkey (Kars; Erzurum, Cukurova, Adapazarı). Starch gel electrophoresis, alkaline polyacrylamide gel electrophoresis and polyacrylamide isoelectric focusing electrophoresis were used to identify genotypic variants of AIB glycoprotein (AIB), Albumin (ALB), Vitamin D binding protein (GC), Hemoglobin-α (HBA), Transferrin (TF), Carboxylesterase (ES), 6-phosphogluconate dehyrogenase (PGD) and Phosphoglucomutase (PGM) Loci. Direct Counting Method was used for the calculation of gene frequencies of blood proteins systems. Homozygosity degrees, effective allele numbers, and efficiencies of genes were also calculated. In albumin system A and B allele frequencies were found equal. The gene frequencies of I allele in ES system; K allele in AIB system, F allele in GC, PGD, PGM systems were determined quite high ranging from 0,782 to 0,994. The highest frequency in Transferrin system was found 0,431 in F2 allele. BI allele frequency (0,534) was determined highest frequency than other allele frequencies in Hb System.

**D032**
**RH mapping of porcine ESTs to improve Human-Pig comparative map at evolutionary breakpoints**

Y. LAHBIB-MANSAIS, T. FARAUT, F. MOMPART, S. LEROUX, M. YERLE
*Laboratoire de Génétique Cellulaire, INRA, Toulouse, France*

Our aim is to define syntenic breakpoints between the human and porcine genome maps through RH mapping of porcine ESTs in order to permit a rapid and efficient exploitation of genetic information between species. Indeed, the construction of high-resolution comparative maps that define gene order within syntenic regions will provide valuable molecular genetic information for mapping agriculturally important traits. The previous comparative map studies based on non dense localisation of genes distributed over all chromosomes and on bi-directional painting between human and swine chromosomes showed conservation of synteny without allowing the precise determination of syntenic breakpoints. To construct high-resolution maps, we proceed as follow. According to the state of the art comparative maps, human genes located near evolutionnary breakpoints are used to search, on a sequence similarity basis, for candidate orthologous pig ESTs. These ESTs are further on processed to design primers for the mapping experiment on a swine 7000 rad radiation hybrid panel (IMpRH). This computer assisted strategy for comparative mapping is integrated in a computer tool, Iccare (Interspecific Comparative Clustering and Annotation for ESTs), developped in our laboratory. An example will be presented, the determination of the precise breakpoints between porcine chr3 and human chr 7, 2 and 18.

**D033**
**Validation of milk origin in goat dairy products by species-specific PCR**

MARIA FELIGINI[1], PIETRO PARMA[1], VLATKA CUBRIC-CURIK[2], GIAN FRANCO GREPPI[1], GIUSEPPE ENNE[1]
[1]*Istituto Sperimentale Italiano 'Lazzaro Spallanzani', Milan, Italy;* [2]*Faculty of Agriculture, University of Zagreb, Departement of Dairy Science, Zagreb, Croatia*

In order to verify the quality and authenticity of dairy products and protect consumers from fraud, adequate control methods are required. Identification of animal species using DNA analysis has become more important to detect adulterations in food products. In this study an adapted DNA extraction in combination with specific polymerase chain reaction-restriction fragment length polymorphism analysis were

applied to validate milk origin in goat dairy products.
The DNA from somatic milk cells was extracted from pasteurised milk, powdered milk, fresh and mature cheeses by phenol and chloroform. The cleared lysate was purified by a commercial kit. The target for PCR amplification and restriction enzyme analysis was a selected partial sequence of the Capra hircus kappa casein region (exon 4). The 167-bp amplified sequence contained the *MAE*III restriction sites for k-CN A and B alleles. The digestion results were analysed by continuous polyacrylamide gel. Specific restriction enzyme recognition sites, exclusively located within the goat PCR product, permitted the accurate validation after digestion. This k-CN polymorphism-based method is applicable to validate the origin of dairy products from goat milk and to characterise k-CN alleles from cheeses in research related to the estimation of polymorphism effects on technological properties and cheese making yield.

**D034**
**Identification of a new haplotype for the *MC1R* gene in Iberian pig**

ANA FERNÁNDEZ, CRISTINA ÓVILO, CARMINA CASTELLANOS, CARMEN RODRÍGUEZ, MIGUEL ANGEL TORO, LUIS SILIÓ
*INIA, Department of Mejora Genética Animal, Madrid, Spain*
Molecular interaction between the G-protein coupled melanocortin receptor 1 (*MC1R*) and the agouti protein is the main regulatory system know to control the synthesis of eumelanin and phaeomelanin. *Extension*(*MC1R*) and *Agouti* loci make up a regulatory system that is known to control this process. In previous studies, the sequence analysis of *MC1R* gene has revealed six *MC1R* alleles, *MC1R**1 needed for the expression of the wild-type color, *MC1R**2 associated with the black phenotype in Large Black and Meishan pigs, *MC1R**3 associated with the black color in Hampshire, *MC1R**4 associated with the red phenotype in Duroc, *MC1R**5 presents in Japanese wild boar, and *MC1R**6 associated with white and red phenotype in Landrace, Yorkshire and Linderöd. We have analyzed DNA sequence of *MC1R* gene by direct sequencing of the PCR product in Iberian pigs (black, red, and chestnut animals), sequence analysis revealed a new allele/haplotype, *MC1R**7, that corresponds to the combination *MC1R**1 and *MC1R**6 alleles. This new haplotype results from a mutation in the form of a 2bp insertion at 23 codon. We have genotyped *MC1R* alleles by PCR-RFLP test and fragment analysis of PCR products on an ABI 3100 in black (n= 31), red (n= 109) and chestnut (n= 79) Iberian pigs, *MC1R**6 and *MC1R**7 alleles were found in red and chestnut pigs, and black pigs were homozygous for the *MC1R**3 allele.

**D035**
**Expression and Characterization of the Bovine Prion-Doppel and Analysis of PRND Gene Polymorphisms**

LAURENT R. CHIARELLI[2], SERGIO COMINCINI[1], MARIA GABRIELLA FOTI[3], UMBERTO AGRIMI[5], GIOVANNI DI GUARDO[4], GABRIELE VACCARI[5], DONATA VERDINELLI[1], MICHAEL TRANULIS[6], INGRID HARBITZ[6], JOERG SCHLAEPFER[7], GAUDENZ DOLF[7], DAVID HILLS[8], JOHN L. WILLIAMS[8], GIOVANNA VALENTINI[2], LUCA FERRETTI[1]
*[1]Dipartimento di Genetica e Microbiologia, Università di Pavia, Italy; [2]Dipartimento di Biochimica, Università di Pavia, Italy; [3]CERSA, FPTP, Lodi, Italy; [4]Instituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, Roma, Italy; [5]ISS, Roma, Italy; [6]Norweagian School of Veterinary Science, Oslo, Norway; [7]Institute ofAnimal Breeding, University of Berne, Switzerland; [8]Roslin Institute, Roslin, Scotland*
The bovine recombinant Dpl(26-155) was purified from E.coli inclusion bodies with yields of 2/3 mg/liter. Edman degradation and Carboxypeptidase Y treatment revealed a single species shortened by 12 aa albeit with the expected Alanine 155. Titration of free thiol groups with Edman's reagent in the presence of urea gave negative results suggesting that cysteines were blocked in disulfide bonds. The comparison of tryptic maps obtained by HPLC of the peptides highlighted two prominent peaks that disappeared after treatment with DTT. Two N-terminal sequences were obtained for each peak, matching the portions of Dpl that are involved in the formation of the two predicted disulfide bonds ($Y^{77}$-$K^{96}$/$H^{146}$-$R^{153}$ for $C^{94}$-$C^{151}$ and $F^{103}$-$R^{122}$/$E^{140}$-$K^{145}$ for $C^{108}$-$C^{142}$). Our data suggest that the rDpl(39-155) is properly folded. The entire

PRND ORF was sequenced in 60 swiss cattle (27 affected, 33 controls), 56 Italian Sarda sheep (32/24), 52 Italian Comisana sheep (12/40), 50 Norwegian Rygja sheep (16/34), and 37 Italian Ionica goat (17/20). Eleven polymorphisms were detected in cattle (I15V; C22S; R50H; D69N; K96E; I105V; A114T; R132Q; T144I; R158Q; R158K). Variation was higher in affected cattle compared to controls, with codons 50, 69 and 158 significantly represented in the diseased animals ($P<0.003$). On the contrary, PRND was largely monomorphic in the sheep breeds investigated, except for two synonimous mutations (I12I; A26A). Eleven polymorphisms were detected in goats (C8W; W9G; L10V; K34T; T47R; T51A; Y77D; L129V; E140D, G156R; Q163H), but variation was apparently unrelated to the diseased animals.

Acknowledgments: This work was supported by EU MASSES (FAIR5 CT97 3311)

**D036**

**Genetic variation of mitochondrial DNA D-loop regions in Japanese Sika deer**

EMIKO FUKUI[1], MASAAKI KOGANEZAWA[2], MIDORI YOSHIZAWA[1]

*[1]Department of Animal Breeding and Reproduction, Utsunomiya University, Utsunomiya, Japan; [2]University Forest, Faculty of Agriculture, Utsunomiya University, Utsunomiya, Japan*

Wild Japanese Sika deer (*Cervus nippon*) are distributed throughout the Japanese Archipelago, including Honshu, Hokkaido, Kyushu, Shikoku and other islands. Some populations of these deer were domesticated and used for the production of venison and velvet. Sequence polymorphism in mitochondrial DNA displacement loop (mtDNA D-loop) regions has been widely used to study phylogenetic relationships at specific and subspecific levels and between various populations. In cattle, nucleotide sequences of mtDNA D-loop regions have been examined by many researchers since the complete sequence of bovine mtDNA was reported. However, the complete sequence of mtDNA in Japanese Sika deer has not previously been reported, although several studies have reported population genetics. The comparison of genetic variation among various populations of Japanese Sika deer is vital for the development of useful management and conservation strategies. In the present study, genetic variation in wild Japanese Sika deer in the areas of Nikko, Gunma, Hokkaido and Tsushima was investigated using sequence polymorphism in the mtDNA D-loop regions, and mother-child relationships between populations were assessed.

DNA samples of 110 Japanese Sika deer were collected from Nikko (Nikko National Park), Gunma, Hokkaido and Tsushima areas between January and March in every year from 1996 to 2001. DNA was extracted from heparinized whole blood or a tooth. The mtDNA D-loop region was amplified using PCR methods with primers constructed by Nagata *et al*. (1998):LD5-AGCCATAGCCCCACTATCAA and HD8-TTGACTTAATGCGCTATGTA. PCR was performed using a PCR reagent kit (TAKARA) according to the instructions provided by the manufacturer. The PCR reaction mixture was heated at 93°C for 2 min, and amplified under 35 sequential cycles at three different temperatures : 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. Nucleotide sequences of PCR products were determined using an automatic sequencer. Alignment of sequences was achieved using the CLUSTALW software package. Haplotypes in the mtDNA D-loop region in Japanese Sika deer were defined by comparison with mtDNA D-loop sequences of Japanese Sika deer from Hokkaido (accession number D50128, a-type) published by Nagata *et al.* (1998). Eight haplotypes for the D-loop region were identified in Sika deer from the Nikko area, and homogeneity among these haplotypes was 82.4~99.7%. Six haplotypes were identified in the population in the Gunma area, with homogeneity of 95.0~98.0%. The phylogenetic tree constructed from these results demonstrated that the Tsushima population is the oldest of the four populations examined.

**D037**

**Coat colour and polymorphism in the MC1R gene of the Hérens cattle breed**

HANNES W. JÖRG[1], STEPHAN J. RIEDER[1], ELLIE FELLAY[2], JEAN-JACQUES LAUVERGNE[3], CLAUDE GAILLARD[4]

*[1]Swiss Federal Institute of Technology, Institute of Animal Sciences, Zürich, Switzerland; [2]Fédération d'èlevage de la race d'Hérens, Sion, Switzerland; [3]INRA CRJ, Laboratoire de Génétique Factorielle, Jouy-en-Josas, France; [4]University of Bern, Faculty of Veterinary*

*Medicine, Bern, Switzerland*
The Hérens cattle breed has three different coat colour phenotypes: red, black with a red dorsal strip, and black. In mammals, dominant alleles of the Extension (E) locus are associated with black pigment, while the recessive alleles lead to production of the red pigment. However, in the Hérens breed, the red coat colour is not completely recessively inherited. Molecular studies have revealed that the E locus encodes a melanocyte stimulating hormone receptor (MC1R). In this study, 11 animals of various coat colours were selected. Analysis of the MC1R gene showed three polymorphic sites. A deletion of a G nucleotide at position 311 resulting in a frameshift was identified in two animals. This frameshift is associated with the red coat colour in Holstein cattle. However, the low frequency of the allele in our sample confirms that red is not only due to this recessive allele in the Hérens breed. A nucleotide substitution of a T to an A at position 296 was identified only and in each black animal. This allele encodes a constitutively active receptor and results in the black phenotype. A duplication of 12 nucleotides starting at position 650 was found in two of the four black animals with a red dorsal stripe and is not informative for the described phenotypes. Our results show that the black phenotype is due to an allele of the MC1R gene. The red and the black with a red dorsal stripe phenotypes are controlled by another gene, which likely interacts with the function of MC1R.

**D038**
**Genetic characterization of two alpine autochthon cattle breeds**

MICHELE BLASI[1], ADELE LANZA[1], ELENA GENZINI[1], ANDREA ROSATI[1], DANIELA IAMARTINO[2], FABIO PILLA[2]
*[1]Laboratorio Gruppi Sanguigni,Potenza-Cremona, Italy; [2]Università degli Studi del Molise, Campobasso, Italy*
The autochthon cattle breed named Barrà is reared in Piedmont, a northern Italian region. This is a breed producing both milk and meat. The goal of this research is to verify the genetic origin of Barrà cattle and particularly to control the possibility to distinguish this breed from the Pustertaler Sprinzer cattle breed, from which, most probably, the Barrà originated. 64 samples of Barrà and 29 samples of Pustertaler Sprinzer were used. Genotypes, obtained by 16 microsatellites loci, were analyzed by estimating the useful parameters to evaluate the genetic profile of the populations. The obtained frequencies were used to estimate the expected heterozygote percentage following Hardy-Weinberg equilibrium. Frequencies of heterozygote animals, actually observed, were estimated by using the software ARLEQUIN 2.0. Genetic distances were estimated following the model proposed by Bowcock about the proportion of shared alleles. The obtained distances matrix was used to build a Neighbour-Joining diagram using the software PHYLIP. From the obtained results there is no evidence of significant difference among the two groups therefore Barrà animals are not a separated group from the Pustertaler animals.

**D039**
**Reliability of DNA certificates in parentage verification for cattle**

MARIE-LOUISE GLOWATZKI-MULLIS, JEANNETTE MUNTWYLER
*University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, Switzerland*
Since April 1999, STR profiling is routinely used for parentage verification and identity testing in our lab. In some cases the genotype of a progeny has to be verified with data from DNA certificates. 9 STR markers were selected as international ISAG standard: BM1824, BM2113, ETH10, ETH225, INRA023, SPS115, TGLA122, TGLA126, TGLA227. For DNA certificates, the results are adjusted to a reference sample. Projects exist to establish DNA databases which would serve as central platforms for storage and comparison of data. Thus, the reliability of DNA typing results is of crucial interest. However interlab concordance is not always attained. 1) Reports of ISAG Comparison Tests. For none of the 9 ISAG markers the participating labs reported absolutely identical results in 1999/2000. Best marker was BM1824: 2 labs out of 33 (40 samples tested) differed in more than one allele, 2 labs failed in typing at least 39 samples, 5 labs made false adjustments to the reference. 2) Casework experience. 90 DNA certificates were requested: in 21 of them the results were either not adjusted to the reference or allele designation was erroneous. 3) Allelic ladders are not usual in cattle casework. This requires caution when comparing results

obtained from different types of DNA Sequencers. ETH225: difference between allele 1 and 2 (same PCR product) is 12bp (sample 1 ISAG CT 2001/2002) when the fragments are run on the 373A slab gel system and 16bp when run on the capillary 3100 system from ABI.

**D040**

**Characterization of the prion protein gene region in Swiss sheep breeds based on genetic polymorphisms**

ANDREAS GMÜR, CLAUDE GAILLARD, GAUDENZ DOLF
*University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, CH*

Polymorphisms at *PRNP* in sheep seem to be associated with the incubation time of both scrapie and BSE. In the light that sheep can be experimentally infected with BSE, the question whether Swiss sheep potentially are susceptible to the disease becomes extremly important in the context with public health. The aim of this study was to characterize 50 animals of each of the four major Swiss sheep breeds, namely Swiss Oxford Down (BFS), Swiss Black-Brown Mountain (SBS), Valais Blacknose (SN) and Swiss White Alpine (WAS) with respect to polymorphisms (codons 112, 136, 137, 138, 141, 151, 154, 171 and 211) in the exon 3 of the *PRNP*. First we amplified part of exon 3 using Goldmann's primers (upper 5'-CCGCTATCCACCTCAGGGA-3' and lower5'-TCTCATAGTAGGATAGGGGCAA-3') and then we sequenced the PCR products from both ends using the primers (5'-AAGGTGGTAGCCACAGTC-3' and 5'-CAGGAGGGGAAGAAAAGAGGAT-3') which allowed us to double check the sequences. At codon 143 a novel polymorphism was found. Based on the genotypes allele frequencies were estimated at the polymorphic codons. Substantial polymorphism was found only at codon 171 in all breeds and to a lesser extent at codon 112 and 154 in SBS, and at codon 136 in WAS. At other codons or in other breeds polymorphism is rather low or was not observed. If codon 171 indeed plays a major role in susceptibility to BSE, we expect all four breeds to be potentially susceptible for BSE.

**D041**

**Further studies on amplification of peccary microsatellites using porcine primers**

JAIME GONGORA, CHRIS MORAN
*Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2006, Australia*

There are many examples of microsatellite primers designed from one species being used to amplify products from another. Interspecific amplification has been demonstrated among cattle, sheep, goats and deer, among chickens, quail and turkeys and among domestic pigs and wild Suiformes. Here 61 porcine microsatellite primer pairs were tested for amplification of microsatellite products from seven Colombian Collared peccaries (*Tayassu tajacu*), with 47 (74%) yielding products. Fluorescent genotyping of the PCR products revealed that 10 (16%) were polymorphic. All observed peccary alleles fall within the range of allele sizes found in Australian commercial pigs. The high success rate with porcine primers on peccaries suggests low nucleotide divergence between Suidae and Tayassuidae. The amplification efficiency agrees reasonably well with our previous study (16/18; 89%), although the polymorphism level here is lower than in the previous study (11/16; 69%) By contrast, the only other reported study on peccaries (Lowden, pers. comm) found an amplification success rate of only 29% (9/31) using porcine primers. Perhaps surprisingly, many porcine microsatellites primers promise to be useful tools for population and phylogenetic studies of peccaries.

**D042**

**Polymorphism of pig breeds for the presence of an active full-length endogenous retrovirus (PERV) at position 1q24.**

M. GORBOVITSKAIA[1], N. BOURGEAUX[1], Z. LIAN[2], N. LI[2], P. CHARDON[1], C. ROGEL-GAILLARD[1]
[1]*Laboratoire de Radiobiologie et d'Etude du Génome, UMR INRA-CEA 13.314, Jouy-en-Josas, France;* [2]*Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China*

Pig (*Sus scrofa*) is a potential organ donor for man. However, the porcine endogenous retroviruses (PERVs) represent a major concern for the patients and it has not yet been clearly established whether these sequences are safe or not. A major goal would be to select potentially active PERV-free pigs. We have previously identified 24 integration loci for type C

PERVs using porcine BAC clones. It was further shown that at least three loci contained PERVs, that were replication competent upon transfection of the corresponding BAC clones into susceptible human cells (J Virol, 76:2714-2720). We have thus started to study the polymorphism of distinct pig breeds for the presence of a PERV at one of these loci, at position 1q24. The PERV genomic flanks were subcloned from the BAC clone using a viral probe specific for the Long Terminal Repeats (LTRs). Two DNA fragments of 2021 and 2445 base pairs were recovered for the LTR 5 and 3 prime junctions, respectively. The sequencing results indicated that the PERV interrupted a tandem of 8 repetitions of a 196 bp element within the second repeat at position 181, leading to the duplication of a small motif AGAC. PCR primers were designed to amplify both PERV junctions. Fourteen pig breeds were tested, including 11 national Chinese breeds. Amplification products were obtained for some but not all the breeds, clearly suggesting a polymorphism for the presence of the active PERV at position 1q24 and the possibility of genetic selection.

**D043**
**Weitzman approach and components of diversity in Northern European sheep breeds**

ILMA GRIGALIUNAITE[1], MIIKA TAPIO[2], LARS-ERIK HOLM[3], SVEN JEPPSSON[4], JUHA KANTANEN[2], ILONA MICEIKIENE[1], INGRID OLSAKER[5], HALDJA VIINALASS[6], EMMA EYTHORSDOTTIR[7]
*[1]Lithuanian Veterinary Academy, Kaunas, Lithuania; [2]MTT Agrifood Research Finland, Jokioinen, Finland; [3]Danish Institute of Agricultural Science, Tjele, Denmark; [4]Swedish Board of Agriculture, Jönköping, Sweden; [5]The Norwegian School of Veterinary Science, Oslo, Norway; [6]Institute of Animal Science of Estonian Agricultural University, Tartu, Estonia; [7]Agricultural Research Institute, Reykjavik, Iceland*

Weitzman marginal loss of diversity and the contribution to total diversity described by Petit *et al.* (1998) were applied to a set of 32 sheep breeds from Northern Europe that were genotyped for 22 microsatellite markers. Conservation values given by the methods for individual populations were compared. Weitzman diversity values for populations were positively correlated with divergence components and negatively with within population components of Petit *et al*. (1998). The comparison of Weitzman marginal loss of diversity and the contribution to total diversity defined by Petit *et al*. (1998) were only weakly positively correlated. The results suggest that setting conservation priorities for the studied sheep breeds only with the Weitzman approach can be misleading, if intrabreed genetic variation is ignored.

**D044**
**Eurofins-TAG™ for meat DNA traceability and a cattle rustling case**

HÉLÈNE PFITZINGER, NATHALIE ARCIDIACONO, STÉPHANIE GUILLET
*Eurofins Scientific, 44323 Nantes, France*

Since the BSE crisis, professionals have to restore consumer confidence in beef products and to guarantee maximum food safety and transparency. In this context, the DNA technology using genetic markers such as microsatellites provides a very efficient mean for the identification of the animal of origin.
Based on this molecular technology but also on an important logistic and dedicated sampling tools, the Eurofins-TAG™ system assures the traceability of cattle from farm to fork using a combination of microsatellite markers recommended by ISAG. This system is based on the comparison of DNA profiles of reference and check samples (muscle, hair, cartilage…) that can be taken at any stage during animal's life or along the production chain.
A large scale pilote project involving 14000 cows was conducted with the first beef producing french region "Pays de Loire". Systematic sampling and DNA fingerprinting were realized throughout the beef production chain. The DNA profiling results allowed to show a good correlation with the paper-based traceability.
In addition to the validation of beef traceability schemes, Eurofins-TAG™ also permits to realize parentage testing or to certify a breed origin, and has been used to elucidate a cattle rustling case.

**D045**
**Evaluation of 26 microsatellites for paternity testing in dog**

GAUDENZ DOLF[1], KARINA GUZIEWICZ[1], BRIGITTA COLOMB[1], CLAUDE SCHELLING[2]
[1]*University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, Switzerland;* [2]*Federal Institute of Technology and Faculty of Veterinary Medicine, Department of Animal Sciences, Zürich, Switzerland*

The microsatellites ZuBeCa1 to ZuBeCa23, ZuBeCa25, ZuBeCa26 and CanBern6 have been typed in 37 Cairn Terriers (FCI 3), 50 Dachshunds (FCI 4), 31 Flat-Coated Retrievers (FCI 8), 30 Papillons (FCI 9), 69 Siberian Huskys (FCI 5), 52 Vizslas (FCI 7) and 42 Whippets (FCI 10). Average PIC values across the 7 breeds (designated PIC-B) ranged from 0.000 (ZuBeCa8) to 0.845 (ZuBeCa2). Microsatellites with a PIC-B < 0.4 tend to be monomorphic in single breeds. As an exception ZuBeCa21 with a PIC-B of 0.512 was monomorphic in Flat-Coated Retrievers. Microsatellites with PIC-Bs > 0.7 (ZuBeCa2, ZuBeCa4, ZuBeCa6, ZuBeCa12 and ZuBeCa16) were difficult to type due to the high numbers of alleles (26 to 40). The average PIC value over all microsatellites, without ZuBeCa8, (designated PIC-M) was lowest in Flat-Coated Retrievers with 0.400 where 4 microsatellites (ZuBeCa5, ZuBeCa10, ZuBeCa21 and ZuBeCa22) were monomorphic, and highest in Siberian Huskys with 0.591 where only ZuBeCa3 was monomorphic. PIC-Ms may rather reflect population size and breeding effects than sample size. Based on these findings CanBern6 (CFA33, 11 alleles, PIC-B 0.596), ZuBeCa1 (CFA10, 9 alleles, PIC-B 0.586), ZuBeCa14 (CFA24, 5 alleles, PIC-B 0.491), ZuBeCa18 (CFA9, 8 alleles, PIC-B 0.417), ZuBeCa25 (CFA17, 9 alleles, PIC-B 0.610) and ZuBeCa26 (CFA27, 7 alleles, PIC-B 0.642) are proposed to be evaluated for inclusion in an international panel for paternity testing.

**D046**

**Physical mapping, sequencing and detection of VNTR of the porcine milk proline-rich protein gene**

ANDREW J. HALL[1], HELEN ARTHUR[1], T. KEVIN BELL[1], RYAN MIDDLETON[2], CHRIS MORAN[2]
[1]*Australian Equine Genetics Research Centre, The University of Queensland, St. Lucia, QLD, Australia;* [2]*Centre for Advanced Technologies in Animal Genetics and Reproduction (Reprogen), The University of Sydney, NSW, Australia*

Proline-rich proteins (PRPs) account for 70% of the protein in human saliva and have been attributed with a variety of functions including the ability to bind fimbriae of various pathogenic organisms (e.g. *Porphyromonas gingivalis, Candida albicans*), to precipitate tannins and to act as a masticatory lubricant. We have previously reported the identification of a protein homologous with the human salivary PRPs (*Hs*PRPs), isolated from porcine milk. The porcine milk PRP (*Pm*PRP) gene is at least 5kb and, like the human genes, consists of four exons with the third exon comprised almost entirely of tandemly repetitive sequence. The tandem repeat unit in the *Pm*PRP gene sequence is 33 nucleotides in length and is repeated between 41 and 45 times, as determined by PCR and nucleotide sequencing. *Hs*PRPs are the products of a six-gene cluster that spans approximately 700kb of HSA12p13.2, a chromosomal region that has been shown to have conserved synteny with the proximal part of the SSC5 q arm. Physical mapping of the *Pm*PRP gene with a pig/rodent somatic cell hybrid panel is currently underway.

**D047**

**Genetic analysis of Korean native horses using microsatellite markers**

S. K. HAN[1], H. D. BYUN[1], E. R. CHUNG[2]
[1]*Laboratory of Molecular Genetics, Department of Dairy Science, College of Animal and Life Science, Konkuk University, Seoul, Korea;* [2]*Laboratory of Animal Molecular Genetics, Division of Applied Animal Science, College of Life Science and Natural Resources, Sangji University, Seoul, Korea*

Korean native horse (KNH) is the oldest breed of domestic animals at Jeju island in Korea. This breed classified two population as pure breed (KNH I) and crossbreed (KNH II). KNH I has been conserved as natural monument. The genetic variation of KNH was measured using data from 9 microsatellite loci (AHT5, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10 and VHL20) and compared with those of Przewalski's horse (EPR) and the Thoroughbred horse (TH). Allele frequencies and heterozygosities were calculated using CERVUS Ver. 2.0 (Marshall, 1998). The number of alleles of those varied from 5 to 11 with an average number of 7.22. When the results were

compared with those of TH and ERP by 6 loci, HMS3, HMS6, HMS7, HTG6, HTG10 and VHL20 (Breen et al., 1994), the number of alleles of KNH I (8.00) and KNH II (7.666) were more than those of TH (5.66) and EPR (4.33). The average heterozygosities are 0.697 and 0.773 in KNH I and II, respectively. And the average heterozygosity of KNH I (0.699) at the 6 loci was higher than that of EPR (0.681) but lower than those of KNH II (0.786) and TH (0.715). The most frequent alleles of KNH I were different from those of KNH II at 5 loci, AHT5, HMS6, HTG6, HTG7 and VHL20. In HMS6 and HTG6 loci, the most frequent alleles of KNH II were the same as those of TH. 12 alleles were presented at 4 loci (HMS3, HMS6, HTG10 and VHL20), which are unique to KNH I and II, and especially, the new allele of 108bp was identified in VHL20 locus of KNH I. The genetic distance was estimated among the 4 breeds, with the result that KNH I was closer to EPR than KNH II, and KNH II was closer to TH. This result was similar to that of Han et al. (1998) using data from protein markers and we found the gene introgression of TH to KNH II by crossbreeding. These results suggest that KNH represent unique native breed as a horse genetic resource and preservation efforts should be continued, although the genetic variation of KNH population has been deduced.

**D048**

**Cattle mitochondrial DNA introgression in yak (*Poephagus* or *Bos grunniens*)**

QI XUEBIN[1,2], HAN JIANLIN[2,4], EDWARD O. REGE[3], OLIVIER HANOTTE[2]

*[1]State Key Laboratory of Arid Agroecology, Lanzhou University, Lanzhou, China; [2]International Livestock Research Institute, Nairobi, Kenya; [3]International Livestock Research Institute, Addis Ababa, Ethiopia; [4]International Yak Information Centre, Gansu Agricultural University, Lanzhou, China*

To assess the level of cattle mtDNA introgression in yak, a 357 bp cattle specific D-loop and a 590 bp cattle-yak conserved 16S rDNA fragments were PCR amplified in a single multiplex reaction. The amplification of the 16S rDNA was used as an internal control (T. Ward *et al.* (1999) Animal Conservation 2, 51-57). PCR products were separated on a 2% agarose gel. A total of 963 samples from 24 yak populations were analysed. Twenty-one individuals from 13 yak populations had a cattle mtDNA with the frequencies of cattle introgression ranging from 1.5% (Tianzhu White) to 10.6% (Tianzhu Black). More particularly, cattle mtDNA were present in seven yak populations from China (Tianzhu White (1/68), Tianzhu Black (5/47), Sunan (2/35), Luqu (1/32), Datong (2/84), Jiulong (1/24) and Maiwa (2/38)), three yak populations from Mongolia (Gobi Altai (1/40), Arkhangai (2/60) and South Gobi (1/30)), two yak populations from Bhutan (west (1/35) and central (1/36)) and one yak population from Nepal (1/25). No cattle mtDNA introgression were detected in Maqu (0/65), Xiahe (0/17), Jianzha (0/33), Jiali (0/50), Pali (0/48) and Sibu (0/50) yak populations from China; in Hovsgol (0/20) and Ubs-Khovd (0/19) yak populations from Mongolia; in the east Bhutanese yak population (0/40); in the Indian (0/23) and the Pakistani (0/44) yak populations. In general, the introgression of cattle mtDNA in yak is rare and it is mostly limited to marginal and agro-pastoral areas where yak and cattle coexists.

**D049**

**Evaluation of Linkage Disequilibrium in commercial PIGS's populations**

NATACHA HARMEGNIES, FRÉDÉRIC FARNIR, NADINE BUYS, WOUTER COPPIETERS, MYRIAM MNI, PATRICIA SIMON, MICHEL GEORGES

*University of Liège, Department of Genetics, Liège, Belgium*

Extensive linkage disequilibrium (LD) has been demonstrated in cattle, offering possibilities to exploit this information for mapping and fine-mapping purposes using the presently available medium density maps. To verify whether the same possibilities might exist in pigs, we have measured pair-wise LD between 13 microsatellite markers *(S0369, SW1989, SW1401, S0284, S1263, SWR1002, SW1683, SW1309, S1004, S1006, SW2608, SW1510 and SW1262)* mapping on SSC15. The distance between adjacent markers ranged from 0.9 to 9.6 cM. Fourthly and 33 unrelated individuals were respectively sampled in a Large White derived line (Line A) and in a synthetic line (Line B). Multiplex microsatellite genotyping was performed using automatic capillary sequencers. Haplotype frequencies maximising the likelihood (L) of the genotype data were estimated using an EM algorithm. LD was

measured using (i) the statistical significance of the L ratio (Ldata|LD/Ldata|linkage equilibrium (LE)) under the null hypothesis of LE as determined by genotype permutation, (ii) the normalised D' measure of LD computed from the maximum L haplotype frequencies, and (iii) the square of the correlation coefficient between loci ($r^2$) measured from the same haplotype frequencies. Preliminary results suggest that sufficient LD will be present in these pig populations to devise mapping approaches (association studies on DNA pools or individual DNAs as well as TDT approaches using individual DNAs) based on LD only.

**D050**

**Two polymorphisms detected in equine *APAF1* and possible linkage to ECA28q**

JULIA HENNER[1,2], CAROLA MAU[1,2], GERALD STRANZINGER[1,2], STEFAN RIEDER[1]

*[1]Institute of Animal Science, Breeding Biology, Swiss Federal Institute of Technology, Zürich, Switzerland; [2]Faculty of Veterinary Medicine, University of Zürich, Switzerland*

Apoptotic protease activating factor 1 plays an important role in the apoptosis pathway and is of particular interest in human melanoma research. *APAF1* maps to HSA12q23. This region also contains *IGF1*, *TMPO* and *KITLG*. The equine homologs of those genes belong to ECA28q. Two fragments of equine *APAF1* were amplified from horse genomic DNA. Sequence analysis and polymorphism screening revealed two single nucleotide polymorphisms (*APAF1* SNP I3 and *APAF1* SNP I6). In order to test whether equine *APAF1* would map to ECA28q, a linkage analysis was carried out using known microsatellite markers from ECA28q (*IGF1*, *HTG30*, *UM003*, *TKY333*) and the two detected *APAF1* SNP's. A family of 66 Franches-Montagnes horses, a native breed from Switzerland, was genotyped. Linkage between microsatellites on ECA28q was confirmed and a tendency for linkage between *APAF1* SNP's and marker *IGF1* was found (*APAF1* SNP I3: Z=1.50 θ=0.0; *APAF1* SNP I6: Z=0.72 θ=0.14). Microsatellite *IGF1* was only poorly informative (PIC=0.4) in our family. We could not show linkage between *IGF1* and *HTG30*, even linkage between these two markers is known. However, the available comparative mapping data and the tendency for linkage between *APAF1* SNP's and *IGF1* we found, indicate mapping of equine *APAF1* proximal to *IGF1* on ECA28q. This result perfectly fits to the gene order known from the human gene map and is helpful for the orientation of the group of microsatellite markers and genes on ECA28q.

**D051**

**Temporal changes of gene frequencies in three breeds of horses**

UWE HERTNER[1], IVICA MEDUGORAC[2], MARTIN FÖRSTER[1,2]

*[1]Tierzuchtforschung e.V. München, Grub, Germany; [2]Institut für Tierzucht der Ludwig-Maximilians-Universität, München, Germany*

In order to develop a suitable sport horse, original breeds of German horses have been modified by incrossing of breeds like Arabian, Thoroughbred and East Prussian Trakehner since the middle of the 20th century. In contrast, Thoroughbreds have had a closed studbook for about two centuries. In this study we compare two age groups of Hanoverian, Holsteinian and Thoroughbred horses. Animals are grouped in these classes by their year of birth: Hanoverian I, 1949 – 1970 (N = 134); Hanoverian II, 1993 – 1999 (N=1851); Holsteinian I, 1955 – 1971, (N = 123); Holsteinian II, 1993 – 1998 (N = 464); Thoroughbred I, 1948 – 1966 (N = 111); Thoroughbred II, 1999 (N = 1221). Changes in gene frequencies are evaluated using 5 polymorphic protein loci (A1B, ALB, ES, GC, TF). Laboratory results for age group I have been obtained by retyping of stored plasma samples sent to our laboratory before the year 1978. Results for age group II are from routine paternity testing. As expected, lowest differences are observed between the two classes of Thoroughbreds, whereas the differences of allele frequencies between the two classes of Holsteinians show the highest significancy.

**D052**

**An *Msp*I polymorphism in the GHRH-Receptor gene and its association with growth traits in Angus beef cattle**

QUN ZHAO, M. E. DAVIS, H. C. HINES

*Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA*

Growth hormone releasing hormone (GHRH) plays a major role in stimulation of both synthesis and release of GH in the anterior pitui-

tary through its receptor. Therefore, the GHRH-receptor gene is a candidate gene for growth traits in domestic animals. Genetic marker information on this gene could be used to facilitate selection and breeding through marker assisted selection. An *Msp*I polymorphism was detected in exon 6 of the GHRH-receptor gene. Sequencing result showed a point mutation of A in allele M to G in allele N. The polymorphism was examined in 194 Angus beef cattle, which were divergently selected for high or low blood serum IGF-I concentration. The genotypic frequencies were .79 for MM, .19 for MN, and .02 for NN. The associations of the polymorphism with growth traits and IGF-I concentration were analyzed using the GLM procedure in SAS. A linear model was fitted for birth weight, weaning weight, weight at d 28 and 56 of the 140-d postweaning test, off-test weight, weight gain during the 20-d period between weaning and the beginning of the postweaning test, postweaning gain, serum IGF-I concentration on d 28, 42, and 56, and mean serum IGF-I concentration. No significant associations were found in these animals. More genotyping is needed to further study this polymorphism.

**D053**

**Allelic variation of the dog dopamine receptor D4 gene polymorphic region and its relation to behavioral traits**

MIHO INOUE-MURAYAMA[1], NAOTO MATSUURA[1], HIDEYUKI ITO[1], YUKO UEDA[1], KEIICHI TONOSAKI[2], YUICHI MURAYAMA[3], MITSUO MORITA[4], HITOSHI KITAGAWA[1], TOSHIROH IWASAKI[5], SHIN'ICHI ITO[1]

*[1]Gifu University, Gifu, Japan; [2]Meikai University, Sakato, Japan; [3]National Institute of Animal Health, Tsukuba, Japan; [4]Livestock Improv. Assoc. of Japan, Maebashi, Japan; [5]Tokyo University of Agricul-ture and Technology, Fuchu, Japan*

The dog (*Canis familiaris*) has the longest history among domestic animals and more than 400 breeds have so far been established all around the world. Purebred dogs are significantly different from each other in their behavioral traits, suggesting that some behavioral traits are under genetic control. The dopamine receptor D4 (*DRD4*) gene polymorphic region in exon 3, which is possibly related to the personality trait known as novelty seeking in humans, was examined in 860 dogs from 31 breeds and 9 alleles were identified by DNA sequencing. We then investigated the relationship between behavioral profiles and *DRD4* exon 3 allele frequency in each dog breed. Significant correlation was recognized between the allele *498* and score of aggression-related behaviors such as 'aggression to dogs', 'dominance over owner' and 'territorial defense'. Furthermore, we examined the association of *DRD4* genotype and behavioral traits in the Labrador Retriever trained as drug dogs. Seven behavioral traits were evaluated by trainers and exon 3 polymorphism was found to be related to 'aggression to dogs' and 'affection demand'. Another polymorphism was also found in exon 1 of *DRD4* gene and this polymorphism was related to 'excitability'. This information might be of use in the effective training program for working dogs such as police dogs and guide dogs.

**D054**

**Diversity of mitochondrial DNA in Asian native goats**

MISAKO ISHIDA[1], HIDEYUKI MANNEN[1], KEN NOZAWA[2], SOICHI TSUJI[1]

*[1]Kobe University, Faculty of Agriculture, Kobe, Japan; [2]Koto University, Primate Research Institute, Aichi, Japan*

Domestic goats (*Capra hircus*) were the first herbivores to be domesticated. Despite their importance, the origins of domestic goats remain uncertain and controversial. Recent mitochondrial DNA study revealed three highly divergent mitochondrial lineages in goat (*C.hircus* A-C). In this study, we determined complete sequences of mitochondrial D-loop region in 12 Myanmar, 36 Mongolian and 33 Chinese native goats. Phylogenetic analysis was performed with these sequences and published complete D-loop sequences of 16 domestic goats and three wild goats (two markhor, and one bezoar). The phylogenetic tree revealed four distinct major clusters, three were consistent with *C.hircus* A-C and one consisted of markhor sequences was located as outgroup. Subsequently we assessed the frequency of the lineages by PCR-RFLP and mismatch PCR methods in 180 Myanmar, 33 Chinese, 96 Mongolian, 25 Laotian and 60 Northern Vietnam natives. The results were follows; China (A:0.88, B:0.03, C:0.09), Mongol (A:0.91, B:0.02, C:0.07), Myanmar

(A:0.55, B:0.45), Laos (A:0.36, B:0.64) and Northern Vietnam (A:0.35, B:0.65). The *C.hircus* B lineage was detected at high frequencies in Southeast Asia. The *C.hircus* C lineage was observed only in Chinese and Mongolian native goats at low frequencies.

**D056**

**Usefulness of a set of six microsatellites for parentage control in horses in Slovakia**

DANIELA JAKABOVÁ[1], JOZEF TRANDŽÍK[1], JOZEF CHRASTINA[1], LUDMILA HUDECOVÁ[1], ERIKA ZETOCHOVÁ[1], JOZEF BULLA[2], PETER KOZLÍK[1], FRANTIŠEK JAKAB[3]

*[1]State Breeding Institute, Nitra, Slovak Republic; [2]Research Institute of Animal Production, Nitra, Slovak Republic; [3]State Breeding Inspection, Nitra, Slovak Republic*

Parentage control in horses has been performed using microsatellites tests (DNA typing). The DNA typing panel consisted of 6 dinucleotide repeat microsatellites (ASB2, HMS3, HMS6, HMS7, HTG4, VHL20). In this study, the polymorphism of six microsatellites was investigated in population of Thoroughbred (n=303 ), Slovak Warmblood (n= 16) and Trotter (n= 26). We applied a combination of microsatellite PCR and semiautomatic fluorescence - based detection. Amplified PCR products were separated and visualised by an Automated Laser Fluorescent DNA sequencer (A.L.F. DNA sequencer, Pharmacia). Allele frequencies, observed and expected heterozygosity and probability of exclusion are presented for each STR locus and for all combined loci in these three populations. The results showed high polymorphic information content PIC (0,63 - 0,86). The combined exclusion probabilities (CPE) estimated using six microsatellites were in Thoroughbred CPE=0,988, Slovak Warmblood CPE= 0,996 and Trotter CPE= 0,995. These results demonstrate that the present DNA typing is useful and sufficient for individual identification and parentage verification of Thoroughbred, Slovak Warmblood and Trotter.

**D057**

**Genetic structure of synthetic line 990 pigs defined on the basis of polymorphism of the blood groups, lipoprotein allotypes and erythrocyte enzymes**

ANDRZEJ JANIK[1], MARIAN KAMYCZEK[2], ANNA KWACZYŃSKA[2], MARIAN RÓŻYCKI[1], MONIKA SIKORA[2], TOMASZ ZĄBEK[1], BARBARA BOJCZUK[3], ANNA RADKO[1]

[1]*National Research Institute of Animal Production, Kraków/Balice, Poland;* [2]*National Research Institute of Animal Production, Experimental Station, Pawłowice, Poland;* [3]*Świętokrzyska Academy, Institute of Biology, Kielce, Poland*

Synthetic line 990 was formed from 6 breeds: Polish Large White, Belgian Landrace, German Landrace, Welsh Landrace, Duroc and Hampshire. Total of 548 pigs line 990 was genotyped for 11 blood group systems (A, B, D, E, F, G, H, K, L, M, N), 2 lipoprotein systems (*LPB*, *LPR*) and 2 erythrocyte enzyme systems (*GPI*, *PGD*) and plasma protein (*TF*). It was proved that blood groups frequencies in synthetic line 990 was different from results in the earlier study in the increased frequency particulary in alleles: $E^{edgh}$ (0,344→0,393), $F^{bd}$ (0,836→0,914), $G^{b}$ (0,605→0,681), $H^{a}$ (0,526→0,650), $K^{bf}$ (0,403→0,450) and $N^{a}$ (0,389→0.566). The frequency of the $Lpb^{5}$ (0,945) was found to be the highest in comparison to the all the remaining Lpb alleles. In *GPI* system the higher frequency was for $GPI^{B}$ (0,761) than for $GPI^{A}$ allele (0,239). The frequency of $PGD^{A}$ allele (0,641) was higher than for $PGD^{B}$ allele (0,359). Three alleles controling the heterogeneity of transferrin ($Tf^{A}$, $Tf^{B}$, $Tf^{C}$) were found in the pigs of line 990 with respective frequencies (0,193, 0,713, 0,094).

**D058**

**Detection of polymorphism in the pig heat shock protein 70 gene by PCR-SSCP**

HYUN J. JIN[1], IN C. KIM[1], JUN C. PARK[1], DONG S. SON[1], CHOONG H. YOO[1], HO Y. CHUNG[2], IL C. CHEONG[2], SHU T. FENG[3], ZAN D. LI[4], CHOUNG I. KIM[5]

*[1]National Livestock Research Institute, RDA Cheonan 330-800, Korea; [2]National Livestock Research Institute, RDA Suwon 441-350, Korea; [3]Institute of Animal Science, CAAS Beijing 100094, China; [4]College of Biological Science, China Agricultural University, Beijing 100094, China; [5]College of Animal Resource Science, Kangwon National University, Chunchon 200-701, Korea*

Polymorphism of expressed pig major histocompatibility complex (MHC) class Heat

shock protein 70 (*HSP 70*) gene was investigated in seven different breed groups (Duroc, Yorkshire, Landrace, Korean native pig, Minzhu, Xiangzhu, Wuzhishanzhu). The primer sequences were selected based on the cDNA sequence (GenBank accession No. X68213), and segments for the primer were conducted in 290~512, 830~1424 and 1363~2041 of *HSP 70* gene. The polymorphism of *HSP 70* was found by polymerase chain reaction–single strand conformation polymorphism (PCR-SSCP) analysis in amplified fragment of 223bp, 595bp and 679bp. *HSP 70* genotypes were detected as AA, AB, and BB for *HSP 70* (290~512), *HSP 70* (830~1424) and *HSP 70* (1363~2041). The frequency of alleles in seven breed groups was *HSP 70* (290~512) $^{A}$ = 0.367, *HSP 70* (830~1424) $^{A}$ = 0.414 and *HSP 70* (1363~2041) $^{A}$ = 0.109. The allele frequencies were used to estimate the genetic distances and to construct both a neighbor joining tree and a unweighted pair group method with arithmatic mean (UPGMA) tree by the *Phylip* programs. The two closer breeds were Wuzhishanzhu and Xiangzhu, while the two more different were Xiangzhu and Korean native pig. *HSP 70* (1363~2041) genotypes were associated with significant effects ($P<0.05$) on litter size (total born) from Artificial Insemination (AI) of frozen boar semen, but failed to provide evidence for significant effects of pork quality traits(color, firmness, pH and cooking loss) in Duroc, Landrace and Yorkshire breed.

**D059**

**Some genetic markers and Halothane susceptibility in Yugoslav Wild pigs (*SUS SCROFA FERUS*)**

SLOBODAN J. JOVANOVIC, MIROSLAV SARAC, MILA SAVIC, RUZICA TRAILOVIC, VLADIMIR DIMITRIJEVIC
*Faculty of Veterinary Medicine University of Belgrade, Department of Animal Breeding and Genetics, Belgrade, Yugoslavia*

A total of 56, 3 moths, old wild pigs, were tested for PSS by 5 minute exposure to 3% Halothane inhalant solution. Blood samples with $K_3$EDTA were collected from all tested animals and genetic variations at PHI and 6-PGD loci were tested by starch gel electrophoresis, while C-T mutation at nucleotide 1843 of RYR1 gene was tested by PCR-RFLP genotyping. Halothane test revealed 6 positives out of 56 pigs. All $Hal^{+}$ animals had increased serum CPK concentration. All tested pigs were homozygous at 6-PGD and only A allele was established. Two allelic genes A and B, in frequencies 0.29 and 0.71, respectively, were segregated within PHI locus. All animals had normal C/C genotype at RYR1 locus. The results obtained show that halothane sensitivity and PSS are present in Yugoslav wild pig even in absence of C/T mutation at RYR1 locus. Possible multifactorial influence as the base of PSS (MH) phenotype in pigs has not been confirmed yet.

Acknowledgement: The invetigation was prepared thanks to the support of NORAGRIC, in the scope of Norwegian program for updating academic education and research in agriculture in South Eastern Europe.

**D060**

**Genetic analysis of three Lithuanian native horse breeds**

R. JURAS[1], E. G. COTHRAN[2]
[1]*Lithuanian Institute of Animal Science, Baisogala, Lithuania;* [2]*University of Kentucky, Lexington, KY, USA*

Genetic variation in three Lithuanian native horse breeds (Zemaitukai, large-type Zemaitukai and Lithuanian Heavy Drought) was investigated using serological and electrophoretic procedures to detect genetic variation in seven blood groups (*A, C, D, K, P, Q, U*) and 10 protein systems (*A1B, ALB, ES, GC, HBA, PGD, GPI, PGM, PI, TF*) and DNA typing of 16 microsatellites (*ASB17, VHL20, HTG10, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7*, *ASB23, LEX3, LEX33, ASB2, HTG6, KTG7, HMS2*). A total of 83 animals were tested. No significant deviations from Hardy-Weinberg equilibrium were observed. Rodger's genetic similarity and Nei's genetic distance were calculated from the gene frequencies between Lithuanian native horses and other horse breeds. Populational inbreeding level was estimated by Wright's *Fis*. Restricted maximum likelihood (RML) analysis was used to construct the dendograms. Genetic variability of the Zemaitukai breeds based upon blood groups and biochemical loci was higher than average for domestic horse breeds. For microsatellite loci, the variability of the large-type Zemaitukai and the Heavy Draught breeds also were high compared to the mean variation for domestic breeds, however,

for the Zemaitukai breed, variation levels were very near the average for horses. Zemaitukai horses showed greatest resemblance to the American Appaloosa breed and the large-type Zemaitukai to Morgan horses. However, phylogenic analysis paired Lithuanian horses with the Skyros pony from Greece.

**D061**

**Equine osteoarthrite (OA) chondrocytes contribute to the OA matrix metalloproteinase pattern of the synovial fluids more than OA synoviocytes**

EVGUENI KARAKINE, IGOR PONOMAREV, DIRK BARNEWITZ, INGO WILKE

*Research Center for Medical Technique and Biotechnology, Department of Tissue Engineering and Department of Veterinary Chirurgie, Bad Langensalza, Germany*

The predisposing genetic factors have been shown to play an important role in the osteoarthritic (OA) diseases (Vikkula et al., 1994), although the etiology of the OA remains obscure. For instance, it was demonstrated that the matrix metalloproteinases (MMPs), namely MMP-9 of the OA synovial fluids, is a preradiological molecular marker of the OA disease in horse (Clegg et al., 1997), as in a man (Koolvijk et al., 1995; Ahrens et al., 1996). But both the causes and the sources of the increasing of the content of MMP-9 are unclear. Normal (N) and OA (inflammation, trauma, chips) equine chondrocytes from the different joints were cultivated, their MMP patterns including the secretion ones, were screened by the reverse zymography technique and were compared with the MMP patterns of the cultivated synoviocytes and with those of the same synovial fluids. The extracts from both the N ($P_o$) cultivated chondrocytes and N ($P_o$) synoviocytes contain the only MMP-2, their secretion patterns reveal usually MMP-2 and MMP-9. The MMP patterns of the N synovial fluids contain MMP-2, MMP-9, MMP-85 kDa, MMP-220 kDa and also three additional minor MMP fractions, namely MMP-105 kDa, MMP-120 kDa and MMP-145 kDa. By the contrast, the extracts from the OA ($P_o$) cultivated chondrocytes express MMP-2 or MMP-2/MMP-9 patterns, but their secretion ones contain 7– 9 MMP fractions including a set of the synovial MMPs and also MMP-53 kDa, MMP-68 kDa and MMP-74 kDa. It was also surprisingly, that the extracts from the OA($P_o$) cultivated synoviocytes reveal MMP-2 and their secretion pattern contains MMP-2/MMP-9 only. MMP patterns of the OA synovial fluids contain whole set of the normal ones, but, firstly, the relative contents of MMP-9 are 1.5–2.5 times more as compared to N ones, and secondly, the summarysing MMP patterns may increase until 8 - 12 MMP fractions. We conclude that the OA chondrocyte cells *in situ* may contribute to the OA MMP pattern through the secretion into the OA synovial fluids more than the OA synoviocytes.

**D062**

***GUJ0040*: a Z chromosome-specific microsatellite marker in Japanese quail**

BONIFACE B. KAYANG[1], MIHO INOUE-MURAYAMA[1], TAKUYA HOSHI[1], KOJI MATSUO[1], HIDEAKI TAKAHASHI[2], MITSURU MINEZAWA[2], MAKOTO MIZUTANI[3], SHIN'ICHI ITO[1]

[1]*Faculty of Agriculture, Gifu University, Gifu, Japan;* [2]*National Institute Of Agrobiological Sciences, Tsukuba, Japan;* [3]*Laboratory Animal Research Station, Nippon Institute for Biological Science, Kobuchizawa, Japan*

Japanese quail *(Coturnix japonica)* is one of the economically important members of the family Phasianidae used widely as a laboratory research animal and a pilot animal for poultry because of its advantages of small body size, rapid generation turnover, high egg production and inexpensive rearing requirements. In order to construct a molecular genetic linkage map for Japanese quail we isolated and characterized 100 microsatellite markers. Genotyping revealed that one marker, *GUJ0040,* was heterozygous in males only. In a random sample of 26 males and 58 females, heterozygosity was observed in 8 males, while all females were hemizygous. These results suggest that *GUJ0040* is on the Z chromosome. Furthermore, cross-species amplification of this marker was observed in chicken *(Gallus gallus*), guinea fowl (*Numida meleagris*), ring-necked pheasant (*Phasianus colchicus*), and Asian blue quail (*Coturnix chinensis*), and the genotyping results in these species were consistent with those in Japanese quail. This indicates that *GUJ0040* would be useful for mapping the Z chromosome in the family Phasianidae. Recently, two plumage colour loci in Japanese quail, albino (*AL*) and

brown (*BR*), have been reported to be linked to the Z chromosome with a recombination frequency of 38.1 cM. Thus, to determine the relative position of *GUJ0040* by linkage analysis, three-point cross experiments among *AL*, *BR* and *GUJ0040* loci are now in progress.

**D063**
**Characterisation of the bovine *kappa* casein promoter**

AILEEN F. KEATING, MICHAEL CAIRNS[1], TERRY SMITH[1], PAUL ROSS[2]
[1]*National Diagnostic Centre, National University of Ireland, Galway, Ireland;* [2]*Dairy Products Re- search Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland*

Milk serves as a complete food source for the neonate. It contains a rich source of vitamins, minerals, lipids, carbohydrates and amino acids. Bovine milk proteins are extremely well characterised, both genetically and chemically. The caseins account for 80% of milk protein, and expression of the *kappa* casein gene accounts for approximately 10.4% of total milk protein. In this study it is proposed that single nucleotide polymorphisms (SNP's) in the *kappa* casein promoter region account for variations in the expression of *kappa* casein between different bovine breeds. Ten breeds of lactating cows were chosen, and the *kappa* casein promoter amplified and sequenced. Mutations in three transcription factor binding sites were identified in 28.5% of animals screened. Both the wild type and mutant forms of the kappa casein promoter have been cloned into the pGL3-basic reporter vector. Differences in expression between full-length promoter constructs, and also between truncated forms of the promoters were investigated in a number of transiently transfected mammary cell lines, by measuring luciferase levels.

**D064**
**The genetic structure of Vyatka horse populations**

L. A. KHRABROVA, A. M. ZAITCEV
*Laboratory of Immunogenetics, The All-Russian Institute of Horse Breeding, Divovo, Russia*

The Vyatka is ancient native horse breed known since the XIVth century. The Vyatka horse is typical northern wood breed distinguished by good disease resistance, adaptive and exterior qualities. By the end the XXth century the number of these animals was sharply reduced, and in 1996 Vyatka was given the status of disappearing breed (WWL-DAD:2). Now areas of Vyatka horses have two nuclei - Udmurt and Kirov populations. Gene frequencies at 9 blood group and protein polymorphism loci (A, D, C, K, Al, Tf, Es, 6-PGD and Ca) are given for basic populations of Vyatka horses. Investigated loci had a sufficient polymorphism level. 124 Vyatka horses were used in a study designed for blood group and protein polymorphism analysis. The polymorphism analysis indicated that a distribution allele frequency was characteristic for native northern wood breeds in the whole. The distinctions on the investigated systems in genetic structures of the Vyatka horse populations from different regions were revealed. So horses from Kirov area have no alleles TfD and TfH in Tf locus and differ sufficiently by distribution allele frequencies in loci 6-PGD and Alb. There is a tendency to accumulation of gomozygotes on a number of loci in some subpopulations. The highest degree of genetic heterogeneity was found in a large farm of Udmurtiya, where it is kept the basic populations of the breed.

**D065**
**Diversity of African domestic sheep (*Ovis aries*) revealed by mitochondrial D-loop sequence differences**

G. KIERSTEIN[(1)], J. HIRBO[(1)], P. WAFULA[(1)] J.E.O. REGE[(2)] & O. HANOTTE[(1)]
[(1)]*International Livestock Research Institute (ILRI), Nairobi, Kenya, and* [(2)]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia*

To better understand the origin and diversity of sub-Saharan African domestic sheep, we characterized the molecular genetic diversity of the mitochondrial D-loop sequence of 37 individuals belonging to twelve different populations from seven African countries (Ethiopia, Nigeria, Mali, Senegal, South Africa, Botswana). These sequences were compared with European, New Zealand, Near/Middle East domestic sheep sequences and wild sheep sequences obtained from the GenBank database. As is the case with the European haplotypes, the African D-loop consensus sequence is 1180 nucleotides (nt) long, including four tandemly

repeated motifs of 75 nt length. All African sequences represent new unique haplotypes. We observed 15 indels and found 130 variable sites, including 122 transitions and 11 transversions. The pairwise distance between the African haplotypes under the HYK85 model, ranged from 0.0 to 0.05 with an average of 0.02. Interestingly, within the conserved central domain of the D-loop, we observe an A → T transversion separating all New Zealand sequences from all other sequences analysed. Network analysis of the central domain suggests two 'groups' of haplotypes, previously described as A and B (Wood and Phua (1996) Animal Genetics, 27: 25-33), with the majority of African haplotypes closer to subgroup B. However, in Africa we found intermediate haplotypes connecting both groups.

**D066**

**Network analysis demonstrates new insights into water buffalo (*Bubalus bubalis*) phylogeny**

GEROLD KIERSTEIN[(1)], MARCELO VALLINOTO[(2)], ARTUR SILVA[(2)], MARIA P. SCHNEIDER[(2)], LEOPOLDO IANNUZZI[(3)] & BERTRAM BRENIG[(4)]

*[(1)]International Livestock Research Institute (ILRI), Nairobi, Kenya, [(2)]Centro de Ciencias Biológicas, Laboratorio de Polimorfismo de DNA, Universidade Federal do Pará, Belém, Brazil, [(3)]National Research Council (CNR), IABBAM, Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy and [(4)]Institute of Veterinary Medicine, Georg-August University, Göttingen, Germany*

The phylogeny of water buffaloes (*Bubalus bubalis*) is still a matter of discussion. In the past the divergence time of river and swamp buffaloes calculated by different authors varied between 10,000 and 1.7 million years ago, due to different analysis methods. To obtain more insight, we analysed the complete mitochondrial D-loop region of 80 water buffaloes of four different breeds. We demonstrate here a comprehensive network analysis, which is of great interest when analysing genetic differences between individuals of one species, as e. g. the median-joining algorithm takes into account the different genetic separation within a species compared to the situation between different species. Our results not only confirming the clear divergence between swamp and river buffalo, but also give evidence for ancestral mitochondrial sequences. The average pairwise sequence divergence (uncorrected "p") between river and swamp buffalo was calculated to be around 7 %. Similar genetic differences have been reported between Afro-European and Indian cattle. The average divergence within the two buffalo types was each less than one percent. Assuming an evolutionary rate of 7.5 x $10^{-8}$ substitutions per site and year and using the Tamura-Nei model with gamma correction, we calculated that swamp and river buffaloes diverged at least 913,000 years ago. This time interval clearly exceeds the time since their domestication around 7,000 to 9,000 years ago.

**D067**

**Molecular cloning and chromosomal assignment of three type I markers on porcine chromsome 7**

SONJA KIERSTEIN[1], HIAM AL-BAYATI[2], SONJA KOLLERS[3], FELIX A. HABERMANN[3] ,BERTRAM BRENIG[2]

*[1]International Livestock Research Institute (ILRI), Nairobi, Kenya; [2]Institute of Vetrinary Medicine, Göttingen, Germany; [3]Institute of Animal Breeding, Muenchen-Weihenstephan, Germany*

In course of a genome analysis project with the aim to map and characterize type I markers on porcine chromosome 7 we analysed two genomic PAC clones designated F11 and B5. Fluorescence *in situ* Hybridisation (FISH) and PCR analysis of a somatic as well as a radiation hybrid panel assigned both PAC clones to porcine chromosome 7q13-14 and 7q11-13, respectively. On PAC F11 we sequenced the gene coding for methylmalonyl-CoA mutase (*mut*), which we consider as a candidate gene for neuro-muscular disorders in pig. Sequencing of PAC B5 revealed several regions that are homologous to a human BAC sequence localized on HSA 6q21-21.3. By means of comparative genome analysis we were able to map the exon-intron boundaries of the porcine orthologue of the recently in humans described GPIM gene. The GPIM gene (GPI and MAM protein) is highly conserved between man and pig. The gene is coding for a MAM-, Ig- and fibronectin-domain containing protein with the capability to anchor to cell membranes by the GPI motif. Similar structural features are found in different types of cell adhesive molecules (CAM). Additional se-

quence analysis led us to postulate a second, still unknown gene on the same PAC clone. Although no corresponding cDNA could be obtained by database search, computational analysis of the porcine and human genomic DNA sequences indicate at least six intron-separated exons. Several regions of high homology, which do not refer to known repetitive elements, support our hypothesis of the presence of a transcribed gene.

**D068**

**The first linkage groups in the red fox (*Vulpes fulvus*) and arctic fox (*Alopex lagopus*) genomes**

JOLANTA KLUKOWSKA, MACIEJ SZYDŁOWSKI, MAREK SWITONSKI
*Department of Animal Genetics and Breeding, August Cieszkowski Agricultural University of Poznan, Poland*

The dog (*Canis familiaris*), the arctic fox (*Alopex lagopus*) and the red fox (*Vulpes fulvus*) belong to the family *Canidae* but diverged from a common ancestor some 10 million years ago. It is well known that majority of canine primers can be successfully used for PCR amplification of the microsatellites from the red fox and arctic fox DNA.

Polymorphism and linkage analysis of nineteen canine-derived microsatellite loci (CPH3, CPH6, CPH8, CPH11, 2004, 2010, 2019, 2140, 2168, 2206, 2281, 2319, 2320, 2541, 2594, ZuBeCa4 i ZuBeCa6) in the silver fox and the arctic fox genomes were carried out. Altogether, 14 silver fox families (87 animals) and 17 arctic fox families (145 animals) were included into this study. Ten markers appeared to be linked in the red fox genome, with a lod-score $4 \geq Z \geq 3$. Two linkage groups were identified: LGV 1: CPH8-2004-2319-2019 and LGV 2: 2010-2168-2281-2541-ZuBeca4. Eight markers were linked in the arctic fox genome. Three linkage groups were identified with a lod score $\geq 5.0$: LGA2: ZuBeCa6-2594-2140; LGA 3: ZuBeCa4-2320 and LGA 4: 2010-2168-2281 and one group with a lod score $4 \geq Z \geq 3$ : LGA 1: CPH3-CPH6. Using the physical mapping data we could assigned one linkage group (LGV2) to the red fox chromosome 14 and two linkage groups to the arctic fox chromosomes: 10 (LGA2) and 4 (LGA3). The identified linked microsatellite loci are also linked in the dog genome.

(This study was supported by the Foundation for Polish Science, contract 13/2000)

**D069**

**Comparison of two microsatellite panels for parentage verification in pigs in Czech Republic**

ALEŠ KNOLL, LENKA PUTNOVÁ
*Mendel University of Agriculture and Forestry Brno, Brno, Czech Republic*

The genotype determination and parentage verification in pigs in Czech is performed according to Law No. 154 (2000). This preliminary study compares the utilization of two DNA microsatellite panels for parentage verification in Czech Republic. The first panel (A) is based on our assembled and at present for genotyping used set of 9 porcine microsatellites (MS) and second from set of 10 MS described by Nechtelberger et al. (panel B). We tested unrelated animals from different farm and breeds Large White (LW, N=30), Landrace (Lc, N=30) and Czech gene reserve Black Pied Prestice (BPP, N=20). Genotyping was done on ABI 310 genetic analyser. We described the number of founded alleles (NA) and their heterozygosity (H):

| Panel A | LW | | Lc | | BPP | |
|---|---|---|---|---|---|---|
| MS | NA | H | NA | H | NA | H |
| S0068 | 7 | 0.83 | 5 | 0.63 | 5 | 0.75 |
| S0107 | 8 | 0.90 | 5 | 0.70 | 7 | 0.85 |
| SW24 | 5 | 0.70 | 5 | 0.73 | 7 | 0.90 |
| SW840 | 3 | 0.13 | 3 | 0.10 | 4 | 0.60 |
| SW353 | 5 | 0.57 | 5 | 0.73 | 4 | 0.35 |
| SW936 | 4 | 0.70 | 6 | 0.70 | 6 | 0.80 |
| S0070 | 6 | 0.80 | 8 | 0.67 | 8 | 0.80 |
| SW72 | 6 | 0.73 | 3 | 0.63 | 5 | 0.90 |
| TNFB | 8 | 0.90 | 9 | 0.97 | 6 | 0.90 |
| S0005 | 8 | 0.88 | 6 | 0.86 | 8 | 0.88 |
| S0090 | 4 | 0.67 | 5 | 0.88 | 5 | 0.93 |
| S0101 | 6 | 0.67 | 4 | 0.70 | 4 | 0.65 |
| S0155 | 4 | 0.64 | 4 | 0.60 | 5 | 0.92 |
| S0355 | 6 | 0.77 | 3 | 0.58 | 6 | 0.79 |
| S0386 | 3 | 0.52 | 5 | 0.48 | 5 | 0.84 |
| SW24 | 5 | 0.70 | 5 | 0.73 | 7 | 0.90 |
| SW240 | 6 | 0.57 | 5 | 0.77 | 7 | 0.80 |
| SW857 | 7 | 0.63 | 9 | 0.79 | 7 | 0.85 |
| SW951 | 4 | 0.63 | 3 | 0.36 | 2 | 0.50 |

The results from testing of larger set of tested animals will be available in time of poster presentation.

**D071**

**Genetic diversity of cattle from Mongol, Korea and Japan using microsatellite analysis**

MIKI KONO[1], HIDEYUKI MANNEN[2], KOU NOMURA[3], TAKASHI AMANO[3], JUNG S. YEO[4], SOICHI TSUJI[2]

*[1]Kobe University, Graduate School of Science and Technology, Kobe, Japan; [2]Kobe University, Faculty of Agriculture, Kobe, Japan; [3]Tokyo University of Agriculture, Tokyo, Japan; [4]Yeungnam University, College of Natural Resources, Kyeungbuk, Korea*

Information on the genetic structure and distance among breed is necessary for meaningful conservation of genetic diversity. The objective of this study is to characterize the genetic variability of native cattle breeds in the Northeastern Asia using microsatellite markers. The 15 microsatellite markers (INRA063, INRA005, ETH225, ILSTS005, HEL5, HEL1, INRA035, BM1824, HEL13, INRA037, BM1818, HAUT24, TGLA122, TGLA53, SPS115) were analyzed for three cattle breeds in Mongol (n=50), Korea (n=50) and Japan (n=50). The average numbers of allele were 8.0 in Mongolian, 6.4 in Korean and 5.5 in Japanese native cattle. Mean heterozygosity and PIC values were calculated at 0.71 and 0.67 in Mongolian, 0.63 and 0.59 in Korean, and 0.68 and 0.63 in Japanese native cattle, respectively, indicating the highest genetic diversity in Mongolian cattle. In addition, some microsatellite markers revealed Mongol-specific alleles. Neighbor-joining tree using the genetic distance showed close genetic relationship between Mongolian and Korean natives, and Japanese native as outgroup. These results are useful information not only for conservation of genetic diversity, but also for studies on the origin and the history of the Northeastern Asian cattle.

**D072**

**Gene frequencies of blood group and blood proteins in the Thoroughbred horses in Turkey**

AHMET KOPAR[1], OKAN ERTUĞRUL[2]

*[1]Institute of Etlik, Veterinary Control and Research Center, Laboratory of Blood Groups and Genetics, Etlik, Ankara, Turkey; [2]Ankara University, Veterinary Faculty, Department of Genetics, Ankara, Turkey*

Blood group and sera protein polymorphisms were investigated in 4905 Thoroughbred horses in Turkey. Blood groups systems (A, C, D, P, Q, U) were analyzed by Hemolytic and agglutination tests. Starch gel electrophoresis, alkaline polyacrylamide gel electrophoresis and polyacrylamide isoelectric focusing electrophoresis were used to identify genotypic variants of AIB glycoprotein, Albumin, Carboxylesterase, Vitamin D binding protein, Hemoglobin-α, Transferrin loci. The gene frequencies of the A, D, Q blood group systems were calculated by Maximum likelihood method. Direct Counting Method was used for the calculation of other blood groups and plasma proteins systems gene frequencies. Five alleles (D, F, H, O, R) were identified for Tf. The highest frequency in this system was found in F allele. Two alleles (F, S) were identified in GC system. Three alleles (F, I, S) have been detected in Carboxylesterase system. Frequency of I was the highest among all alleles. Two alleles (K, S) were detected in the AIB (Xk) system. F allele, which is present in some breeds of the horse, was not found in this study. The estimated frequency of K in the study material of the thoroughbred horses was the highest. In Hemoglobin-α system, the frequency of BII was the highest. However AII allele was not found. The gene frequencies in blood groups and protein polymorphism in thoroughbred horses in Turkey were similar to those thoroughbred horses in Europe than the once reported in other countries.

**D073**

**A full genome scan panel of horse (*Equus caballus*) microsatellite markers applied to different equid species**

KARIN KRÜGER[1,2], GERALD STRANZINGER[1,2], STEFAN RIEDER[1]

*[1]Institute of Animal Science, Breeding Biology, Swiss Federal Institute of Technology, Zürich, Switzerland; [2]Faculty of Veterinary Medicine, University of Zürich, Switzerland*

Conservation and homology among mammalian genomes is widely recognized. Thus, molecular approaches using sequence and marker information from one species to establish specific sequence and marker information in a second or third species, belong to the "state of the art" techniques in animal genetic research. We applied a panel of microsatellite markers covering all 31 horse autosomal chromosomes

and the X chromosome to different members of the equid family: Equus caballus, Equus asinus, Equus africanus somaliensis, Equus hemionus, Equus kiang. Horse microsatellites were found polymorphic in all mentioned equids. PCR conditions for equine markers needed adjustment in all species other than Equus caballus itself. Allele sharing statistics for population demarcation and a maximum likelihood approach to assess allocation success of individual animals to a specific population cluster were proceeded. The results demonstrate the application of these molecular tools in evolutionary biology and as an instrument for conservation programs of endangered species.

**D074**

**A new silent mutation in German Simmental and Brown cattle could be used as a marker for a BSE case control study**

OLIVER KUPPINGER, STEFAN KREBS, RENATE DAMIAN, MARTIN FÖRSTER
*University of Munich, Department of Animal Breeding, Munich, Germany*

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease caused by accumulation of abnormal prion protein (PrP) in cattle. Mutations within the *PrP* coding region from amino acid 100 to 231 in mice, sheep and humans are associated to incubation period and resistance in experimental and natural TSE. However, such an association is not known for the bovine PrP. Our present work aims at identifying a genetic marker in the bovine gene in order to conduct a case control study about its association to disease. The genomic DNA encoding PrP 95-231 was analysed by nucleotide sequencing. Initial screening of 100 German Simmental and 100 Brown Swiss cattle yielded a silent point mutation for asparagine at position 173. All three possible genotypes: AAC/AAC (wildtype), AAC/AAT and AAT/AAT could be detected and verified by either MALDI or RFLP. Further 294 German Simmental and 300 Brown Swiss cattle were genptyped by HincII-RFLP. Frequency of the mutant was 12,93 % for German Simmental and only 7,17 % for Brown Swiss cattle breed. Despite the high sequence identity to sheep, mice and human no other mutations could be detected. However, PrP genotyping of the identified mutation could give hints to a yet unidentified factor that may influence susceptibility and resistance to BSE. In order to carry out an automated screening for this polymorphism a MALDI-assay was established that allows genotyping of a larger sample number. Furthermore, a case control study with BSE affected animals is under way to investigate the association between the polymorphism and BSE.

**D076**

**Genetic polymorphism of *GPI* and *PGD* in the pigs of Polish Large White, Polish Landrace and synthetic line 990 breeded in Poland**

ANNA KWACZYŃSKA[1], MARIAN RÓŻYCKI[2], MARIAN KAMYCZEK[1]
*[1]National Research Institute of Animal Production, Experimental Station, Pawłowice, Poland; [2]National Research Institute of Animal Production, Kraków/Balice, Poland*

The studies on the genes located on chromosome 6 in pigs, which determine polymorphism of erythrocyte enzymes glucose phosphate isomerase (*GPI*) and 6-phosphogluconate dehydro-genase (*PGD*) were carried out on the material tested at Pig Performance Stations. A total of 709 Polish Large White (PLW), 1341 Polish Landrace (PL) and 535 synthetic line 990 (SL990) pigs were used. The analysis of genotypes of the *GPI* and *PGD* loci was conducted on blood samples using the agarose gel electrophoresis according to method Gahne and Juneja. Only two alleles, A and B, were identified within both systems. There were estimated genotypes frequency of *GPI* and *PGD* and of *GPI-PGD* linked loci. In *GPI* system frequencies of the AA, AB, BB genotypes were 0,259, 0,504, 0,237 respectively in PLW pigs, 0,031, 0,300, 0,669 in PL pigs and 0,069, 0,400, 0,531 in SL990 pigs. As regards the *PGD* locus in PLW frequencies of the AA, AB and BB genotypes were 0,536, 0,395 and 0,069 in PL 0,310, 0,496 and 0,194 and in SL990 pigs 0,387, 0,467 and 0,146. Analysis of *GPI-PGD* linkage reveal that the highest frequencies were in PLW AB-AA (0,294), in PL BB-AB (0,327) and in SL990 pigs BB-AA (0,238). In each breed the lowest frequency was for AA-BB genotype linkage. Taking into consideration both loci, the PL gene frequencies were more similar to those calculated for SL990 than the PLW pigs.

**D077**
**Genetic diversity of European cattle**

THE EUROPEAN GENETIC CATTLE DIVERSITY CONSORTIUM (J. A. LENSTRA[1], I. J. NIJ-MAN[1], G. MOMMENS[2], G. DOLF[3], P. WIENER[4], D. BURTON[4], J. L. WILLIAMS[4], K. MOAZAMI-GOUDARZI[5], D. LALOË[5], S. DUNNER[6], J. CANON[6], P. ZARAGOZA[7], C. RODELLAR[7], I. MARTIN-BURRIEL[7], A. SANCHEZ[8], G. ERHARDT[9], O. JANN[9], C. WEIMANN[9], P. AJMONE-MARSAN[10], R. NEGRINI[10], A. VALENTINI[11], M. C. SAVARESE[11], M. ZANOTTI[12], F. PILLA[13], A. BRUZZONE[13], D. IAMARTINO[13], I. OLSAKER[14], I. MICEIKIENE[15])
*[1]Faculty of Veterinary Medicine, Utrecht Univerity, The Netherlands; [2]PolyGenLab, Malle, Belgium; [3]Institute of Animal Breeding, Nutrition and Housing, University of Berne, Switzerland; [4]Roslin Institute (Edinburgh), Roslin, UK; [5]INRA, Jouy-en-Josas, France; [6]Facultad Veterinaria, Madrid, Spain; [7]Facultad Veterinaria, Zaragoza, Spain; [8]Facultad Veterinaria, Barcelona, Spain; [9]Department of Animal Breeding and Genetics, Justus Liebig Universität, Giessen, Germany; [10]Institute of Zootechnics, Catholic University of S. Cuore, Piacenza, Italy; [11]Istituto di Zootecnica, Universitá della Tuscia, Viterbo, Italy; [12]Instituto di Zootecnica, University of Milano, Italy; [13]Facoltà di Agragria, Campobasso, Italy; [14]Division of Genetics, The Norwegian School of Veterinary Science, Oslo, Norway; [15]Gyvunu Genetikos Laboratorija, Kaunas, Lithuania*

The EU-sponsored project *Towards a strategy for the conservation of the genetic diversity of European Cattle* (Resgen CT98-118, 1999-2001) carried out a molecular assessment of the genetic diversity of European Cattle and formulated recommendations for conservation policies. Estimates of relative genetic distances were based on two different types of genetic marker, microsatellites and AFLP polymorphisms. Microsatellite typing was completed for 68 breeds with 30 markers and 25 to 50 animals per breed. AFLP typing has been done on 49 continental breeds, 20 animals per breed. Factors that influence the relative estimates of distance measures (typing methodology, history of the species or of the breed, geographical origin of the breed) will be discussed, as will how these data are of relevance for guiding the conservation of genetic diversity.

**D078**
**Genetic characterisation of Istrian sheep by microsatellites**

MARIA LONGERI[1], MARIA GIUSEPPINA STRILLACCI[1], ROBERTA LEONARDUZZI[2], FABIO PILLA[3], ROBERTO RASERO[4], ANDREA FRAGHI[5], MARTA ZANOTTI[1]
*[1]University of Milan, Faculty of Veterinary Medicine, Istituto di Zootecnica, Milan, Italy; [2]University of Udine, Dep. Scienze della Produzione Animale, Udine, Italy; [3]University of Molise, Dep. SAVA, Campobasso, Italy; [4]University of Turin, Faculty of Veterinary Medicine, Dep. Produzioni Animali, Turin, Italy; [5]Ist. Zootecnico Caseario per la Sardegna, Sassari, Italy*

Two small Istrian sheep populations, Istrian Pramenka and Carsolina bred in Istria (Croatia) and in Carso (Italy) respectively, have been analysed in order to define their genetic identity. The two groups, originally coming of the same stock, have been divided following to the politic partition of the breeding area after the Second World War. Samples randomly chosen out of the two breeds have been compared each other and with three Italian sheep breeds, different both for origin and morphology: Sarda, Comisana and Delle Langhe. Microsatellites CSSM31, OARAR119, OARCP34, OARFCB11, MILVET07-08, ILSTS005-11-29, MAF214 have been analysed by automatic fluorescent methods. Allelic frequencies and Hardy-Weinberg *equilibrium* by GENEPOP software and heterozygosity index according to Nei (1979) have been calculated. Genetic distances within populations according to Nei by DISPAN software and Principal Component Analysis (PCA) by SAS' PRINCOMP procedure have been performed. High homozygosity due to high level of inbreeding, particularly in Carsolina has been recorded. Comparing allelic frequencies, significant differences among the breeds and between Carsolina and Istrian have been shown. Genetic distances and PCA confirmed wide differences among all the populations. Despite morphological homogeneity between the two Istrians, genetic markers showed strong differences due to the bottleneck and genetic drift occurred after the political partition.

**D079**
**Identification of SNPs in the interleukin-2 gene (*IL2*) of cattle and goat**

GESINE LÜHKEN, GEORG ERHARDT
*Justus-Liebig-University of Giessen, Department of Animal Breeding and Genetics, Giessen, Germany*

Polymorphisms in cytokine genes and cytokine receptor genes have shown to influence genetic disease resistance and susceptibility. In a previous study, we characterized the gene of the cytokine interleukin-2 in sheep and found 12 SNPs comparing sequence data derived from seven breeds (GeneBank AF287479). Here we describe the sequence analysis of *IL2* in one sample each of *Bos taurus breeds* (German Angus, German Simmental, Holstein-Friesian, Pinzgau, N'Dama, Anatolian Black), of the *Bos indicus* Nelore, of five *Capra hircus* breeds (Saanen, Toggenburg, Boer, Norwegian, Orobica) and of wild goat (*Capra aegagrus*) and Markhor (*Capra falconieri*). After PCR amplification and cloning of the approximately 4.8 kb gene, sequencing was done by primer walking using two vector primers and eight specific primers. Interestingly, all of the specific primers developed for sequencing of ovine *IL2* were useful in goat, while in cattle, half of the primers had to be replaced by primers with cattle-specific sequences. We found more than six SNPs in the 5'flanking and intronic region of caprine *IL2* and more than three SNPs in the intronic region of bovine *IL2*, and more potential polymorphisms are in progress to be verified in both species.

**D080**
**An enhanced sheep linkage map comprising 240 gene and EST associated markers**

JILLIAN F. MADDOX, IAN FRANKLIN[1], CYNTHIA D.K. BOTTEMA[2], UDAYA DESILVA[1], DAVID L. ADELSON[3], CRISTINA DIEZ-TASCÓN[4], GREG NATTRASS[5], CLARE GILL[3], GRAHAM WEBB[2], KEN DODDS[4] & DANIEL VAIMAN[6]
*Centre for Animal Biotechnology, University of Melbourne, Victoria, Australia; [1]Commonwealth Scientific & Industrial Research Organisation (CSIRO) Livestock Industries, Blacktown, Australia; [2]Department of Animal Science, University of Adelaide, Roseworthy, Australia; [3]Department of Animal Science, Texas A& M University, College Station, USA; [4]AgResearch , Dunedin, New Zealand, [5]Livestock Systems - Molecular Biology, South Australian Research and Development Institute (SARDI), Glenside, Australia; and [6]Department de Génétique Animale, Institut National de la Recherche Agronomique (INRA), Jouy-en-Josas, France.*

The most recent sheep linkage map, published in 2001, comprises 1,091 markers representing 1,062 unique loci. One hundred and twenty-one of these loci are genes, and as such represent potential links between the sheep and human maps, while the remaining 941 loci are anonymous. One way to increase the usefulness of the sheep linkage map, and take advantage of more of the information generated by the human genome project, is to increase the number of links between the sheep and human maps. This can be achieved by positioning more genes on the sheep linkage map. We have developed a number of new ruminant gene and expressed sequence tag (EST) associated microsatellite markers following physical screening of ovine EST and bacterial artificial chromosome (BAC) libraries, and *in silico* screening of ruminant EST and gene GenBank entries. These markers have been characterised, and their sheep linkage map locations determined by genotyping the International Mapping Flock (IMF, AgResearch, NZ). This mapping information, together with mapping information for new anonymous loci, has been integrated with data for the existing map to construct a new sheep linkage map. The new map, containing 240 genes and EST sequences, will be presented at ISAG 2002 and can be found at http://rubens.its.unimelb.edu.au/~jillm/jill.htm.

**D081**
**North European Cattle Diversity (N-EURO-CAD)**

JOLANTA MALEVICIUTE[1], ILONA MICEIKIENE[1], INGRID OLSAKER[2], SIRJE VÄRV[3], HALDJA VIINALASS[3], ERLING FIMLAND[4], ZIEDONIS GRISLIS[5], JUHA KANTANEN[6]
*[1]Lithuanian Veterinary Academy, Kaunas, Lithuania; [2]The Norwegian School of Veterinary Science, Oslo, Norway; [3]Institute of Animal Science of Estonian Agriculture University, Tartu, Estonia; [4]Nordic Genebank for Farm Animals (NGH), Aas, Norway; [5]Latvian University of Agriculture, Jelgava, Latvia;*

[6]*Agrifood Research Finland MTT, Jokioinen, Finland*

The aim of the N-EURO-CAD project is to analyse genetic diversity within and between North European and Baltic cattle breeds and to promote conservation of cattle genetic resources (http://www.neurocad.lva.lt/). At present 16 microsatellites have been typed in 31 cattle breeds. Expected heterozygosities, pairwise $D_A$ genetic distances and $G_{ST}$ were calculated and correspondence analysis was performed. The heterozygosities varied from 0.57 to 0.71. About 11% of the total genetic variability was due to differences between breeds, indicating a moderate subdivision. A neighbour-joining tree was constructed by using the $D_A$ genetic distances. The branching pattern of the tree and correspondence analysis suggested a grouping of the cattle breeds into four main groups.

**D082**

**Development of microsatellite markers from a heart cDNA library in Japanese quail**

HIDEYUKI MANNEN[1], KAYO MURATA[1], MAKOTO MIZUTANI[2], SOICHI TSUJI[1]
[1]*Kobe University, Faculty of Agriculture, Kobe, Japan;* [2]*Nippon Institute of Biological Science, Kobuchizawa, Japan*

Japanese quail (*Coturnix japonica*) has been used not only for meat and egg producer but also as a model animal for poultry because of its small size, short generation intervals and high egg production. However, linkage map of Japanese quail has not been developed yet. Nevertheless, the expressed sequence markers are becoming important for comparative mapping studies. In this study we isolated microsatellites from a Japanese quail heart cDNA library using a (CA/GT)n repeat as probe. After screening from 200,000 cDNA clones, 37 of the clones gave strongly positive signals and subsequently were isolated, purified and sequenced. From the 37 positive clones 26 clones showed a high percentage of sequence homology with sequences from other species present in the International DNA database. Of the 26 clones 18 occurred only once. Seventy-three percent of the clones were complete (CA/GT) repeats, and 27% were incomplete repeats. The number of (CA/GT)n repeats in these clones varied from 11 to 31. The average number of repeat units was 17.6. These markers will be tested for polymorphism to Japanese quail reference families, which we are constructing. These expressed functional genes including microsatellite would be useful markers for studying comparative mapping when the linkage map of Japanese quail is constructed in the near future.

**D083**

**Age of myostatin alleles causing double muscling in some cattle breeds**

CINZIA MARCHITELLI, MARIA CARMELA SAVARESE, ALESSANDRA CRISÀ, ALESSIO VALENTINI
*Dipartimento di Produzioni animali, Università della Tuscia, Viterbo, Italy*

Myostatin (or GDF8), a negative regulator of muscle cell growth, is highly conserved across species. The loss of functional myostatin causes the "double muscled" phenotype in several cattle breeds. Targeted disruption of the myostatin gene in mice and mutations in the third exon in Belgian Blue, Piedmontese and Marchigiana cattle breeds, results in skeletal muscle hyperplasia. For nearly 200 years, double-muscled animals have captured the attention of livestock breeders and researchers The aim of this work has been to estimate the onset of mutations in myostatin gene using linked genetic markers. The estimates, based on intra-allelic variation, follow from the exponential decay of linkage disequilibrium due to recombination and mutation.
To maximize the information about allele age of myostatin gene, here we choosed five linked microsatellites that map on chromosome 2 , at varying distance from GDF-8 locus. We analysed both "double muscled" and normal cattle breeds.
In Marchigiana breed the mutation in myostatin gene appeared recently, while in Belgian Blue and in Piedmontese thousands of generations seem to have elapsed from its appearance.

**D085**

**PrP genotyping in autochthonous Spanish sheep: Genetic characterisation of healthy and scrapie flocks**

CRISTINA ACÍN[1], INMACULADA MARTÍN-BURRIEL[1,2], CLEMEN RODELLAR[2], JUAN J. BADIOLA[1], PILAR ZARAGOZA[2]
[1]*University of Zaragoza, National Reference*

*Centre for TSEs, Faculty of Veterinary, Zaragoza, Spain; [2]University of Zaragoza, Bichemical Genetics and Blood Groups Laboratory, Faculty of Veterinary, Zaragoza, Spain*
Scrapie is a neurodegenerative disease that affects small ruminants like sheep and goat. It is a Transmissible Spongiform Encephalopathy (TSE). The sheep PrP gene encodes a protein of 256 amino acids. At least 10 different mutually exclusive polymorphism are present in PrP and an association between susceptibility/resistant to natural scrapie and the polymorphism at amino acid codons 136, 154 and 171 has been reported. Valine (V) at codon 136 and Glutamine (Q) at codon 171 are associated with susceptibility and Alanine (A) at codon 136, Histidine (H) at codon 154 and Arginine (R) at codon 171 are associated with resistance. This association has been proved in certain breeds, we report the study of these polymorphisms in autochthonous Spanish sheep breeds from the region of Aragón (Rasa Aragonesa, Ojinegra, Roya Bilbilitana and Ansotana). Fifty animals, belonging to the regional genotyping plan, from each breed were genotyped by PCR/RFLPs. Allelic and genotypic frequencies were calculated for each breed. Moreover, a natural scrapie flock (250 sheep and 3 goats) of Rasa Aragonesa has been genotyped. Three scrapie postitive animals were diagnosed in this flock showing all of them the ARQ/ARQ genotype. Allelic and genotypic frequencies of this scrapie flock were compared with the results obtained in the Rasa Aragonesa population from the Regional Genotyping Plan, in order to stablish possible differences between both groups.

**D086**
**The use of microsatellite markers to detect bottlenecks in a Chato Murciano Pig population**

JOSE L. VEGA-PLA[1], AMPARO M. MARTÍNEZ[2], ANGEL POTO[3], BEGOÑA PEINADO[3], JUAN V. DELGADO[2]
*[1]Laboratorio de Grupos Sanguíneos, Servicio de Cría Caballar, Córdoba, España; [2]Unidad de Veterinaria del Departamento de Genética, Universidad de Córdoba, España; [3]Centro de Investigaciones Agroalimentarias, Consejería de Agricultura, Murcia, España*
Genetic variability at 26 microsatellite loci was analysed in two populations of Chato Murciano pig. These populations were part of a selection programme to recover an old and rare pig variety located near the extinction. When the effective number of reproducers in a population is small, the allele frequencies will be different in males and females, which causes an excess of heterozygotes in the progeny with respect to Hardy-Weinberg equilibrium expectations. It is important to detect population bottlenecks in threatened and managed species because bottlenecks can increase the risk of population extinction. Early detection is critical and can be facilitated by statistically powerful monitoring programs. We evaluate the accuracy and precision of the heterozygote-excess method using two data sets from a Chato Murciano pig. One of them is a original population and the other is a F3+F4+F5 generations of a line created from the mating of one Chato Murciano stallion with one LargeWhite female, followed of an absorption programe based in backcrosses. Analysis of highly polymorphic loci detects the experimental bottleneck and is a very good reference to estimate the magnitude of the bottleneck severity in the original population.

**D088**
**The AMPK gene family in cattle: Mapping and SNP detection**

STEPHANIE D. McKAY, JAMES E. WOMACK
*Dept of Veterinary Pathobiology, Texas A&M University, College Station, Texas*
The 5`-AMP-activated protein kinase (AMPK) family is an ancient stress response system whose primary function is regulation of cellular ATP. Activation of AMPK, which is instigated by environmental and nutritional stresses, initiates energy conserving measures that protect the cell by inhibition and phosphorylation of key enzymes in energy consuming biochemical pathways. Initially the seven genes that compose the bovine AMPK family were mapped in cattle using a radiation hybrid panel. Seven genes mapped to six different chromosomes in cattle, each with a LOD score greater than 10.0. PRKAA1 mapped to BTA20, PRKAA2 and PRKAB2 to BTA3, PRKAB1 to BTA 17, PRKAG1 to BTA5, PRKAG2 to BTA 4 and PRKAG3 to BTA 2. Five of the seven genes mapped to regions expected from human/cattle comparative maps. PRKAB2 and PRKAG3, however, have not been mapped in humans. We predict these

genes to be located on HSA 1 and 2, respectively. Additionally, one synonymous and one non-synonymous single nucleotide polymorphism (SNP) have been detected in PRKAG3. Various herds of mixed breed cattle have been tested for these SNPs by use of high resolution electrophoresis of allele specific restriction enzyme digests. Due to the physiological importance of this gene family, we believe that its individual genes are candidate genes for conferring differential disease resistance in cattle.

**D089**

**Evolution of the αs1-casein polymorphism in Orobica goats undergoing the standardisation process**

DANIELA MEGGIOLARO, PAOLA CREPALDI, MARTA MARILLI, LUISA VERDOGLIA, LUCIA FRANCESCHI, MARIO CICOGNA

*Istituto di Zootecnia Generale, Facoltà di Agraria, Università degli Studi di Milano, Italy*

The *Orobica* is a goat breed raised on the Lombardy Alps, protected by the regional measures for the conservation of autochthonous breeds. The standard of this breed was approved in 1992 and the flock book started in 1995. This population now consists of about 4200 subjects, including 2000 animals from 190 farms registered in the flock book. In order to evaluate if with the breed standardisation the polymorphism at the *CSN1S1* locus remained unchanged, in the year 1999-2000 milk and blood samples were taken on 193 registered goats from 31 farms. The polymorphism of αs1-casein was analysed, at protein level, by IEF, for the detection of the alleles A, B and C and, at genomic level, by three PCR protocols for the detection of *CSN1S1* F, E and $0_1$. To assess the evolution of the polymorphism at this locus, the results were compared with those of our previous study based on the typing of *CSN1S1* variants by IEF on 198 Orobica's milk samples collected in 1993-1994. The present investigation shows that, in about six years, a relevant reduction of the studied polymorphism occurred, as the number of observed genotypes decreased from 11 to 6. Concerning the gene frequencies, a decrease was observed for all the alleles except for F that, on the contrary, increased significantly ($P< 0.001$). The frequency of the null allele $0_1$ did not show significant variation. It thus appears that the breed standardisation, mainly based on visible traits, was accompanied by αs1-casein polymorphism reduction.

**D090**

**Variation of genetic markers in the Göttingen Minipig after 40 years of selection on low body weight.**

JAN- N. MEYER[1] , HORST R. BRANDT[2] , ROSEMARIE CLEMENS[1] , CLAUDIA KALTWASSER[1] , PETER LUDEWIG[1] , PETER GLODEK[1], HENNER SIMIANER[1]

[1]*University of Göttingen, Institute of Animal Breeding and Genetics, Albrecht-Thaer-Weg 3, 37075 Göttingen, Germany;* [2]*University of Giessen, Institute of Animal Breeding and Genetics, Ludwigstr. 21b, 35390 Giessen, Germany*

The breeding of the Göttingen Minipig has started in 1960 by Fritz Haring and Ruth Gruhn with the Minnesota miniature pig and the Vietnamese pot-bellied pig.

The first identification was done 1967 by Schahmirzadi with 41 defined blood group factors and 6 not determined antisera, which were tested in more than 500 minipigs.

In 1969 Peter Glodek established an SPF population in the new breeding center on the experimental station Relliehausen. The white and the spotted line was tested in 1971 with 9 blood group systems and 35 factors. Additional 7 biochemical systems were included. These preliminary results were compared with analysis of blood groups and biochemical systems of 1980, 1990 and 2002. In the 1980 and 1990 analysis 6 new biochemical systems were added.

In addition to the blood group and biochemical systems, two sets of 15 microsatellites were tested for the remaining white line in 2002, to get first results of molecular genetic markers in the Göttingen minipig population.

**D091**

**Genetic variability of 5 honeybee populations from the Basque Country (Western Pyrenees) inferred by mitochondrial and microsatellite markers**

IRATI MIGUEL[1], LIONEL GARNERY[2], MIKEL IRIONDO[1], ANDONE ESTONBA[1]

[1]*University of the Basque Country, Dpt. of Animal Biology and Genetics, Bilbao, Spain;* [2]*Laboratoire populations, génétique et evolution, Centre national de recherche scientifique*

*(CNRS), Gif-sur-Yvette, France*

A total of 639 colonies from 5 populations of the local honeybee (*Apis mellifera*) from the Basque Country (Western Pyrenees), including 2 localities where conservatories are to be established have been analysed using mitochondrial (DraI RFLP of COI-COII region) and nuclear (10 microsatellite loci) markers. Our aim is to study the genetic variability of these populations, to detect the introgression level from north Mediterranean lineage, and to analyze the phylogenetic relationship of our honeybees with the Iberian (*A. m. iberica*) and French populations (*A. m. mellifera)*. The introgression detected is quite low (0-4%). Nuclear and mitochondrial results are well correlated in the most of populations, except in one population where the level of introgression in mtDNA is zero while the nuclear level up to 2'1%. In this population the practice of transhumance is habitual, so genetic hybridation could be due to foreign drones' genetic contribution. The genetic diversity detected is higher than described in French populations, but similar to Iberian populations. The distribution of mitochondrial haplotypes is similar in the 5 populations with high percentage of M haplotypes (80-100%), confirming that this populations are at the extreme of the distribution area of haplotype A. The haplotypes M31 and M32 are described for the first time. Their sequences indicate that they are closely phylogenetically related to haplotype M7 and their low frequency suggest that they are rare. The pairwise $F_{ST}$ values point to a gradient of differentiation correlated with geographic distance, indicating that these populations are a product of "natural" evolution with low influence of modern apicultural management as the importation of foreign queens by bee-keepers. Phylogenetic analyses indicate that Basque Country's populations are intermediate between Iberian (*A.m.i*) and French (*A.m.m*) populations, in agreement with their geographical location and are in concordance with the evolutionary history of the West European honeybee. These results show that these populations are good candidates to preserve local population of honeybee due to their low introgression level and the relatively high genetic diversity detected.

**D092**

**African derived mitochondria in South American native cattle breeds (*Bos taurus*). Evidence of a new taurine mitochondrial lineage**

M. M. MIRETTI[1,2], H. A. PEREIRA JR.[1], M. A. POLI[3], E. P. B. CONTEL[2], J. A. FERRO[2]
*[1]Depto. de Tecnologia, FCAV, UNESP, Jaboticabal, SP, Brazil; [2]Depto. de Genética, FMRP, USP, Ribeirão Preto, SP, Brazil; [3]Instituto de Genética, CNIA-INTA, Castelar, Argentina*

We report the nucleotide diversity within the control region of 42 mtDNA sequences from an Argentinean and four Brazilian (AB) native cattle breeds. The analysis in conjunction with African (A), European (E), Near Eastern (NE), Japanese *B.taurus* and Indian *B.indicus* (n=550), allowed the recognition of 8 new haplotypes and their relative positions in a phylogenetic network. The structure of genetic variation among different hypothetical groupings was tested through the molecular variance decomposition and pairwise Fst distance, which were best explained by haplotype-groups components. We then classified *B.taurus* haplotypes into NE, A and E taurus-derived haplotypes (haplogroups) based on the sequence state at five positions (16050-16057-16113-16189-16255). As AB, as well as Portuguese cattle, is in fact an admixture of highly divergent haplotypes, more confident information could be obtained from a gene-tree instead of a population-based analysis. Two haplotypes within the A cluster were more divergent from the A consensus than the latter from the E consensus. A NJ tree confirms haplogrouping and shows the position of two haplotypes relative to the E/A lineage splitting, which divergence might have occurred subsequent to that between AA and A. This putatively ancestral mitochondrial lineage (AA) is supported by the calibration of sequence divergence based on the Bos-Bison separation. These data could reflect the haplotype distribution of the Iberian cattle five centuries ago.

**D093**

**Characterization of a novel *Ssp*I-family repetitive sequence on the chicken W chromosome**

YUICHIRO ITOH[1], SHIGEKI MIZUNO[2]
*[1]Gene Research Center, Tohoku University, Sendai, Japan; [2]Department of Agricultural and Biological Chemistry, College of Bioresource Sciences, Nihon University, Fujisawa*

*252-8510, Japan*

A clone λWS44 containing a 223-bp insert from the chicken W chromosome-specific genomic library was noticed because it showed repetitive sequence-like and female-specific signals in Southern blot hybridization, yet was different from *Xho*I- and *Eco*RI-family sequences. A major repeating unit was a 0.5-kb sequence produced by digestion with *Ssp*I. A genomic clone pWPRS09 containing a 508-bp *Ssp*I unit was subsequently obtained. The 0.5-kb units showed sequence diversity, as demonstrated by occasional absence of a single *Ear*I site in the unit. Slot blot hybridization demonstrated that the 0.5-kb *Ssp*I unit repeated about 11,300 times on the chicken W chromosome. The 0.5-kb unit contains a number of tandemly repeated GGAGT and GGAGA elements but does not consist of regular internal repeats and is a non-curved DNA. The *Ssp*I-family is unique to the genus *Gallus* (chickens and jungle fowls). FISH to the W lampbrush chromosome demonstrated that the *Ssp*I family was located to the chromomere-6 near the terminal non-heterochromatic region on the short arm, and thus served as a good positional marker for functional genes. FISH to interphase nuclei demonstrated that the *Ssp*I-family sequence was much less heterochromatic comparing to *Xho*I- and *Eco*RI- family sequences. The presence of *Ssp*I-family (5.8 Mb) in addition to *Xho*I- (21 Mb) and *Eco*RI-(11Mb) families narrows down non-repetitive DNA regions to at most 16 Mb on the W chromosome (about 54Mb).

**D094**

**An AFLP genetic map of Atlantic salmon**

THOMAS MOEN[1], KJERSTI T. FJALESTAD[1], HEGE MUNCK[1], BJÖRN HOYHEIM[2], LUIS GOMEZ-RAYA[3]

*[1]AKVAFORSK, Aas, Norway; [2]Norwegian School of Veterinary Science, Oslo, Norway; [3]Àrea de Producció Animal, Centre UdL-IRTA, Spain*

A genetic map of Atlantic salmon has not yet been published, in spite of the economic importance of the species. 33 microsatellites, distributed on 22 linkage groups, were used as a framework to construct an AFLP/microsatellite map. Two full-sib families sired by the same male were genotyped with respect to these microsatellites and 64 AFLP primer combinations. The number of animals genotyped per full-sib family varied from 20 to 100. 350 polymorphic AFLP markers were found, and in 90 % of these, the segregation of AFLP markers could be followed from offspring to parents in at least one of the full-sib families (i.e. one parent was heterozygous, and the other homozygous for the null allele). Linkage analysis was performed using CRIMAP, disregarding the cases for which both parents of a family were heterozygous for the marker. The proportion of the markers segregating in a non-Mendelian fashion was larger than what was expected by chance, which is consistent with previous studies showing that the salmonids have remains of tetraploid inheritance.

**D095**

**Allozyme variability in wild and domesticated common carp (*Cyprinus carpio* L.) populations from Germany, Uzbekistan and East Asia**

KLAUS KOHLMANN[1], ASIYA MURAKAEVA[1,2], PETRA KERSTEN[1]

*[1]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Inland Fisheries, Berlin, Germany; [2]Institute of Biochemistry, Tashkent, Uzbekistan*

Common carp is among the most important cultured fish species worldwide. The natural distribution of its wild ancestor is believed to range from Western Europe throughout Eurasia to China, Japan and South-East Asia. Representative wild and domesticated populations from these regions (five from Germany, six from Uzbekistan and three from East Asia) were selected to describe the genetic variability within and differentiation between them by examining eight enzymatic systems (= 22 loci). The observed level of polymorphism was high: 1.4 to 1.9 alleles per locus, 26.3 to 47.4% polymorphic loci and expected heterozygosities from 0.098 to 0.191. Highly significant differentiation based on $F_{ST}$ values from pairwise comparisons was found between Uzbek and German, Uzbek and East Asian, German and East Asian populations, and between all Uzbek wild and Uzbek domesticated and some of the German feral and domesticated carp. The dendrogram based on 13 populations showed a general grouping of populations according to their geographic origin: Europe, Central Asia and East Asia. High bootstrap support was found for groups consisting of German domes-

ticated carp (87.4%), German feral carp (89.5%), Uzbek wild carp (95.2%) and East Asian wild carp (86.2%). About 30.5% of total variation could be attributed to the variability among the three geographic regions, about 11% could be explained by variation among populations within regions and 58.5% represented the within population component of variation.

**D096**
**Length standardization of microsatellites used in analysis of biodiversity in cattle**

M. C. SAVARESE[1], L. PARISET[1], J. A. LENSTRA[2], I. J. NIJMAN[2], G. MOMMENS[3], P. WIENER[4], D. BURT[4], J. L. WILLIAMS[4], K. MOAZAMI-GOUDARZI[5], S. DUNNER[6], C. RODELLAR[7], G. ERHARDT[8], C. WEIMANN[8], M. ZANOTTI[9], F. PILLA[10], A. BRUZZONE[10], A. VALENTINI[1]
*[1]Department of Animal Production, Universitá della Tuscia, Viterbo, Italy; [2]Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; [3]PolyGenLab, Malle, Belgium; [4]Roslin Institute, Roslin, Midlothian, Scotland, UK; [5]INRA, Jouy-en-Josas, France; [6]Dpto. Produccion Animal, Facultad Veterinaria, Madrid, Spain; [7]Lab. Genetica Bioquimica Y Grupos Sanguineos, Facultad Veterinaria, Zaragoza, Spain; [8]Department of Animal Breeding and Genetics, Justus Liebig Universität, Giessen, Germany; [9]Istituto di Zootecnia, Universitá di Milano, Milan, Italy; [10]SAVA, Facoltà di Agraria, Campobasso, Italy*

Conservation of genetic variation is recognized as a crucial concern at international level to preserve a basis for selection. Microsatellite markers are valuable tools for assessing genetic diversity and phylogeny in many species. This work is part of a project aimed to facilitate the comparison of European breeds using a panel of 30 microsatellites originally selected through an EC funded programme and now adopted by the FAO. DNA from a set of reference individuals has been distributed among the participating laboratories. To standardise the allele calling, the reference individuals were typed independently by the laboratories and the local allele size of the microsatellites reported. Subsequently, three different reference animals were used for the sequence analysis. Homozygous individuals were chosen to minimize problems due to stutter bands. Microsatellites loci were PCR amplified, the products were then purified, cloned and sequenced in both strands using a LICOR 4200 sequencer to determine their exact length. So far, we have sequenced 25 loci and calculated the correspondence of the sequence with the mean allele length obtained by the collaborating laboratories. The full sequencing of microsatellite loci represents an absolute standard for the laboratories working on cattle. The sequence can also provide information on the structure and the type of repeats of the locus and definition of allelic variation not revealed by apparent mobility of PCR products.

**D097**
**Study of polymorphism of leptin receptor gene in Iberian and Landrace pigs**

CRISTINA ÓVILO[1], BÁRBARA MELÉNDEZ[2], JAVIER BENÍTEZ[2]
*[1]INIA, Dpto Mejora Genética Animal, Madrid, Spain; [2]CNIO, Dpto Genética Humana, Madrid, Spain*

The leptin receptor gene is candidate for fatness and body composition traits, as it is involved in food consumption and it maps on pig chromosome six, in a region where several QTL have been found for these traits. The aim of this work was to identify SNP on coding regions of this gene, which could be causal mutations of phenotypic variations. In first place genomic DNA was analyzed by sequencing of the largest exons (4, 6, 9, 15 and 20, 1604bp) in Iberian and Landrace samples. This resulted in the identification of three SNP, located on exons 4 (C/T), 9 (C/A) and 20 (G/A). Among them, the ones on exons 4 and 9 produce a change in the aminoacid composition of the protein (thr/met and ala/asp respectively). Due to the complex structure of this gene (20 exons, some of them very small), cDNA was also analyzed. RNA was obtained from muscle samples of Iberian and Landrace origin and cDNA was amplified and sequenced on five fragments covering exons 4 to 17 (2376bp). Seven new SNP were identified from cDNA. Four of them, located on exons 4, 6, 15 and 16, have the new allele at low frequency. The other three SNP, on exons 13, 14 and 16, are more informative, with different frequencies on Iberian and Landrace samples. Moreover, the one on exon 14 (C/T) produces a change in the aminoacid coded (leu/phe). Missense polymorphisms on exons 4, 9 and 14 are being genotyped by pyrosequencing on pig

populations with phenotypic records, for association studies.

**D098**
**Use of AFLP markers for the evaluation of genetic diversity of *Coregonus lavaretus* (L. 1758) after restocking in Bolsena Lake, Italy**

LORRAINE PARISET[1], MARIA CARMELA SAVARESE[1], PAOLA BONDANELLI[2], PAOLO COLOMBARI[2], ALESSIO VALENTINI[1]
[1]*Department of Animal Production, Università degli Studi della Tuscia, Viterbo, Italy;* [2]*Department of Environmental Sciences, Università degli Studi della Tuscia, Viterbo, Italy*
*Coregonus lavaretus* (L. 1758) has been introduced in Italian lakes since the second half of the XIX century and nowadays it represents one of the main fishery resources of these deep lakes of the country. Programs finalized to the evaluation of the genetic effects caused by restocking have not been proposed yet. The current reproduction techniques can bring negative effects on natural populations due to a drop in genetic variability that can result in fitness reduction. With the aid of molecular markers, the genetic diversity of the populations in the Bolsena Lake, Italy, has been studied in order to evaluate if reared *C. lavaretus* are suitable to be employed for restocking in the wild. The amplified fragment length polymorphism (AFLP) technique has been used to investigate the level of genetic diversity within and between wild (adults) and reared (from Marta and Bolsena incubators) populations. AFLP markers, generated by EcoRI/TaqI digestion and amplified with 8 primer combinations on 20 individuals per population, produced polymorphic bands that were analysed using a Licor 4200 sequencer. To evaluate genetic variability, the percentage of homozygosity and the Jaccard index have been calculated and PCA analysis has been performed. Results are useful for a management of restocking finalized to conservation, by maintaining the genetic diversity of the populations.

**D099**
**Genetic diversity in pointing Dogs**

D. PARRA, S. DUNNER, J. CAÑON
*Dpto. Producción Animal, Facultad de Veterinaria, 28040 Madrid, Spain*
Dogs show the most diverse phenotypes among the mammalian species and in the special case of the pointing dogs, most breeds are morphologically quite different, although their behaviour is alike. This study focuses on the determination of the genetic relationship and genetic diversity between 5 pointing dogs breeds: German Short-haired Pointing (GSP, n=30), Deutsch Drahthaar (n=8), Epagneul Breton (n=15), English Pointer (n=48) and English Setter (n=63) by typing 21 microsatellites, and sequencing 652 bp from the mitochondrial D-loop. Highest levels of genetic variability at the mitochondrial level are shown by Drahthaar with 7.14 variable sites over a total of 30 contained in 17 haplotypes. In the other hand, GSP only shows 1.77 variable sites and also the lowest heterozygosity (56.7%) towards Pointer (67.6%). For the genome loci, Drahthaar had the lower number of effective alleles (3), and Pointer the highest (3.8). Despite their common origin, distance between some of them, e.g. Drahthaar and Breton is specially high either at the genome ($F_{ST}$=0.15) nor at the mitochondrial level where sequence diversity estimated by Kimura-2 distance was 0.014. The overall Fst was 0.11, which shows a high variability level compared to other domestic species (e.g.0.08 in horses, 0.064-0.082 in bovine breeds) but lower than 0.23 shown in studies for other canine breeds.

**D100**
**Analysis of the variability at mtDNA in three indigenous Spanish sheep**

SUSANA PEDROSA, JUAN J. ARRANZ, YOLANDA BAYÓN, FERMÍN SAN PRIMITIVO
*Departamento de Producción Animal, Universidad de León, León, Spain*
Genetic variation at the mitochondrial DNA level was investigated in three Spanish sheep breeds: Churra, Castellana and Alcarreña. The study was performed on 1209 pb of the mtDNA control region, which is known to be more variable than other sequences. On the basis of published information about this region in sheep, different primers were designed which allowed for an analysis of the whole selected sequence, divided into three overlapping segments. Total DNA was extracted from blood samples and each of the three segments amplified through PCR and subsequently cy-

cle-sequenced using an ABIPRISM 377 DNA sequencer. The alignment of the resulting sequences was performed to obtain the consensus sequence. Comparison of individual sequences revealed a total of 219 single nucleotide substitutions, among which transitions predominated over transversions, in accordance with the general patterns known in mammalian mitochondrial evolution. Moreover, two of the positions revealed insertion/deletion mutations. Phylogenetic analysis of mtDNA haplotypes was performed in order to derive genetic relationships within and between groups of animals.

**D101**
**Characterisation of bovine dopamine receptors 1, 4 and 5**

ANDY HAEGEMAN, ALEX VAN ZEVEREN, LUC PEELMAN
*Department of Animal Nutrition, Genetics, Breeding and Ethology, Ghent University, 9820 Merelbeke, Belgium*
The balance of calorie uptake and expenditure is of vitable importance for each living organism. Chronic excessive calorific uptake or fasting will lead to tipping of that balance and result in starvation or obesity with the resulting severe health risks. Neurotransmitters and their receptors play a major role in determining and modelling feeding behaviour, feeding rewards and satiating effects. A prime candidate is the dopaminergic system. The prominent role of dopamine receptor (DRD) 2 in the several aspects of feeding behaviour has been extensively documented in human and rat models. Although a lot is known about DRD2, much less information about the potential role of DRD1, DRD4 and DRD5 is available. We report here for bovine, the isolation and sequencing of *DRD1*, the localisation of *DRD1* and *DRD4* by RH mapping, mutation screening and identification (using SSCP and/or sequence comparison) in *DRD1, DRD4* and *DRD5*.

**D102**
**Evaluation of SNPs in the Pig genome**

ANETTE H. PETERSEN, LONE B. MADSEN, FRANK PANITZ, HENRIK STENGAARD, CHRISTIAN BENDIXEN
*Danish Institute of Agricultural Sciences, Research Centre Foulum, Dept. of Animal Breeding and Genetics, Tjele, Denmark*
Genomic maps are becoming more and more detailed as new technologies for genetic research are developed. The numbers of known genes and genetic markers are increasing at a tremendous speed creating high density chromosome maps. A speciel attention is paid to single nucleotide polymorphisms (SNPs) which are predicted to replace traditional markers (microsatellites etc.) in most future genetic assays. A lot of time and effort is invested in detection and localisation of SNPs and the resulting high-density chromsome maps will ease detection of genes and QTLs.
In this project we detect and evaluate SNPs in the Pig genome. The SNPs are detected in overlapping EST-sequences which are processed through a pipeline including Phred, Phrap, PolyBayes, and Consed (see also abstract by Frank Panitz et al.). Consensus sequences of clusters containing SNPs are BLASTed against the human RefSeq to get an idea of the identity of the gene, the chromosomal location, the exon-intron boundaries and if the SNPs are synonomous or non-synonomous.

**D103**
**Quantitative analysis of *KIT* copy numbers in domestic pigs (SUS SCROFA) using Pyrosequencing technology**

GERLI PIELBERG[1], ANDERS ALDERBORN[2], MONICA PETTERSSON[2], LEIF ANDERSSON[1]
*[1]Swedish University of Agricultural Sciences (SLU), Department of Animal Breeding and Genetics, Uppsala, Sweden; [2]Pyrosequencing AB, Uppsala, Sweden*
Mutations in the *KIT* gene, encoding the mast/stem cell growth factor receptor (MGF), are responsible for coat color variation in domestic pigs. The dominant white phenotype is caused by at least two mutations, a gene duplication and a splice mutation in one of the copies leading to skipping of exon 17. We have recently shown an unexpectedly high allelic diversity at the *KIT* locus in the domestic pig (Pielberg *et al*. 2002). By applying minisequencing and pyrosequencing for quantitative analysis of the number of copies with the splice form, we found evidence for at least two new *KIT* alleles in pigs, both with a triplication of the gene. With such a high allelic diversity at the *KIT* locus, the determination of genotypes at the *KIT* locus in domestic pigs is com-

plicated. Here we present a new approach for the analysis of *KIT* copy numbers. The recently identified unique duplication breakpoint (Giuffra *et al*. submitted) is amplified by PCR and gene copy number estimated using Pyrosequencing technology. Our new method confirms the existence of *KIT* haplotypes with triplication. The results imply that *KIT* alleles with the duplication are genetically unstable and new alleles are most likely generated by unequal crossing-over. This study provides an excellent method for genotyping the complicated *Dominant white/KIT* locus in pigs and a general approach for determining gene copy numbers.

**D104**

**A microdissected whole canine X chromosome paint applied for 3 related canid species**

ALDONA PIEŃKOWSKA[1], MARIOLA ZAWADA[2], GERALD STRANZINGER[3], CLAUDE SCHELLING[3]

[1]*Agricultural University, Department of Animal Genetics and Breeding, Poznań, Poland;* [2]*Institute for Human Genetics, Polish Academy of Science, Poznań, Poland;* [3]*Federal Institute of Technology and Faculty of Veterinary Medicine, Department of Animal Sciences, Zurich, Switzerland*

A whole chromosome painting probe was developed after microdissection of canine X chromosomes. This canine X chromosome paint was applied by FISH to the chromosomes of three other canid species: red fox (*Vulpes vulpes*), arctic fox (*Alopex lagopus*) and Chinese raccoon dog (*Nyctereutes procyonides p.*). In the raccoon dog and the red fox the FISH-staining was restricted to the entire X chromosome. In the arctic fox, beside the staining of the entire X chromosome, chromosome arms carrying constitutive heterochromatin were also stained.

This work has been supported by the Polish State committee for Scientific Research (6PO 6D 028 21).

**D105**

**Genetic variation and phylogenetic relationships in some dog breeds, Italian *wolf (Canis lupus italicus)* and in red fox *(Vulpes vulpes)* based on microsatellite DNA polymorphisms**

MICHELE POLLI[1], PAOLA MAGNETTI[2], MARIA LONGERI[1], STEFANO MARELLI[1], MARTA ZANOTTI[1], LUIGI G. CAVALCHINI[1]

[1]*University of Milan, Istituto di Zootecnica, Faculty of Veterinary Medicine, Milan, Italy;* [2]*University of Milan, Departement of Biology, Faculty of Science, Milan, Italy*

Genetic variation at 10 canine microsatellite loci (AHT121, CXX20-123-263-403, FH2159-2137-2138-2001-2132) was estimated to determine the genetic diversity and phylogenetic relationship in 15 different dog breeds (Bergamasco, Bolognese, Bracco Italiano, Cirneco dell'Etna, Corso, Fonnese Italiano, Volpino Italiano, Akita-inu, Czech Wolfdog, German shepherds, Dobermann), in Italian wolf *(Canis lupus italicus)* and in red fox *(Vulpes vulpes).* All the samples have been collected from animals unrelated in two generations and were analysed by automatic fluorescent methods. Microsatellite allele frequencies were examined and evaluated by GENEPOP statistic package. Our data showed heterozygosities ranging from 0,47 (Dobermann) to 0,80 (Maremma sheepdog) and PIC (polymorphism information content) from 0,36 (Dobermann) to 0,92 (Volpino Italiano). Mean number of alleles per breed ranged from 3 in Dobermann to 8 in Maremma sheepdog. Genetic distances between populations were measured according to Nei's standard genetic distances by DISPAN statistic package. Both UPGMA dendrogram, based on Nei's genetic distances and Principal Component Analysis showed all dog breeds and Italian wolf closely related, and clearly distant from wild fox. Data confirm the existence of a lower degree of genetic differentiation among the breeds of dog considered with the Italian wolf and high genetic distance with other canids.

**D106**

**Comparison among three methods to study the double-muscling locus (*mh*) in Piedmontese cattle**

A. POZZI, G. BONGIONI, A. GALLI

*Istituto Sperimentale Italiano "Lazzaro Spallanzani", Milano, Italy*

The Piedmontese bovine is one of the most important and finest Italian beef breed. Its interest is determined by the exceptional development of muscle mass, known as "double-muscled", given by a point mutation (G938A)

of the myostatin gene (*GDF8*). The aim of this work was to develop a fast test to genotype a large number of samples comparing one classical and two innovatives method for the identification of SNPs. The first one was represented by a simple PCR-based allele detection system with fluorescent genotyping technology on 377 DNA Sequencer (ABI Prism-Applied Biosystems), which required several manipulations and was time consuming. The second one used a SNaPshot methodology by single nucleotide primer extension executed in different steps. For the last one an allelic discrimination in PCR Real-Time was employed in only one step with a MGB TaqMan, a specific short probe to identify a single base mismatch. Piedmontese animals (324 semen or hair samples) were analized using the three methods: 315 homozigotes and 9 heterozigotes for the mutation were found. The PCR Real-Time shown the following advantages: easy execution, less time for manipulations and analysis, therefore represents the faster test to apply to a large number of samples.

**D107**

**Association between new variants in the ovine PrP gene and Scrapie susceptibility**

SIEGFRIED PREUSS, ANDREAS W. KUSS, HONGZHE HE, HEINZ BARTENSCHLAGER, HERMANN GELDERMANN

*University of Hohenheim, Institute of Animal Husbandry and Breeding, Department of Animal Breeding and Biotechnology, Stuttgart, Germany*

Scrapie is a fatal neurodegenerative disease of sheep which belongs to the group of transmissible spongiform encephalopathies (TSE). The host encoded prion protein (PrP) plays a central role in the disease process. Genetic susceptibility to scrapie and pathogenesis are associated with polymorphisms in three different codons (136, 154 and 171) of the ovine PrP gene. Up to 15 different genotypes can be observed in several breeds. These genotypes have been grouped in 5 levels of scrapie risk (Dawson et al. 1998). Efforts have been made to improve resistance against Scrapie by breeding for resistant genotypes. However the determination of these ORF-variants is time consuming and expensive. In a separate investigation several polymorphic sites outside the ovine PrP-ORF were identified which can be determined more easily and cost-saving. In this study we tested 3 of these loci for their association with the 3 ORF-variants. For 573 German breeding sheep from important breeds (Dorper, Gotland, Isle de France, German merino, East Frisien milk, German black headed mutton, Suffolk, Texel) these 3 new loci were genotyped in addition to the 3 ORF-positions. Only breeds of which at least 60 samples had been obtained were included in analysis of variance. In German merino the association of the new markers with risk levels deduced from ORF genotypes was only weak ($r^2$ = 7,6 %). In contrast more than 75 % of the risk variance in East Frisien milk, Suffolk and Texel could be explained by the 3 new loci. Independent from breed the ORF-genotype ARR/ARR, the only genotype in the lowest risk group, was always (n = 76) associated with a specific threefold homozygeous combination of alleles at the new sites. Thus the new variants might be used for predicting scrapie susceptibility at least in some breeds. The precision of risk assessment could be further increased by taking additional informative markers into account. A similar approach could be used in cattle, for which no linkage of genetic markers inside or outside the ORF of the PrP gene with BSE incidence is known so far.

Ref.: Dawson, M., Hoinville, L.J., Hosie, B.D., Hunter, N. (1998): Vet. Rec. 142: 623-625

**D108**

**Variants of *CSN3* in Chinese yak (*Bos grunniens*)**

EVA-MARIA PRINZENBERG[1], HAN JIANLIN[2], GEORG ERHARDT[1]

[1]*Justus-Liebig-University of Giessen, Department of Animal Breeding and Genetics, Giessen, Germany;* [2]*Gansu Agricultural University, International Yak Information Centre, Lanzhou 730070, China*

Variants of κ-casein (*CSN3*) have been extensively studied in cattle and up to 9 alleles have been simultaneously shown by SSCP analysis in *Bos taurus* and *Bos indicus* breeds so far. Evolution of these alleles and a possible common ancestor still remain unclear in some cases. PCR-SSCP analysis of domesticated yak *CSN3* exon IV revealed a two allele polymorphism showing intermediate migration patterns compared to the cattle *CSN3*A* and *B* alleles. PCR products of both yak *CSN3* alleles were cloned and sequenced. All yak had nucleotide sequences corresponding to Thr in amino acid

position 136 (identical to *CSN3*A*) and Ala in position 148 (identical to *CSN3*B*). This is in accordance with a sequence reported from *Bison bonasus* (*CSN3*G*$_{Bison}$) and may represent the common ancestor of *CSN3*A* and *B* variants of cattle. Moreover, a 12 bp insertion resulting in a duplicated nucleotide and amino acid motive was found in one yak allele compared to the other. Position of the insertion could not be unequivocally assigned. The duplication is either corresponding to the codons for amino acids 147 to 150 or 148 to 151 which are repeated identically. In 18 yak typed by SSCP analysis, the long variant was found with frequencies about 70% and the short variant about 30%, implicating the longer variant being the predominant and probably the older allele in yak. The loss of the insertion may have led to the ancestral *CSN3* allele from which all today known variants in *Bos indicus* and *Bos taurus* evolved.

**D109**

**Sequence analysis of *cytb* gene in some species conserved in Poland (*Bison Bonasus, Lynx Lynx, Canis Lupus*)**

BEATA PRUSAK[1], J. REKLEWSKI[2], A. KRZYWIŃSKI[3]

[1]*Institute of Genetics and Animal Breeding, Jastrzebiec, Poland;* [2]*Kampinos National Park, Poland;* [3]*Wild Animals Park, Kadzidłowo, Poland*

The preservation of genetic diversity both within and among natural populations is a fundamental goal of conservation biology. Various molecular markers and PCR techniques have been used in a wide variety of studies in an effort to attain this diversity. Cytochrome b gene is encoded by mtDNA and contains species – specific information. Because of no recombination between different mtDNAs and maternal inheritance, mtDNA can shed light on the evolutionary history of species. Analysis of *cytb* gene sequence can be used to determine taxonomic identity, phylogenetic relationships and genetic distance between species under investigations as well as to monitor number and migrations of threatened species. The aim of this study was to analyse the *cytb* gene sequence in some wild species (*Bison bonasus, Lynx lynx, Canis lupus*) being under conservation in Poland. The next step was to compare derived sequences with adequate sequences existing in GenBank. Applied in present studies organic method for DNA extraction from biological stains (single hair) allows for non – invasive analysis of anonymous genomes, where limited DNA is available. Purified PCR products (313 base pair length) were sequenced with ABI Prism Dye Primer Cycle Sequencing Ready Reaction Kit (PE Biosystems) according to the user's manual. The sequencing products were separated in a DNA sequencer ABI PRISM 377 (PE Biosystems). The electrophoretic data were analysed by the Sequence Navigator v. 1.0.1 software (PE Biosystems). Performed studies revealed that derived sequences of *cytb* gene can be considered as representative for investigated species and can be used for diagnostic species identification from anonymous biological stain.

**D110**

**Fine mapping of the intestinal receptor locus for enterotoxigenic *Escherichia coli* F4ab and F4ac on chromosome 13 in pigs**

PASCAL PYTHON[1], HANNES JÖRG[1], STEFAN NEUENSCHWANDER[1], CHRISTIAN HAGGER[1], CHRISTIAN STRICKER[2], ESTER BÜRGI[3], HANS ULRICH BERTSCHINGER[1], GERALD STRANZINGER[1], PETER VÖGELI[1]

[1]*Swiss Federal Institute of Technology, Institute of Animal Sciences, Zurich, Switzerland;* [2]*Applied Genetics Network, Altendorf, Switzerland;* [3]*University of Zurich, Department of Internal Veterinary Medicine, Switzerland*

Enterotoxigenic *E. coli* (ETEC) with fimbriae of the F4 (K88) family are frequently associated with diarrhoea in neonatal and weaned pigs. A linkage analysis was performed on a pedigree of 200 Swiss Large White pigs to refine the localization of receptor loci F4ab and F4ac. Small intestinal enterocyte preparations from 171 eight-weeks-old pigs were phenotyped by an *in vitro* adhesion test using two strains of *E. coli* representing the variants F4ab and F4ac. The serum transferrin (*TF*) gene and 10 microsatellites on chromosome 13 were linked with F4ac receptor locus (*F4acR*) (recombination rates (θ) between 0.00 and 0.11 and lod score values (Z) between 11.4 and 40.4). The multipoint analysis revealed *S0222-TF-Sw2459-S0068-F4acR-S0075-Sw1030-Sw520-Sw398* as the most likely gene order. The *F4acR* locus was located between *S0068* and *Sw1030*, with a recombination rate (θ) of 0.05 between *S0068* and *F4acR*, and 0.03 with

*Sw1030*. A weak and a strong adhesion receptor were observed for F4ab. The weak adhesion receptor for F4ab was detectable only in pigs lacking F4acR and the strong adhesion receptor for F4ab coincided with the presence of F4acR. No pigs were found expressing only F4acR and lacking F4abR. Therefore, we conclude that the receptor for F4ac binds F4ab bacteria as well, controlled by one gene localized between *S0068* and *Sw1030*. However, due to the limited number of informative animals, the inheritance of the weak adhesion receptor F4ab could not be shown.

**D111**
**Estimation of genetic variability in Mazandaran native fowls using RAPD markers**

GHODRAT RAHIMI[1], ALIREZA KHANAHMADI[1], ARDESHIR NEJATI-JAVAREMI[2], SAEID ESMAEILKHANIAN[2]
*[1]Univ. Mazandaran, Dept. Anim. Sci., Sari, Iran; [2]Anim. Sci. Res. Inst., Karaj, Iran*

Polymorphism at RAPD marker loci is characterized by the presence (+) or absence (-) of specific DNA sequences across the genome. If the individual carries the sequence on at least one of its homolog chromosomes it will show a band. Therefore, RAPD markers follow a complete dominant mode of inheritance and only two phenotypes are visible for each marker locus. Separation of the dominant phenotypes into the two constituting genotypes is only possible by further analysis of the pedigree. In this study blood samples were collected from 10 males and 90 females of Mazandaran Native Fowl Breeding Station and were treated with EDTA. Twenty random decamer primers were used in RAPD-PCR analysis. Any inept DNA pattern generated due to unsatisfactory amplification was excluded from the analysis and only reproducible bands in multiple runs were taken into consideration and scored as present or absent. Eighteen primers yielded satisfactory amplification products. Banding data was analyzed by the RAPD-MGA computer program developed by one of the authors. The program assumed HWE to estimate allelic frequencies. The number of bands displayed for each primer ranged from 6 to 29. Only those bands absent in at least one individual were considered as polymorphic and those present for all birds were taken as monomorphic. In total 12 monomorphic and 269 polymorphic bands were present for the 18 primers. Heterozygosity of polymorphic bands varied from 0.01 to 0.50.

**D112**
**Genetic diversity in Piedmontese cattle breed**

R. RASERO, P. SACCHI, S. MAIONE, S. SARTORE, E. CAUVIN
*University of Torino, Department of Animal Production, Grugliasco, Italy*

The Piedmontese cattle, originally a dual purpose breed, became the main Italian beef cattle owing to the presence of muscular hypertrophy. In spite of this in the last years its number decreased. Furthermore, the adoption of AI is quite widespread and a reduced number of BLUP selected bulls is commonly used. This study is concerned with the analysis of genetic variation to conserve future selection options. Thirty-two microsatellites mapping on 21 different chromosomes were chosen according to their technical properties and potential for detecting polymorphism. A preliminary investigation of 29 unrelated animals allowed to find 22 markers (15 in 2 multiplex PCR) having heterozygosity >0.6 by which 233 more individuals were typed. The HW proportions were tested using an exact test, overall heterozygosity excess or deficiency was estimated using an asymptotic test. Five loci showed disagreement with HW proportions but only one of them (*TGLA53*) showed significant deficiency of heterozygosity. The observed and effective average numbers of alleles/locus were 9.5±3.5 and 4.7±1.6 respectively, the average expected heterozygosity was 0.765±0.069. The probability of identity between randomly chosen pairs was 2.4E-11 and 1.8E-7 for the 2 multiplex PCR respectively. These informations will be used to study the genetic evolution of the breed under the selection pressure.

**D113**
**The allele $e^a$ - a rare mutation in the *MC1R* gene in horse (*EQUUS CABALLUS*)**

MONIKA REIßMANN[1], HEINZ-JÜRGEN WAGNER[2], LÁSZLÓ GULYÁZ[3], SOPHIE SCHUSTER[1]
*[1]Humboldt-University, Institute of Animal Science, Berlin, Germany; [2]Free University, Institute of Veterinary Biochemistry, Berlin,*

*Germany; [3]University of West-Hungary, Institute of Animal Breeding and Husbandry, Mosonmagyaróvár, Hungary*

The coat colour of horses is considerably determined by the *MC1R* gene. A single point mutation in this gene leads to the chestnut allele e. We found another point mutation in the *MC1R* gene that is spread in the Black Forest /1/. This mutation leads to the allele $e^a$ and it has no association to the melanin synthesis but it falsifies the differentiation between E and e using the widespread *Taq I* - RFLP test.

Here we report the detection of the allele $e^a$ in another horse breed. The tests were carried out in 457 individual horses belonging to 18 breeds, amongst them Ardennais, Noriker, Black Forest, Hungarian Coldblood, as well as in 18 Przewalski individuals. Confirming our former result the allele $e^a$ was found in the Black Forest and furthermore only in the Hungarian Coldblood. Obviously within the species horse the allele $e^a$ exists exceptionally rare, whereas within the both breeds it is spread frequently:

Allelic frequency in the Black Forest (n=75): E = 0.01; e = 0.80; $e^a$ = 0.19 and

allelic frequency in the Hungarian Coldblood (n=73): E = 0.07; e = 0.80; $e^a$ = 0.13.

Because there is no evidence of a direct relation between these two breeds it is remarkably that the $e^a$ allele has not been found in Ardennais and Noriker – two breeds which took part in the development of the named breeds. From an evolutionary point of view we suggest that the single base mutation $e^a$ in the *MC1R* gene occurred in a very late time. Its origin remains to be elucidated.

/1/ Wagner, H.-J.; Reissmann, M. (2000), Anim. Genet. 31, 289-290

**D114**

**Genetic characterisation of two Northern Spanish endangered cattle breeds (Betizu and Casta Navarra) using microsatellites: Population assignment of individuals and subpopulation relationships**

INMACULADA MARTÍN-BURRIEL, CLEMEN RODELLAR, EMILIO GARCÍA-MURO, ISAÍAS ZARAGOZA, PILAR ZARAGOZA

*University of Zaragoza, Biochemical Genetics and Blood Groups Laboratory, Faculty of Veterinary, Zaragoza, Spain*

The Casta Navarra (CN) is considered as one of the Spanish Fighting Bull (FB) founding castes. Isolated from the remaining castes during centuries, its ethnic characteristics are different from the others. On the other hand, the Betizu (BET) is considered as one of the most endangered cattle breed in Spain and it is one of the few feral breeds in Europe. Its origin is uncertain, one hypothesis places the BET breed as the precursor of CN but it seems more realistic to consider the BET as the low developed and distant relative of Pyrinean (PYR) breed. Fifty animals from CN and BET were selected for population characterisation using 30 microsatellites, showing both breeds a heterozygosity deficiency. The genetic relationships between these breeds and PYR and FB populations were also analysed. The genotype data from 21 microsatellites analysed in these four populations was used as a baseline for identifying the breed of 300 animals classified as CN and BET. CN cattle belonged to six different farms, as FB is traditionally bred on farms which impose reproductive isolation, we have analysed the genetic relationships between the different CN subpopulations and the BET, PYR and FB populations. Phylogenetic trees constructed on the basis of different genetic distance measures (Da, Gst, Dc) cluster together the different CN farms and BF. Finally, the genetic relationships between 339 CN and 44 FB individual cattle were also studied showing genetic differences between subpopulations.

**D115**

**Genetic mapping porcine EST sequences using length polymorphisms**

GARY A. ROHRER, BRAD A. FREKING, DAN J. NONNEMAN

*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*

The current priority of the MARC swine genome group is to identify and map SNPs (single nucleotide polymorphisms) associated with EST sequences to develop the comparative map and provide a large number of validated SNP markers for the porcine genome. Our approach is based on sequencing amplicons from animal genomic DNA spanning introns and evaluate the sequence files for polymorphisms. While collecting this sequence data, it was noted that approximately 20% of the amplicons possess polymorphic insertion/deletion events (Fahrenkrug, et al., Anim. Genet.,

2002). Some of these insertion/deletion events were actually caused by the presence of a CA/GT dinucleotide repeat. A cost effective method for genotyping these polymorphisms is to design primers flanking the polymorphism, amplify genomic DNA incorporating radioactive nucleotides, separate the fragments with polyacrylamide gel electrophoresis and exposing the gels to film overnight. With this methodology, seven informative microsatellite markers and 36 informative insertion/deletion markers were developed for 42 genes (one intron contained informative microsatellite and insertion/deletion markers). Forty of these genes were placed on the porcine genetic map as two markers did not have enough informative meioses to detect significant linkage. These genes were distributed across 14 of the 19 chromosomes of the porcine genome and develop additional ties between the human and porcine genomes.

**D116**
**Genomic characterization of bovine beta-defensin genes**

SUSANNE ROOSEN, ERNST KALM, CHRISTIAN LOOFT
*Institute of Animal Breeding and Husbandry, Christian Albrechts University, Olshausenstrasse 40, D-24118 Kiel, Germany*

Defensins are cationic peptides of 38-42 amino acids and are part of the innate, unspecific host defense mechanisms of mammals. Within the scope of the research project ″Inquiries of the importance of antimicrobial peptides in the bovine udder″ the relevance of bovine beta-defensin genes concerning udder health is to be analysed. First own studies provided evidence of gene expression of several defensins in epithelial tissue of bovine mammary glands. In order to characterize the genomic organization of bovine beta-defensin genes, we designed primers based on published beta-defensin consensus sequences and screened the primary pools of two bov. BAC libraries. 18 defensin-positive BACs have been isolated by PCR analysis and characterized by digestion with restriction endonuclease and pulsed-field gel electrophoresis. A *Not*I digestion revealed an average insert size of 83 kb. The digested BACs were adapter-ligated and sequenced within the defensin-gene area. Up to now, 11 of 18 BACs have been analysed this way. PCR-products established with primers flanking the defensin-gene region have been subcloned. In order to establish a contig, the inserts of the BAC-clones have been analysed using BAC-end sequencing and oligonucleotides have been designed from each end of insert. Further, we designed primers based on published bovine EST-sequences, which are homologous to *hBD-3*. All BACs have been characterized by PCR to identify overlapping regions. A preliminary contig, based on 9 BAC clones, has been established.

**D117**
**Isolation and mapping of the Mitochondrial Glycerol-3-Phosphate Acyltransferase (*GPAM*) gene in Cattle**

R. ROY[1], M. GAUTIER[2], H. HAYES[2], P. LAURENT[2], P. ZARAGOZA[1], A. EGGEN[2], C. RODELLAR[1]
[1]*Laboratorio de Genética Bioquímica y Grupos Sanguíneos, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain;* [2]*Laboratoire de Génétique biochimique et de Cytogénétique, INRA-CRJ, Jouy-en-Josas, France*

Glycerol-3-phosphate acyltransferase catalizes the first step of glycerolipid biosynthesis. It plays a key role in the regulation of cellular triacylglycerol and phospholipid levels.There are two isoforms of Glycerol-3- phosphate acyltransferase in mammals, a mitochondrial and a cytosolic form. The mitochondrial form (GPAM) prefers saturated fatty acyl-CoA as a substrate, whereas the cytosolic enzyme uses both saturated and unsatured fatty acyl-Co. We used the mouse GPAM gene sequence to design specific primers in order to partially amplify the bovine gene. We assigned GPAM to BTA26 using the INRA hamster-bovine somatic cell hybrid panel and confirmed this assignment by analysis of a 3000rad radiation panel. The primers were used to screen a bovine BAC library and two BAC clones were identified (0522B11 and 0784H09). Both were mapped to BTA 26q22 by fluorescence in situ hybridization. This is in agreement with the corresponding human and mouse localizations on HSA 10q24-q26 and MMU 19 respectively. Two polymorphic microsatellites were isolated from each BAC clone after subcloning and hybridation with a poly (AC) probe.

**D118**

**Assessment of genetic diversity in Mazurian red deer populations by DNA fingerprinting analysis**

J. RUTKOWSKI[1], M. SACHARCZUK[2], K. JASZCZAK[2], M. ŻURKOWSKI[1]

*[1]Research Station for Ecological, Agriculture and Preserve Animal Breeding; [2]Institute of Genetics and Animal Breeding of Polish Academy of Sciences*

Multilocus DNA fingerprinting technique based on screening of many polymorphic loci in the genome is effective tool for determination of genetic heterogeneity and differentiation of population. A study involving the use of DNA fingerprinting was conducted to asses the genetic variation and diversity in Mazurian red deer population.

High molecular weight DNA was prepared from blood samples by proteinase K digestion and phenol/chloroform extraction. The restriction endonuclease *Hinf* I was used to digest individual and pooled DNA samples. Hybridisation was performed with the 33.6 probe.

The individual and representative DNA fingerprinting profiles were analysed with a computer program and band sharing (BS) were measured within four regions and between them.

Higher values of BS between individuals within regions than between regions were observed.

**D119**

**Microsatellite analysis of genetic diversity in Alpine ibex (CAPRA IBEX)**

P. SACCHI[1], R. RASERO[1], E. CAUVIN[1], S. SARTORE[1], S. MAIONE[1], B. BASSANO[2], G. MENEGUZ[1], M. BLASI[3]

*[1]University of Torino, Dipartimento di Produzioni Animali, Grugliasco, Italy; [2]Parco Nazionale Gran Paradiso, Italy; [3]Laboratorio Gruppi Sanguigni, Potenza, Italy*

Genetic diversity at microsatellite loci was examined in three populations of Alpine Ibex (*Capra ibex*) from different geographic locations in Italian Alpes. Sixty individuals were captured in Gran Paradiso area, namely 46 in Val Savarance (A) and 14 in Valle Orco (B); other 21 individuals were captured in Parco Naturale delle Alpi Marittime (C). Thirty microsatellite primer pairs designed in domestic cattle, goat and sheep were chosen. From the set of selected markers twenty-two (73,33 %) amplified a specific PCR product in Alpine Ibex. Seven loci were polymorphic in two populations (A and B), six in the third. The average number of alleles per locus was 2.42, 2.42 and 2.14 for populations A, B and C respectively (ranging from 1 to 3 allele per locus). The average heterozigosity was 0.41, 0.40 and 0.39 respectively. In populations A and B all loci were in Hardy - Weinberg equilibrium. In C population one locus showed significant deviation from Hardy - Weinberg proportions towards an excess of heterozigotes. Calculated *F*st values revealed a low degree of differentiation in allelic frequencies between populations. The reduced genetic variation observed in the three populations confirms the historic data of a severe population bottlenek.

**D120**

**PDME in Brown Swiss cattle: an improved molecular test for Italian livestock**

STEFANO SANGALLI[1], SUE DENISE[2], EMILY OBERG[2], MARIO BERTOLETTI[1], PAOLO AJMONE-MARSAN[3]

*[1]Laboratorio Gruppi Sanguigni, Cremona, Italy; [2]Department of Animal Sciences, University of Arizona, Tucson, USA; [3]Universita' Cattolica del Sacro Cuore, Piacenza, Italy*

Weaver syndrome or PDME (Progressive Degenerative Myeloencephalopathy) is an autosomal recessive disease causing severe neurological defects in Brown Swiss cattle. In Italy, Brown Swiss individuals belonging to carrier families have been tested routinely since 1996, using TGLA 116 and MAF 50 microsatellites that permitted to reach a diagnosis with a likelihood of at least 90% in approximately 50% of the cases investigated. To improve the precision of the diagnosis, we tested the informativeness of 9 additional markers flanking the PDME gene on chromosome 4. Six of these belong to the official set used for diagnostic purposes in the United States, while three have never been tested before. We set up two PCR multiplexes: a) MAF 50, TGLA 116, BMS 2172, BMS 885, DIK 008, INRA 072, BM 6458 and b) BM 1224, BM 6437, RM 232, BMS 779. We genotyped the most important carrier bulls used in Italy. All carriers tested so far share the same haplotype linked to the disease gene at 8 microsatellite loci that span a region of 11.9 cM. This result suggests a recent origin of the mutation and the inheritance of

the recessive PDME gene from a common ancestor, as observed in other bovine genetic defects (CVM, BLAD, SMA). All affected animals tested so far are homozygous for the same haplotype. Recombinant animals allowed to locate the PDME gene between BM 1224 and INRA 072.

**D121**
**Analysis of genetic variation in Agerolese cattle breed**

S. SARTORE[1], P. SACCHI[1], L. DI STASIO[2], R. RASERO[1], V. PERETTI[3], F. CIOTOLA[3], V. BARBIERI[3], G. SARTORE[1]
*[1]University of Torino, Dipartimento di Produzioni Animali, Grugliasco, Italy; [2]University of Torino, Dipartimento di Scienze Zootecniche, Grugliasco, Italy; [3]University of Napoli, Dipartimento Scienze Zootecniche, Napoli, Italy*

The Agerolese cattle is an endangered breed, with less than 100 animals, reared in Naples province. It derives from crosses between Podolian, Brown Swiss, Italian Friesian and Jersey during the XX century. It is a dual purpose breed, well adapted to a mountainous country and fed with products of pruning and undergrowth. This study is concerned with the characterization of local genetic resources. Sixty animals were genotyped for sixteen microsatellites (chosen according to their possibility of multiplexing and potential for detecting polymorphism) and 2 coding genes, *POU1F1* and *GH1*, analysed by PCR-RFLP and AS-PCR respectively. Deviations from the HW frequencies were evaluated with an exact test and overall heterozygosity excess or deficiency was estimated using an asymptotic test. All the selected loci showed polymorphism. The observed and effective average numbers of alleles/microsatellite locus were 8.2 and 4.0 respectively. The *POU1F1* and *GH1* loci were biallelic. The average expected heterozygosity was 0.686. Although statistically significant deviations from HW equilibrium were observed, they occurred at 3/18 loci and only 2 of them showed significant lack of heterozygotes. The results show that, in spite of its very reduced size, the Agerolese maintains a relatively high variability, likely depending on its composite origin. The genotypic information will be used to plan individual mating systems aimed to the maximal maintenance of the existing genetic variability.

**D122**
**Analysis of genetic structure of Japanese Black cattle of Hyogo using microsatellite makers**

SHINJI SASAZAKI[1], TAKESHI HONDA[1], MORIYUKI FUKUSHIMA[2], KENJI OYAMA[3], HIDEYUKI MANNEN[4], FUMIO MUKAI[4], SOICHI TSUJI[4]
*[1]Kobe University, Graduate school of Science and Technology, Kobe, Japan; [2]Northern Hyogo Prefecture Institute of Agriculture, Wadayama, Japan; [3]Kobe University, Experimental Farm, Kasai, Japan; [4]Kobe University, Faculty of Agriculture, Kobe, Japan*

Japanese Black cattle of Hyogo prefecture (Tajima strain) is famous as high quality meat producer and has been maintained as a closed system more than 80 years with a few sires. The average inbreeding coefficient of the strain reaches over 0.2 and is still increasing year by year. Serious inbreeding depressions will be expected. To avoid inbreeding depression, we investigated genetic structure of the strain using microsatellite markers. Here we analyzed representative 252 cows which are kept at Northern Hyogo Prefecture Institute of Agriculture with 21 out-group animals which have lower kinship with Tajima strain. Relationship coefficients were calculated from their pedigree information as each Japanese Black cow or bull has complete pedigree information. The genetic distances between individuals were calculated using the polymorphic information of microsatellite as well as pedigree records. Two dendrograms were drawn, one from pedigree record and another from polymorphic information of DNA markers, using UPGMA method. The dendrogram drawn by using DNA markers is consistent well with that from relationship coefficients. In both dendrograms, all 21 individuals of outgroup constructed one group. Within Tajima strain several sub-groups were clearly detected, so that using this information we can make plan to maintain the strain. This result showed that DNA polymorphic information using microsatellite markers well reflects the pedigree record even in an unusually high inbreeding herd of cattle, from which it is suggested that analysis using microsatellite markers is useful tool to reveal the genetic structure without pedigree information.

**D123**
**Paternity test development in dromedary racing camels with DNA microsatellites**

JÜRGEN SASSE[1], REKHA SHAH[1], SANJEEV PULLENAYEGUM[2]

[1]*Central Veterinary Research Laboratory, PO Box 597, Dubai, UAE;* [2]*Present address: Winterbourne Drive, Oakville, Ontario, Canada*

Dromedary camel racing used to be a traditional pastime of the Bedouin. Today it has developed into a highly organised sport comparable to horse racing. Large sums are paid for exceptional animals. The high monetary value of the camels and the increased use of artificial insemination and embryo transfer has necessitated a paternity and individual certification test. This poster details the compilation of microsatellite markers from two previous publications into one parentage test. Six microsatellite markers from New World camelids and four developed in dromedaries were pooled into two multiplex reactions of five markers each (*LCA33*, *YWLL44*, *CVRL04*, *LCA18*, *CVRL07 / VOLP03*, *YWLL08*, *CVRL02*, *CVRL05*, *YWLL38*). The PCR products were combined into one fragment analysis on an ABI PRISM™310 Genetic Analyser. The system was evaluated in 10 Local, 9 crossbreed and 10 Sudani camels. These camels are raced or used for breeding in the UAE. The combined exclusion probability for all animals was 0.997, while 0.996, 0.986 and 0.987 for the Local, crossbreed and Sudani, respectively. These markers may also be useful for identifying the breeds of individual animals in order to be entered into the correct race categories. Currently, the test is being evaluated in known dromedary families and larger sample sizes of the respective breeds are under study.

**D124**

**Molecular evolution of *ornithine transcarbamylase* gene in uricotelic animals**

TAKESHI SHIMOGIRI[1], KATSUYOSHI KOYANAGI[2], KOTARO KAWABE[1], HIDEYUKI MANNEN[2], SHIN OKAMOTO[1], YOSHIZANE MAEDA[1], YASUO KITAGAWA[3], SOICHI TSUJI[2]

[1]*Kagoshima University, Faculty of Agriculture, Kagoshima, Japan;* [2]*Kobe University, Faculty of Agriculture, Kobe, Japan;* [3]*Nagoya University, BioScience Center, Nagoya, Japan*

*Ornithine transcarbamylase* (*OTC*) is one of ornithine-urea cycle enzymes. During vertebrate evolution, the cycle enzymes have considerably altered expression patterns and function physiologically in ammonia detoxification and osmotic regulation. From a viewpoint of evolutionary change in function, the cycle was thought not to be expressed in reptiles and birds. In a previous study, we reported that *OTC* was expressed in negligible quantities in chicken kidney and cDNA sequence suggests that it was highly conserved compared to mammals. In this study, we provide evidence that functional *OTC* is expressed in reptiles and birds. All uricotelic animal *OTC* genes appear to have the necessary substrate-binding sites and mitochondrial targeted signal sequences. The phylogenetic tree constructed from mature *OTC* coding sequence was largely consistent with vertebrate evolution. However, it was found that the leader peptide regions for *OTC*s are different in length and show little similarity to one another. Therefore, we investigated subcellular localization of *OTC* using immunofluorescence analysis and a conversion from precursor form to mature form using mitochondrial import assay in uricotelic animals. These results showed that precursor *OTC* was transferred to mitochondria and was processed to mature form in uricotelic animals. These results suggest that *OTC* gene products have been expressed in uricotelic animals and function as a urea cycle enzyme.

**D125**

**Molecular analysis of relatedness among five turkey strains**

ED SMITH, ELIZABETH LONG, DEDRA WRIGHT, ISRA ELRAYAH, WILLIAM PIERSON, PHILIP SPONENBERG

*Virginia Tech, Blacksburg, VA 24061, USA*

Over the last five years, we have led efforts to develop resources essential for increasing our understanding of the turkey genome. These resources have been used to establish genetic differences between wild turkeys of different origins and backgrounds and between commercial turkeys. However, there has been very little analysis of non-commercial turkey varieties. Additionally, the relatedness of different non-commercial turkey strains remains primarily based on phenotypic information and speculation. Here, we describe results of molecular analysis of relatedness among five turkey varieties including Blue slate, Spanish black, Bourbon red, Royal palm, and Narragansett. The molecular resources used in the analyses included microsatellites, sequence tagged sites, and RAPD primers. Evidence from these analyses suggests that the royal

palm and narragansett are more closely related but more distant from the other three varieties. When combined with phenotypic information, this data may finally help us understand the value of these varieties to the on going efforts in turkey genome analysis and mapping as well as to resource population development for QTL mapping.

**D126**
**Large scale linkage mapping of bovine ESTs using SNPs**

TIMOTHY P. L. SMITH, ROGER T. STONE, GARY L. BENNETT, EDUARDO CASAS, WARREN M. SNELLING, JOHN W. KEELE
*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*

Considerable variation in production traits exists within commercial populations of cattle, suggesting the presence of allelic variation in important genes that could be exploited using genomics. However, this possibility requires the development of appropriate research tools. In the absence of an effort to completely sequence the genome, a realistic approach to genome science in mammalian livestock species is construction of dense comparative maps with human and mouse. To construct such maps, we have undertaken a program of EST sequencing using pooled-tissue, normalized libraries. The EST sequences are aligned with the human genome via BLASTN analysis to identify probable introns and human map position. This data is used to design primers that amplify intronic sequence within genes corresponding to the ESTs. Currently 2342 primer pairs have been tested on bovine genomic DNA, of which 1619 (69%) produced amplicons appearing to be of sufficient quality for sequencing. Successful sequences were derived from at least one end of 1340 amplicons (83%), and revealed heterozygous positions within the four sires of the mapping population for 839 amplicons (63% of successful sequences). Primer-extension assays for over 500 of these SNPs have been designed, and genotype data for 465 has been collected by MALDI-TOF mass spec genotyping. Presently, linkage mapping has been performed for 399 bovine ESTs, making a substantial contribution to the bovine linkage map and comparative maps with other species.

**D127**
**Genetic differentiation of the three hare species from the Iberian Peninsula using microsatellite DNA**

AINHOA SOLIS[1], MIKEL IRIONDO[1], FERNANDO PALACIOS[2], ANDONE ESTONBA[1]
[1]*University of the Basque Country, Dpt. of Animal Biology and Genetics, Bilbao, Spain;* [2]*Ministery of Science and Technology, CSIC, Department of biodiversity and evolutive biology, Madrid, Spain*

We studied genetic variation in 52 individuals of three hare species of the Iberian Peninsula: *Lepus europaeus* (brown hare), *Lepus granatensis* (Iberian hare) and *Lepus castroviejoi* (broom hare). Although *L. europaeus* inhabit in all Europe, in the Peninsula appears only in the north. *L. castroviejoi* and *L. granatensis* are endemic to the Iberian Peninsula and while *L. castroviejoi* is present only in the northwest, *L. granatensis* can be found in the whole Peninsula. Seven microsatellite DNA markers (SOL8, SOL30, SAT2, SAT8, SOL33, SAT12 and SAT5) were studied automatically by an ABI 310 Genetic Analyzer and their fragment size by Genescan software. A tree diagram showing the genetic relationships among the sampled hare species was built up using UPGMA method based on the Nei genetic distance. The robustness of the tree was evaluated by carrying out 1000 bootstrap iterations with the PHYLIP statistical package. The dendrogram shows that *L. europaeus* is the most differentiated species. Simple allele sharing statistics were applied to investigate the genetic structure of the species. The neighbor-joining phylogenetic tree shows clear differentiation among the three hare species. The genetic diversity is considerably higher in *L. europaeus* than in *L. granatensis* and *L. castroviejoi*. The observed low level of genetic variability in *L. castroviejoi* can be explained by its limited geographical distribution, while the high diversity of *L. europaeus* could be attributed to the fact of being the original species. *L. europaeus* is the oldest species of the three, as pointed out by Pérez-Suárez (1994) and our results suggest that *L. granatensis* and *L. castroviejoi* are closed species, descendent from a single common ancestor.

**D128**

**Characterization of polymorphism and mapping of the porcine *SKI* gene**

ANTONIN STRATIL[1], GERALD REINER[2], LUC J. PEELMAN[3], ROBERTA DAVOLI[4], MARIO VAN POUCKE[3], PAOLO ZAMBONELLI[4], HERMANN GELDERMANN[2]

*[1]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic; [2]Institute of Animal Husbandry and Breeding, University of Hohenheim, Stuttgart, Germany; [3]Ghent University, Department of Animal Nutrition, Genetics, Breeding and Ethology, Merelbeke, Belgium; [4]Sezione di Allevamenti Zootecnici, University of Bologna, Reggio Emilia, Italy*

The SKI oncoprotein (avian sarcoma viral oncogene homolog) is involved in neural tube development and muscle differentiation. SKI affects muscle mass, apparently via activation of MRF genes. In man the *SKI* gene was first localized to HSA1q22-q24 and is recently reassigned to HSA1p36.33. To study the porcine *SKI* gene we designed PCR primers using the human sequence (EMBL X15218). A single fragment was amplified. The fragments from the Pietrain and Meishan pigs were cloned and sequenced. The sequence (364 bp) was 97 % identical to the human sequence. The two pig sequences differed in two bases (positions 325-326: Pietrain, TC; Meishan, CG). The Meishan fragment was cut by restriction enzyme *Alw*26I (*Bsm*AI) (allele *B*), but not the Pietrain one (allele *A*). The base substitutions result in amino acid replacement (serine – arginine). Codominant inheritance of this polymorphism was confirmed in the Hohenheim Meishan x Pietrain pedigree. *SKI* was polymorphic in Pietrain, Black Pied Prestice, Large White, Landrace, Czech Meat Pig, Hampshire and Duroc breeds, while Meishan was monomorphic for allele *B*. By linkage analysis in the Hohenheim pedigree *SKI* was assigned to chromosome 6 and the gene order was: *S0087-RYR-LIPE-EAH-A1BG-**SKI**-FABP3-S0146*. In radiation hybrid mapping (using IMpRH panel) no marker gave a LOD score >4.8 (this value is the treshold level for significant assignment). (Supported by Grant no. 523/00/0669)

**D129**

**Evolution of the leptin gene in the family *Canidae***

M. ZAJAC[1,2], A. CHMURZYNSKA[1], M. SWITONSKI[1]

*[1]Department of Genetics and Animal Breeding, August Cieszkowski Agricultural University of Poznan, Poland; [2]Institue of Human Genetics, Polish Academy of Sciences, Poznan, Poland*

Two exons (2 and 3) of the leptin gene of four species belonging to the family *Canidae*: the dog (*Canis familiaris*), the Chinese raccoon dog (*Nyctereutes procyonides procyonides*), the red fox (*Vulpes vulpes*) and the arctic fox (*Alopex lagopus*) were screened for intra- and interspecies variants with the use of the SSCP and DNA sequencing approaches. Neither in the exon 2 nor in the exon 3 any polymorphic SSCP patterns among 16 canine breeds, included in this study, were found. The patterns of the exon 2 were identical in all studied species, but were distinctly different in case of the exon 3. Thus, DNA sequencing of the exon 3 was performed and several single nucleotide substitutions were identified. Comparison of the DNA sequences revealed existence of the following number of the subsitutions, distinguishing the studied species: one between the dog and the raccoon dog, four between the dog and the arctic fox, three between the raccoon dog and the arctic fox. Data for the red fox are yet incomplete. The obtained results suggest that exon 2 of the leptin gene is highly conservative in the family *Canidae*, but some mutations in the exon 3 were established during the evolution of this family. Moreover, our studies support previous conclusions concerning species divergence in the family *Canidae*.

(This study was supported by the Foundation for Polish Science, contract 13/2000)

**D132**

**Mitochondrial differentiation in Northern European sheep**

MIIKA TAPIO[1], ILMA GRIGALIUNAITE[2], LARS-ERIK HOLM[3], SVEN JEPPSSON[4], JUHA KANTANEN[1], ILONA MICEIKIENE[2], INGRID OLSAKER[5], HALDJA VIINALASS[6], EMMA EYTHORSDOTTIR[7]

*[1]MTT Agrifood Research Finland, Jokioinen, Finland; [2]Lithuanian Veterinary Academy, Kaunas, Lithuania; [3]Danish Institute of Agricultural Science, Tjele, Denmark; [4]Swedish Board of Agriculture, Jönköping, Sweden; [5]The Norwegian School of Veterinary Science, Oslo, Norway; [6]Institute of Animal Science of Estonian Agricultural University,*

*Tartu, Estonia; [7]Agricultural Research Institute, Reykjavik, Iceland*

Differentiation in 32 short- and long-tailed sheep breeds in Northern Europe was studied by sequencing of mitochondrial control region. Breeds were grouped into four categories: Atlantic short-tailed breeds, Atlantic long-tailed breeds, Baltic Sea region short-tailed breeds and Baltic Sea region long-tailed breeds. Analysis of molecular variation showed significant differentiation between the four groups ($\Phi_{CT}$=0.036) and between populations within the groups ($\Phi_{SC}$=0.289). Pairwise comparisons showed that the Baltic long-tailed breed group was not differentiated from the Baltic short-tailed group or the Atlantic long-tailed group. This agrees with prior knowledge about ancestry of breeds belonging to Baltic long-tailed group. The results encourage further dissection of mtDNA variation on Northern European sheep.

**D135**

**Cattle polymorphism revealed using ISSR-PCR at animals from 10-km alienation zone of Chernobyl NPS**

N. V. TRYAPITCINA, V. I. GLAZKO
*Institute of Agriecology and Biotechnology of UAAS,Kiev,Ukraine*

Family analysis of heredity of amplicon (ISSR-PCR markers) in 4 generations of Holstein cattle reproduced in alienation zone of Chernobyl's accident (200ci/km$^2$) was carried out. The analysis covered 40 animals from 8 families. Among them 3 families are derived from 3 cows survived accident in 1986 in this locality and the rest - from cows supplied in alienation zone after accident in1992-94. All animals of F1,F2,F3 generations were born from one bull also survived accident in the same place. It was revealed and exposed to family analysis 124 loci of anonymous DNA sequences obtained using three dinucleotide and seven trinucleotide microsatellite repeat motifs as primers. 57 loci among them were polymorphous. More polymorphic spectra were obtained in 3 families originated from cows survived accident in this locality The family analysis was not revealed any mutational event at animals investigated. It was found that for 7 out of 10 primers studied the quantity of loci in F2 and F3 generation were increased in comparison with F0 generation, that may be considered as response to influence of ecological stress-factor. The heterozygosity observed in this study with ISSR- markers was comparable with those revealed using biochemical markers in earlier studies. It is probably that under of low irradiation dose influence conditions the heterozygous animals have advantages for reproduction.

**D136**

**Development of single nucleotide polymorphism (SNP) markers adjacent to existing microsatellites to increase marker informativeness for detailed QTL analysis**

YOKO UCHIDA[1], NAOE KANAYA[1], ATSUSHI HORIUCHI[2], HIROHIDE UENISHI[3], TAKESHI HAYASHI[3], TAKASHI AWATA[3]
*[1]STAFF Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki, Japan; [2]Shizuoka Prefectural Swine & Poultry Experiment Station, 2780 Nishikata, Kikugawa, Shizuoka 439-0037, Japan; [3]National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan*

QTL analyses have been performed in various animal populations to reveal genetic regions responsible for quantitative economic traits. However, there are some regions where polymorphic microsatellite (MS) markers are difficult to be located, particularly in resource families derived from the cross between closely related breeds, and it may be an obstacle to the precise location of the QTL. Polymorphic markers adjacent to non-polymorphic MS markers may be useful instead of the MS markers in such cases. We report a method for development of single nucleotide polymorphism (SNP) markers in a particular genomic region using BAC library. As an example, we tried to detect SNPs in pig chromosome 16 (SSC16), where QTL (palmitic acid content) has been detected in Jinhua x Large White resource family. BAC clones containing three MS markers around the QTL, SWR2480, SW1897 and S0061, which were non-polymorphic in the family, were obtained from a swine BAC library. Sequences adjacent to the MS markers and both ends of the BAC inserts were determined, so that PCR primers for detection of SNPs were designed inside the sequences. SNPs were detected by comparison of sequences of the PCR products derived from the parents of the family using these primers,

and analyses on the family were performed with SNaPshot (Applied Biosystems). Utilizing these SNPs as markers, a sufficient number of informative markers could be provided on SSC16 and the region of the QTL could be more precisely restricted.

**D137**

**Mapping of 10 genes to porcine chromosome 13 and refining the regions of conserved synteny with human chromosome 3**

MARIO VAN POUCKE[1], MARTINE YERLE[2], FRANÇOIS PIUMI[3], KATHLEEN JACOBS[1], CARINE GENÊT[2], MARC MATTHEEUWS[1], ALEX VAN ZEVEREN[1], LUC J. PEELMAN[1]

*[1]Department of Animal Nutrition, Genetics, Breeding and Ethology, Ghent University, 9820 Merelbeke, Belgium; [2]Laboratoire de Génétique Cellulaire, INRA, 31326 Castanet-Tolosan, France; [3]Laboratoire de Radiobiologie et d' étude du génome, INRA CEA, 78352 Jouy-en-Josas, France*

We report here the localization of *BAIAP1*, *HTR1F*, *PTPRG* and *UBE1C* by FISH, *B4GALT4*, *BAIAP1*, *GATA2*, *IL5RA*, *LMCD1*, *MME* and *RYK* by RH mapping and *B4GALT4* by linkage mapping to Sscr13. The mapping of these 10 different genes (all mapped to Hsap3) not only confirms the extended conservation of synteny between Hsap3 and Sscr13, but also defines more precisely the regions with conserved linkage. The syntenic region of the centromeric part of Sscr13 was determined by isolating BAC clones using primers amplifying porcine microsatellite markers S0219 and S0076 (mapped to this region). Sequence comparison of the BAC end STS sequences with the human working draft sequence showed that the centromeric part of Sscr13 is syntenic with Hsap3p24.

**D138**

**Patterns of blood group antigen diversity in European and Yakutian cattle populations**

SIRJE VÄRV[1], HALDJA VIINALASS[1], SARAH BLOTT[2], INGRID OLSAKER[3], ZOYA IVANOVA[4], ILONA MICEIKIENE[5], ZIEDONIS GRISLIS[6], ERLING FIMLAND[7], JOHN L. WILLIAMS[8], JUHA KANTANEN[9]

*[1]Estonian Agricultural University, Tartu, Estonia; [2]University of Cambridge and Sygen International, Cambridge, UK; [3]Norwegian School of Veterinary Science, Oslo, Norway; [4]Yakutian State Agricultural Academy, Yakutsk, Russia; [5]Lithuanian Veterinary Academy, Kaunas, Lithuania; [6]Latvia University of Agriculture, Jelgava, Latvia; [7]Nordic Genebank for Farm Animals, Ås, Norway; [8]Roslin Institute (Edinburgh), Roslin, UK; [9]Agrifood Research Finland, Jokioinen, Finland*

Genetic variation of 24 erythrocyte antigens, each treated as an independent diallelic locus, was studied to determine the genetic differences between 71 cattle populations. The data from Nordic, Baltic and Polish cattle breeds (31) were pooled with European (37) and Yakutian (3) populations. An iterative procedure was carried out to estimate the allele frequencies. Genetic distances were calculated and a neighbor-joining tree was constructed from the distance matrix. The robustness of the tree topology was evaluated by bootstrapping (1000 replicates over loci). The known historical relationships between breed groups were confirmed. Correspondence analysis was used to reveal the major patterns of genetic variation among the breeds, based on the allele frequency data at the antigen markers. A two-dimensional scaling plot was used to illustrate the breed relationships. The plot area of the European breeds did not overlap with the area of Baltic-Nordic breeds.

**D139**

**Genetic diversity at microsatellite level of the Brazilian Pantaneiro Horse**

FABIANA T. SERENO[1], JOSÉ R. SERENO[2], PEDRO P. RODRÍGUEZ-GALLARDO[3], JOSÉ L. VEGA-PLA[3], JUAN V. DELGADO[1]

*[1]Departamento de Genética, Universidad de Córdoba, España; [2]EMBRAPA Pantanal, Corumbá, Brasi; [3]Laboratorio de Grupos Sanguíneos, Servicio de Cría Caballar, Córdoba, España*

An analysis of 13 microsatellite loci in 101 animals has been used to define the genetic structure of the Pantaneiro Horse from Brasil. This breed was originated form horses introduced by Spanish and Portuguese colons about three centuries ago. Population was selected to resist the adverse conditions of the enviroment and now is submitted to a conservation program where DNA typing will be an important tool to help to identity and paternity control. The genetic variation was estimated by allele frequencies and average breed heterocygosity.

Nei´s DA distances from Thoroughbred, Arabian, Spanish Pure Breed (Andalusian) and Uruguay Creole horses were calculated showing a minimum distance with Spanish Pure Breed (0.228) and similar distance from Thoroughbred and Arabian (0.355 and 0.332). Distances were used to construct an UPGMA dendrogram. Also an individual tree was obtained from distances based in shared alleles algorithm. Results indicate a great diversity level, clear distancing respect to the other breeds and genetic uniformity inside the Pantaneiro Horse.

**D140**

**Using bovine microsatellite primers for assessment of genetic diversity in two Chinese yak (*Bos grunniens*) populations**

WANG MINQIANG[1], STEFFEN WEIGEND[2], ASILI BARRE-DIRIE[2], JOSEPH W. CARNWATH[2], LOU ZHONGLIN[3], HEINER NIEMANN[2]

*[1]College of Chemical Engineering and Technology, Biologic Science, Biological Engineering, Applied Chemistry, Yantai University, P.R. China; [2]Institute for Animal Science and Animal Behaviour (FAL); [3]Lanzhou Institute of Animal Science and Pharmaceutic, Chinese Academy of Agricultural Sciences, Lanzhou, P.R.China*

DNA samples of 48 Chinese domestic yaks were obtained from two separate herds. 20 animals came from a population, classified as "Plateau type", maintained at the Gannan Liqiaru Stock Breeding Farm in Gansu Province, while 28 animals were sampled from a herd of "Huanhu type" yaks maintained at the Datong Yak Farm in Qinghai Province. A subset of 13 bovine microsatellite markers was selected from the cattle diversity database, CaDBase (www.ri.bbsrc.ac.uk/cdiv_www/homepage.htm), and the corresponding bovine PCR primers were applied to the yak samples. The results indicated that priming sites in the flanking regions and the microsatellite repeat sequences themselves are identical in both species. The yak data were compared with data available in the CaDBase for 15 European cattle breeds. All loci were polymorphic in yaks and fragment sizes overlapped those reported for European cattle. The allele size distribution in yaks fit a stepwise mutation pattern. The total number of yak alleles ranged from 4 to 8 per locus; and the mean number of alleles calculated across all loci was 5.4 in the Gannan sample and 5.5 in the Datong sample. Heterozygosity estimates for the two yak populations were comparable to those calculated for the European cattle breeds. Estimated Genetic distance ($D_A$) between the two yak populations was less than the distance between the most closely related of 15 European cattle breeds suggesting that the two Chinese yak populations are very closely related.

**D141**

**Standardised presentation of SNP-genotypes for paternity testing, individual identification and genetic distance analysis**

FABIAN A. O. WERNER[1], GREGOR DURSTEWITZ[1,2], GEORG THALLER[1], JÖRN MOSNER[3], RUEDI FRIES[1]

*[1]Technische Universität München, Lehrstuhl für Tierzucht, Freising-Weihenstephan, Germany; [2]Agrobiogen GmbH, Hilgertshausen, Germany; [3]G.A.G BioScience GmbH, Bremen, Germany*

We propose a standardised set of single nucleotide polymorphisms (SNPs) as an alternative to microsatellites for verification of identity, paternity testing and the analysis of genetic distances in animals. Advantages of SNPs over microsatellites are lower mutation rates, no need for a specific typing platform, suitability for standardisation, straightforward data management and availability of high throughput genotyping systems. Standardisation is achieved by selecting appropriate SNP loci for a species and defining an order in which the genotypes are represented. Each SNP position is queried for the presence or absence of a specific base. The three possible genotypes are coded in a digital form (homozygous allele 1: '10', heterozygous: '11', homozygous allele 2: '01'). The resulting string of digits is termed digital DNA signature. The first position is assigned to a sex-specific SNP for gender testing. So far we have developed a set of 60 SNP loci in cattle with allele frequencies of at least 0.2 for the less frequent allele within the breeds Holstein Friesian, Brown Swiss and Simmental. These markers allow exclusion powers exceeding 99.99% for parentage testing and probabilities of identity lower than $10^{-11}$ for individual identification. We will extend the digital DNA signature to 96 positions for the bovine species. This signature

will also be useful for the assessment of breed specificity and genetic distance analysis.

**D142**
**Genome-wide scan of Anal Atresia in pig (*Sus Scofa*)**

SABINE WIEDEMANN, RUEDI FRIES, GEORG THALLER
*TU München - Weihenstephan, Chair of Animal Breeding, Germany*
Anal Atresia is a rare disorder with an incidence of 0.1-1.0% in swine. The mode of inheritance is not known, but previous studies indicate that the genetic model involves a single locus in addition to polygenic effects. To identify susceptibility loci for Anal Atresia we have performed a genome scan using an affected half-sib design. After editing, a total of 27 paternal families consisting of 72 affected piglets were included in the analysis. The genome scan was carried out with 130 fluorescent labelled microsatellite markers with an average spacing of 20-25 cM over the porcine genome. The PCR amplification products were run on ABI 377 instruments and genotypes analyzed by GeneScan 3.1 and Genotyper 2.5 software. Two-point and multi-point nonparametric linkage analysis of the pedigrees were performed using the computer package Allegro 1.0. Three chromosomal regions with a nominal significance level of p = 0.05 showed maximum NPL scores of 2.57 (p = 0.006), 1.94 (p = 0.028) and 1.90 (p = 0.031), respectively. The transmission disequilibrium test (TDT) was applied to markers in these regions. Significant $\chi^2$ -test statistics with p-values of $7 \times 10^{-7}$ and $3 \times 10^{-3}$ were found for two markers, which proved to be significant after accounting for multiple testing.

**D143**
**Assessment of genetic relationship of Tanzanian sheep ecotypes using RAPD and microsatellite DNA markers**

JOHN STEPHEN[1], CLEMENS B. A. WOLLNY[2], RAMNI JAMNADASS[3], OLIVIER HANOTTE[3], PAUL S. GWAKISA[4]
[1]*Mogabiri Farm Extension Centre, P. 0. Box 134, Tarime, Tanzania;* [2]*Institute of Animal Breeding and Genetics, Georg-August-University, 37075 Göttingen, Germany;* [3]*International Livestock Research Institute, P. 0. Box 30709, Nairobi, Kenya;* [4]*Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, P. 0. Box 3019, Morogoro, Tanzania*
The diversity of Tanzania's livestock population provides important and valuable resources for local farmers and food security. However, information on small ruminants'indigenous genetic resources remains largely incomplete. Two genotyping techniques were used to study the genetic diversity and differentiation of five Tanzanian local sheep populations from geographically separated regions (Arusha, Mwanza, Mtwara, Dodoma and Coast). Four primers were used for RAPD amplification and six microsatellite loci were studied in 94 unrelated Tanzanian sheep. Two reference breeds from West Africa (West African Dwarf sheep) and England (North Ronaldsay sheep) were also included in the microsatellite study. A total of 30 RAPD bands and 198 microsatellite alleles were detected in the Tanzanian populations. RAPD average heterozygosity values ranged from 0.137 (Dodoma) to 0.203 (Arusha) whereas the microsatellite expected heterozygosity values ($H_0$) ranged from 0.702 (Mtwara) to 0.763 (Dodoma). The smaller microsatellite genetic distance (*Ds*), observed within the Tanzania sheep, was between the Mwanza and the Arusha population (0.019); whereas the largest one was between the Mtwara and the Mwanza population (0.221). The highest RAPD band-sharing value was obtained between the Mwanza and Arusha population (0.552) and the lowest one between the Mtwara and the Mwanza population (0.302). Overall, genetic relationships among the Tanzanian sheep population reflects their geographical locations.

**D144**
**Characterisation of new variants and genotyping of the Caprine Kappa Casein gene**

MOHAMED H. YAHYAOUI[1], ANTONELLA ANGIOLILLO[2], FABIO PILLA[2], ARMAND SANCHEZ[1], JOSEP M. FOLCH[1]
[1]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain;* [2]*Department S.A.V.A., Università del Molise, Campobasso, Italy*
The genetic polymorphism of goat caseins is of interest due to its relationship with composition and technological characteristics of milk.

Kappa casein (KCN) is the protein that determines the size and specific function of milk micelles, and its cleavage by chymosin is responsible for milk coagulation. We have previously characterised three variants (A, B, and C) of the KCN in Spanish and French goat breeds by screening the major part of the coding region in exon 4. Two other variants have been recently detected in German and Italian breeds. The full coding region of the KCN gene (exons 3 and 4) has been analysed for polymorphism by the sequencing method. No additional mutations were found, with the exception of a single nucleotide substitution in exon 3, with no amino acid change. However, the analysis of the association between the different mutations resulted in two new haplotypes, designed F and G. A protocol for rapid and simultaneously genotyping of all KCN variants using the primer extension method was described. A total of 176 animals among six European breeds were genotyped. The novel variants are detected only in Teramane and Murciano-Granadina breeds. Alleles A and B are the most frequent variants in the majority of breeds with a prevalence of the B variant, except for the Canaria breed where allele A is more frequent. The C variant is present especially in Saanen breed. All other alleles are found at low frequencies and are specific for some breeds.

**D145**

**Functional gene mapping of chicken chromosome 2q to identify the causative gene of chicken muscular dystrophy**

KANAKO YOSHIZAWA[1], EUN JUN LEE[1], KYOKO INABA[1], TAKEKI KIKUCHI[2], MAKOTO MIZUTANI[3], HIDEYUKI MANNEN[4], SOICHI TSUJI[4]

*[1]Kobe University, Graduate School of Science and Technology, Kobe, Japan; [2]Department of Animal Models for Human Disease, National Institute of Neuroscience, Tokyo, Japan; [3]Nippon Institute of Biological Science, Kobuchizawa, Japan; [4]Kobe University, Faculty of Agriculture, Kobe, Japan*

Muscular dystrophy of chicken has been studied since 1950's, but the causative gene is not yet known. Recent our studies revealed that genetic locus for chicken muscular dystrophy of abnormal muscle (*AM*) was mapped to chromosome 2q using the Kobe University (KU) resource family. Because comparative mapping analysis showed conserved synteny between this region of chromosome 2q and the segment of human chromosome 8q, genes located on human chromosome 8q are possible candidates for this disease. However, there are few available chicken DNA markers to construct the high density map for this resion. In this study, we attempted to map more functional genes, which located on human chromosome 8q, to chicken chromosomes. Segments of chicken orthologues of human selected genes were amplified from parental DNA of the KU resource family, and the parental alleles were sequenced. Sequence polymorphism was identified between resource family parental DNAs. The polymorphism was genotyped the backcross panel by PCR RFLP to place genes on the chicken linkage map. Thirteen genes were mapped to chicken chromosome 2q, indicating the high level of conserved synteny between human chromosome 8q21-24 and chicken chromosome 2q. The *AM* locus was mapped between DECR1 (6.9cM, 8q21) and SDC2 (11.5cM, 8q22). These results suggested that more genes located on human chromosome 8q21-22 should to be mapped to chicken chromosomes in order to identify the causative gene of chicken muscular dystrophy.

**D146**

**Polymorphism of 12 microsatellites in the Silesian and Thoroughbred horses raised in Poland**

TOMASZ ZĄBEK, MARIAN DUNIEC, EWA SŁOTA

*National Institute of Animal Production, Department of Immuno- and Cytogenetic, Poland*

The polymorphism of 12 microsatellites was investigated in the Polish population of 106 Silesian and 114 Thoroughbred horses. The Silesian breed derives from the heavy German draft horses with the main influence of the Oldenburg stallions. The Thoroughbred horses had a great influence on the Silesian breed as the result of broad crossing the Silesian dams with the Thoroughbred stallions during the 60s and 70s in the past age.

In the study of microsatellite polymorphism the automated DNA sizing was applied. PCR products of 12 microsatellite markers (*VHL20, HTG4, HTG6, HTG7, HTG10, AHT4, AHT5, HMS2, HMS3, HMS6, HMS7, ASB2*) were amplified with the fluorescently labelled primers and then analyzed on the automated DNA

sequencer (ABI 377). 94 alleles were identified in the Silesian breed and 73 in the Thoroughbred horses. The frequencies of most variants in both breeds were similar, despite the ones specific for the Silesian breed, which occurred very rarely. The mean heterozygosity values were close in both breeds (0.740 for the Silesian horse and 0.680 for the Thoroughbred) suggesting the near level of genetic variation of studied populations. Calculated standard Nei's genetic distance (0.28) indicate the small degree of differences in the genetic structure of both horse breeds, which remains in agreement with historical and studbook data. The combined exclusion probability (PE>0.999) in both breeds confirms the usefulness of this set of markers in parentage verification.

**D147**

**Survey of prion gene polymorphisms influencing scrapie resistance in Hungarian flocks**

ATTILA ZSOLNAI, ISTVAN ANTON, LASZLO FESUS

*Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary*

Our aim is to survey prion gene polymorphisms in different flocks and to identify animals having natural resistance against scrapie. This task requires analysing the known genetic variances. As for the analysis we would like to present a faster and more robust method than PCR-RFLP. Moreover we hope, breeders will use our data and initial results in selection.

**D149**

**The study of genetic differentiation of some representatives of subfamily Bovinae using different types of markers**

V. I. GLAZKO

*Institute of Agriecology and Biotechnology of UAAS, Kiev, Ukraine*

The comparative analysis of genetic differentiation between three species of subfamily Bovinae - *Bos taurus, Bison bonasus, Bison bison* with the use of different types of molecular-genetic markers - biochemical (35 loci) and DNA markers (RAPD-PCR, ISSR-PCR) was carried out. The part of polymorphic loci revealed on the base of biochemical markers in investigated species were 28,5; 17,3 and 17,1 and level of heterozygosity in average per locus were 0,131; 0,065; and 0,071 for *Bos taurus, Bison bonasus* and *Bison bison* respectively. Among 33 biochemical systems investigated 15 polymorphic ones were revealed. The quantity of polymorphic loci for *B. taurus, B.bonasus* and *B.bison* were 9, 6 and 6 respectively. Ceruloplasmin, amylase-I and peptidase B were polymorphous in all species tested. The genetic distances calculated on base of different RAPD-PCR, ISSR-PCR markers were higher than ones calculated on the base of biochemical markers. The evaluation of interspecies genetic relationships in subfamily Bovinae was essentially depended from the peculiarities of separate molecular genetic marker (locus), included in analysis, in comparison with type of marker (protein polymorphism, variability of DNA repeat distribution).

**D150**

**Recommendation for allele calling for high throughput genotyping of horses**

SEAN CORLEY[1], IAN FINDLEY[2], IVAN BIROS[1]

[1]*The University of Queensland, Australian Equine Genetics Research Centre, Brisbane, Australia;* [2]*The University of Queensland, Australian Genome Research Facility, Brisbane, Australia*

DNA-based genetic profiling using microsatellite markers has become the internationally accepted method for individual identification and parentage verification both in humans and many other wild and domesticated animals including horses. For horse typing, we have adopted laboratory protocols and two panels of microsatellite markers developed by VGL, University of Davis, California. Over a 7 month period, we have tested the accuracy and reliability of these markers by comparing allele calling in both control DNA samples and 10,000 test samples on the 3700 DNA Analyser *(Applied Biosystems)*. We also compared the accuracy of allele calling in 400 tested samples on three different DNA analysers (3700 DNA Analyser, 377 DNA Analyser *(Applied Biosystems)* and MegaBACE *(Amersham Biosciences)*. Data have been statistically analysed using standard methods (arithmetical average, standard deviation and student t-test).
Our data showed that :

- variation in allele calling was < 0.1 $s_D$ in control DNA samples over 7 months

- variation in allele calling was < 0.1 $s_D$ for all alleles in each test marker
- there is a statistically significant difference (P<0.001, up to 1.5 bp) between empirical and theoretical allele calling between smaller and larger alleles
- that both capillary systems (3700 and MegaBACE) have noticeable allele callingvariation compared to the slab gel-based ABI 377

Our results demonstrate the importance of defining the range of sizes of individual alleles across different DNA analyzers particularly for parentage verification and sharing results between

**D151**
**Sequence variation in the mitochondrial dloop region of Dahomey and other cattle breeds**

SIBYLLE HAHN, INGER VOELKEL, INA PFEIFFER & BERTRAM BRENIG
*University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany*

The dloop region belongs to the most evolving segment in the mitochondrial genome. Its sequence provides a sensitive assay of residual genetic variation in cattle breeds. 400 bp from this region were sequenced and analysed for 10 Dahomeys. These sequences were compared with published sequences from other cattle breeds (*Bos indicus* and *Bos taurus*). We found only a small intra-specific genetic variation in Dahomeys. However, variation was present and the haplotypes of the Dahomeys correspond more to *Bos indicus* than to *Bos taurus*. In the examined region we were able to detect four diagnostic nucleotide positions. Two of this nucleotide positions might suggest a convergent evolution of *bos indicus* and Dahomey.

**D152**
**Study of mitochondrial d-loop DNA sequence variation in some Italian horses**

MARIA C. COZZI, MARIA G. STRILLACCI, MARIA LONGERI, MARTA ZANOTTI
Istituto di Zootecnica, Faculty of Veterinary Medicine, Milan, Italy

The genetic variability of mitochondrial d-loop DNA sequence in seven horse breeds (Giara horse, Haflinger, Italian trotter, Lipizzan horse, Maremmano horse, Thoroughbred and Sarcidano horse) was analysed. Five unrelated horses were chosen for each breed. The mitochondrial DNA from peripheral blood was extracted by standard methods. Two primers in the conserved region flanking d-loop were chosen to specifically amplify the polymorphic region using the kit *ReadyMix*$^{TM}$ (SIGMA). Direct sequence of amplicons, carried out by Big Dye Terminators chemicals (Applied Biosystems) and automatically analysed by ABI PRISM 377 equipped with GeneScan® and Navigator® softwares (Applied Biosystems), allowed to obtain a consensus sequence of 397-bp between sites 15382 and 15778 (GenBank X79547). The obtained sequences were aligned and compared with a reference sequence (GenBank X79547) by BLAST and DNASIS programs. A phylogenetic tree was constructed using d-loop sequence differences using DNAMLK software of PHYLIP package. Only for Giara horses we could find a high degree of similarity, which can be ascribed to one maternal line. On the contrary, Lipizzan horse, Maremmano and Haflinger showed at least two different patterns which can be ascribed to two main maternal lines. The remaining breeds showed a wider diversity suggesting the presence of many maternal lines.

**D153**
**Identification, characterisation and linkage mapping of SNPs in porcine skeletal muscle genes**

ROBERTA DAVOLI, LUCA FONTANESI, SILVIA BRAGLIA, MAURIZIO CORSARO, BARBARA LAMA, MASSIMO CAGNAZZO, VINCENZO RUSSO
*DIPROVAL – Sezione di Allevamenti Zootecnici, Faculty of Agriculture, University of Bologna, Reggio Emilia, Italy*

Porcine skeletal muscle genes can be considered candidates for meat quality and production traits as meat derives mainly from skeletal muscle tissues. With the aim to identify these genes, we isolated about 1000 ESTs from an adult porcine skeletal muscle cDNA library. Then, to develop DNA markers that could be useful in association studies with meat quality and production traits, we started a sistematic approach to identify single nucleotide polymorphisms (SNPs) in these ESTs. PCR primers were designed to amplify fragments of 34

genes expressed in this tissue. To search for polymorphisms, we carried out PCR-SSCP analyses on genomic DNA pooled from 20 pigs and on individual DNA. Moreover sequencing was performed to confirm the presence of DNA mutations. On the whole we analyzed ~6100 bp and identified SSCPs in 13 genes. So far, sequencing was performed for 12 genes (*ATP1A2*, *CA3*, *CTSL*, *DECR1*, *HUMMLC2B*, *MYH4*, *Myopalladin*, *PSMA4, SLN*, *TNNT3*, *TTN*, and *ZASP*) and we identified 15 SNPs, 1 insertion/deletion and 2 microsatellites. Allele frequencies at these loci were studied in six different pig breeds (Large White, Landrace, Duroc, Belgian Landrace, Piétrain and Hampshire). Moreover, linkage mapping was obtained for *TNNT3* (Sscr 2), *HUMMLC2B* (Sscr 3), *ATP1A2* and *DECR1* (Sscr 4), *PSMA4* (Sscr 7), *CRYAB* and *SLN* (Sscr 9) and *MYH4* (Sscr 12).

**D154**
**Development of a 17-plex Microsatellite PCR Kit for the Genotyping of Horses**

PERO DIMSOSKI
*Applied Biosystems, Foster City, USA*
Microsatellite-based molecular markers are used for the genotyping of horses, primarily for pedigree verification. The only commercially available kit, StockMarks® for Horses (Applied Biosystems, Foster City, USA), consists of 12 primer sets labeled with 3 different fluorescent dyes, which are amplified in two multiplex PCR reactions. The recent introduction of medium- and high-throughput genotyping instruments (ABI PRISM® 3100 DNA Genetic Analyzer and ABI PRISM® 3700 DNA Genetic Analyzer) required the development of a new equine genotyping kit that would match the increased performance of the instruments. This new equine kit contains five extra loci (ASB17, LEX3, HMS1, CA425, and ASB23) in addition to the twelve original ISAG-recommended loci (VHL20, HTG4, AHT4, HMS7, HTG6, HMS6, HTG7, HMS3, AHT5, ASB2, HTG10, and HMS2). This is accomplished through the use of a new set of five fluorescent dyes developed by Applied Biosystems (DS-31) with four of the dyes being used to label the forward amplification primers (6-FAM™, VIC™, NED™, and PET™) in each primer set. An in-lane size standard labeled with the fifth dye (LIZ™) provides accurate size determination for genotyping. These seventeen loci are combined and amplified in a single PCR reaction, dramatically improving the power of statistical tests for pedigree analysis while reducing the time and labor required to perform such tests.

**D155**
**Critical parameters for the optimization of short tandem repeat multiplex polymerase chain reactions**

ANTHONY FISHBACK, MATTHEW SHAUNESSY, TYLER ARCHBOLD, WAYNE MURRAY
*Maxxam Analytics, Genetic Identification Division, Guelph, Canada*
Short Tandem Repeat (STR) loci have become the genetic marker of choice for parentage determination and population differentiation. Several STR loci are required for such studies in order to obtain an appropriate amount of genetic polymorphism. Fortunately, genotypic data collection has become efficient via the co-amplification of multiple loci (multiplexing) in a single Polymerase Chain Reaction (PCR) using fluorescently labeled DNA primers. However, the development of a robust multiplex system presents many challenges. Allele-scoring difficulties can result due to the production of stutter bands during the amplification of dinucleotide repeat STR loci, and through variability in "plus A" modifications (non-templated adenylation of the 3' end of the amplified sequence by *Taq* polymerase) of PCR products. Nucleotide substitutions in the DNA sequence of a primer-binding site can also result in allele scoring difficulties. The manual editing required to correct misidentified alleles greatly inhibits the efficiency of automated genotyping systems. We will show that adjusting thermocycling parameters and various concentrations of PCR reagents can eliminate the production of misidentified alleles in PCR multiplex systems containing up to 15 STR loci.

**D156**
**Radiation hybrid map of bovine genome using microsatellites and AFLPs**

C. GORNI[1], R. NEGRINI[1], M. J. T. VAN EIJK[2], H. HEUVEN[2], A. VALENTINI[3], P. AJMONE-MARSAN[1], J. L. WILLIAMS[4]
*[1]Istituto di Zootecnica, Università Cattolica del S. Cuore, Piacenza, Italy; [2]Keygene N. V., Wageningen, The Netherlands; [3]Dipartimento*

*di Zootecnica, Università della Tuscia, Viterbo, Italy; [4]Roslin Institute (Edinburgh), Roslin, United Kingdom*

Radiation hybrid (RH) cell panels are a very efficient tool for ordering monomorphic markers therefore they are particularly useful for mapping genes and ESTs and building comparative maps. A 3000 Rad bovine-hamster RH panel has been recently developed through an EC sponsored collaboration. We have investigated the use of AFLP technology to increase the number of markers available on the RH map as anchor points for the construction of a physical map of BAC or YAC contigs. The 94 cell lines of the RH panel were typed with 37 AFLP EcoRI/TaqI primer pairs carrying respectively 4 and 3 selective nucleotides at their 3' end. A total of 740 bovine specific AFLP bands were clearly distinguishable from the hamster profiles. Their retention frequency averaged 0,179 and ranged from 0,011 to 0,775. AFLP markers were incorporated into the existing 1200 microsatellite RH framework map using the program Carthagene. More than 600 AFLPs were linked to the framework map at LOD 6 or more. Unlinked AFLP markers may represent i) bands heterozygous in the donor bovine cell line and therefore only a single allele is detected; ii) bands originating from repetitive DNA templates; iii) bands too far away from nearby mapped markers to show significant linkage in a high resolution map, which is unlikely, or iv) bands that are hard to distinguish and hence with high typing errors.

**D158**

**The cDNA sequence and polymorphisms of bovine procathepsin-D**

MIKITO HIGUCHI[1], NORIKAZU MIYASHITA[2], YOSHITAKA NAGAMINE[1], AKIRA WATANABE[1], TAKASHI AWATA[2]
*[1]National Agricultural Research Center for Tohoku Region, Department of Animal Production and Grassland Farming, Morioka, Japan; [2]National Institute of Agrobiological Sciences, Institute of Insect and Animal Sciences, Tsukuba, Japan*

The cDNA sequence of the bovine cathepsin-D precursor, procathepsin-D was determined by RT-PCR with primers designed according to the human procathepsin-D cDNA sequence. Both the bovine procathepsin-D cDNA sequence and deduced protein sequence exhibited higher homology to their counterparts in sheep than other animals. A comparison of the cDNA sequence among cattle revealed ten single nucleotide polymorphisms (SNPs) in the encoding region and one nucleotide deletion in 3'-untranslated region. Among these SNPs, a G to A substitution in the pro-fragment region and a change from G to C in the heavy-chain region resulted in a glycine to serine and a glycine to alanine substitution, respectively. Nine different cattle breeds were screened for the presence of both polymorphisms using PCR-RFLP analysis. Three Japanese beef breeds (Japanese Black, Japanese Shorthorn and Japanese Brown) and two zebu breeds (Brahman and Santa Gertrudis) contained these polymorphisms, whereas four European breeds (Holstein-Friesian, Jersey, Aberdeen-Angus and Hereford) did not.

**D159**

**A genetic map for Atlantic salmon (*Salmo salar*).**

KATRINE HANES[1], SIGBJØRN LUNNER[1], JOHN TAGGART[2], RICHARD POWELL[3], LARS-ERIK HOLM[4], RENÉ GUYOMARD[5], ROY DANZMANN[6] & BJØRN HØYHEIM[1]
*[1]Norwegian School of Veterinary Science, MGA-Genetics, Oslo, Norway, [2]University of Stirling, Institute of Aquaculture, Stirling, Scotland, [3]National University of Ireland, Department of Microbiology, Galway, Ireland, [4]Danish Institute of Agricultural Sciences, Department of Animal Breeding and Genetics, Tjele, Denmark, [5]INRA, Laboratoire de génétique des poissons, Jouy-en-Josas, France and [6]University of Guelph, Department of Zoology, Guelph, Canada*

Building a genetic map in salmonids requires special attention when compared to developing a map in mammals. The salmonid genome has undergone duplication in relatively recent evolutionary time and therefore shows residual tetraploidity. Although many genes have been extinguished after the duplication event the fact that areas of the genome is duplicated in an almost identical manner makes the development of a genetic map challenging. Atlantic salmon also show a considerable difference with respect to recombination frequencies between the male and female map. There is little or no recombination in the males while the females seems to exhibit normal recombination. This makes assigning a specific marker to a linkage-group relatively easy using male data

while the data from females must be used to construct a linkage-map. A first generation genetic map for Atlantic salmon was constructed in the EU-project: Generation of highly informative DNA markers and genetic marker maps of salmonid fishes (SALMAP). In this project a total of 447 microsatellite loci was fully charcterised (363 from Atlantic salmon, 57 from rainbow trout and 27 from brown trout). 300 markers (299 DNA-markers + sex) were genotyped in the reference families and subsequently mapped by linkage-analysis. We have expanded this map by developing 520 new microsatellite markers and to date we have added 150 of these to the map.

**D160**

**Mapping of over 1100 bovine polymorphic microsatellite markers to the USDA-MARC cattle linkage map**

NAOYA IHARA[1], AKIKO TAKASUGA[1], KAZUNORI MIZOSHITA[2], HARUKO TAKEDA[1], MAYUMI SUGIMOTO[3], GARY L. BENNETT[4], KENT M. REED[5], CRAIG W. BEATTIE[6], YOSHIKAZU SUGIMOTO[1]

*[1]Shirakawa Institute of Animal Genetics, Fukushima, Japan; [2]Cattle Breeding Development Institute Kagoshima, Kagoshima, Japan; [3]National Livestock Breeding Center, Fukushima, Japan; [4]USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA; [5]University of Minnesota, Department of Veterinary PathoBiology, St. Paul, MN, USA; [6]University of Nevada, Department of Animal Biotechnology, Reno, NV, USA*

Polymorphic microsatellite markers are particularly important to construct a genetic linkage map. In cattle, a second-generation linkage map has been constructed with 1236 DNA markers (Kappes et al., 1997). The linkage map has been useful for mapping loci of genetic diseases as well as economically important traits in cattle. Furthermore, an important aspect of strategy for physical map construction, such as an RH map, is the use of many, ordered DNA markers on a linkage map to determine the relative orders of linkage groups. To further facilitate the power of linkage map, we have developed microsatellites and mapped them to the USDA-MARC cattle linkage map. We have newly developed over 800 polymorphic microsatellites and mapped approximately 400 microsatellites. The remaining 400 microsatellites and 300 already developed, but not mapped microsatellites are being genotyped. With the addition of these 1100 microsatellites alone, the USDA-MARC cattle linkage map will contain at least 2300 DNA markers with 1.3 cM average intervals.

**D161**

**Development and typing of Single Nucleotide Polymorphism markers in a QTL region for fatness traits on porcine chromosome 2**

BART JUNGERIUS[1], ANNEMIEKE RATTINK[1], BARBARA HARLIZIUS[1,2], BERNARD VAN OOST[3], MARINUS TE PAS[4], MARTIEN GROENEN[1]

*[1]Wageningen University, Animal breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands; [2]Present adress: Institute for Pig Genetics, Beuningen, The Netherlands; [3]Utrecht University, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, The Netherlands; [4]Institute for Animal Science and Health (ID-Lelystad), The Netherlands*

A maternally imprinted quantative trait locus (QTL) for backfat thickness (BFT) has been identified on the p-arm of porcine chromosome 2 (SSC2p). To reduce the size of the interval of the QTL and eventually be able to identify the underlying genes responsible for the observed effects additional DNA markers are needed. In order to increase the marker density on SSC2 single nucleotide polymorphism (SNP) markers were developed by sequencing PCR products amplified from genomic DNA of 8 individuals from different breeds. Until now over 200 SNPs were identified in 36 sequence tagged sites (STS) covering 80 cM of SSC2 including the entire P-arm. Currently these SNP markers are being used for detailed haplotyping of animals from the experimental QTL mapping cross. The SNPs are assayed in multiplex single base extension reactions using the ABI Prism SnapShot kit and analysed on an automated sequencer (ABI377). After gel analysis using Genescan 3.1.2 and creating a table containing all signal peaks, the genotypes are assigned in a highly automated way in a MS Excel workbook designed for this purpose. The output from this workbook can be easily formatted to match database import file or files for linkage analysis using CRIMAP.

**D162**
**Screening of *Bos indicus* and *Bos taurus* cattle breeds for *DGAT1* polymorphism**

B. KAUPE[1], A. WINTER[5], E. IBEAGHA[1], C. ÖZBEYAZ[2], J. L. WILLIAMS[3], P. AJMONE-MARSAN[4], R. FRIES[5], G. ERHARDT[1]
[1]*Justus-Liebig-University of Giessen, Department of Animal Breeding and Genetics, Giessen, Germany;* [2]*Department of Zootechnics, University of Ankara, Turkey;* [3]*Roslin Institute Edinburgh, Scotland, United Kongdom;* [4]*Institute of Zootechnics, Catholic University of S. Cuore Piacenza, Italy;* [5]*Technische Universität München, Lehrstuhl für Tierzucht, Freising-Weihenstephan, Germany*

In cattle the diacylglycerol O-acyltransferase gene (*DGAT1*), which maps to the centromeric end of BTA14, is suspected to be a positional candidate gene with a major effect on milk fat content and other milk characteristics, as reported recently for *German Simmental* and *Dutch/ New Zealand Holstein Friesian* populations. We genotyped 821 DNA samples from 17 cattle breeds including *Bos taurus* and *Bos indicus* from 5 different countries on 3 continents (Europe, Africa, Asia), by applying a PCR-RFLP test based on the K232A (*AAG→GCG*) substitution. The lowest frequency of the *AAG* allele was found in *Bos taurus* type cattle e.g. *Hereford* (0.00), while highest *AAG* allele frequencies were harbored by *Bos indicus* type cattle, like *Banyo Gudali* (0.86) and *White Fulani* (0.92). Beef breeds had lower *AAG* allele frequencies, ranging from *Hereford* (0.00) through *Piemontese* (0.03) to *Chianina* (0.38) cattle, compared to dairy breeds with range from *Ayrshire* (0.02), *British Friesian* (0.03) through *Holstein Friesian* (0.56) to *Jersey* (0.69). Non-selected breeds like *South Anatolian Red* (0.20), *East Anatolian Red* (0.25), *Turkish Grey Steppe* (0.36) and *Anatolian Black* (0.51) had intermediate allele frequencies. It remains to be seen if the frequency of haplotypes carrying the *Lysine* residue increased in recent years due to changes in selection criteria. It is conceivable that the *AAG* allele could have been introgressed from *Bos indicus* cattle into *Bos taurus* cattle after domestication and more recently through introduction of zebu cattle into southern Europe.

**D163**
**Construction a dense comparative map between HSA1p35-p36 and SSC6 by using human STS markers directly for RH mapping**

SACHIKO KIUCHI[1], YING ZHANG CHEN[2], HIROHIDE UENISHI[1], TAKESHI HAYASHI[1], EIICHI SOEDA[2], HIROSHI YASUE[1]
[1]*National Institute of Agrobiological Sciences, Genome Research Department, 2 Ikenodai, Kukizaki, Inashiki, Ibaraki 305-0901, Japan;* [2]*Institute of Physical and Chemical Research (RIKEN), Tsukuba Institute, Gene Bank, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan*

Since the human genome organization was elucidated, much effort has been made to construct comparative map between swine and human. The determination of the synthenic relationship between swine and human chromosomes is extremely important for the selection of candidate genes responsible for traits. In the present study, we have attempted to use 910 STSs in HSA1p35-p36 (35Mb) for construction of a denser comparative map between human and swine chromosomes. Thirteen primer pairs of 910 STSs, which are evenly located in the HSA1p35-p36 spanning 35Mb, could be used for assignment of the corresponding STSs to the IMpRH. Eleven STSs were assigned to the framework markers of the IMpRH framework map in SSC6 with lod scores greater than 5 by two-point analysis. WI-20819 was revealed to link to SW1044 localized in SSCX with a lod score of 7.40; this result was consistent with that obtained by analysis using somatic cell hybrid panel. The remaining one, R91D18R, was suggested not to link to the framework markers of SSC6 but to link to MX1 localized in SSC13 with a lod score of 2.37. In order to elucidate most likely position of the 11 STSs in the framework map of SSC6, RH mapping score data of the 11 STSs together with those of SSC6 framework markers were subjected to the calculation using Carthagene software. This revealed that the alignment of the 11 STSs with a direction from SSC6cen to q-arm telomere was conserved in HSA1 with a direction from pter to centromere.

**D164**
**Integrated physical and linkage mapping of bovine chromosome 24**

ERCAN KURAR[1,2], JAMES E. WOMACK[3],

BRIAN W. KIRKPATRICK[1]
[1]*University of Wisconsin-Madison, Department of Animal Sciences, Madison, WI, USA;* [2]*Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey;* [3]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA*

We present herein a bovine chromosome 24 (BTA24) radiation hybrid (RH) map using 40 markers scored on a panel of 90 radiation hybrids. Of these markers, 29 loci were ordered with odds of at least 1000:1 in a framework map. An average retention frequency of 17.4% was observed, with relatively higher frequencies near the centromere. The length of the comprehensive map was 640 $cR_{5000}$ with an average marker interval of approximately 17.3 $cR_{5000}$. The observed locus order is generally consistent with currently published bovine linkage and physical maps. Nineteen markers were either Type I loci or closely associated with expressed sequences and thus could be used to compare the BTA24 RH map with human mapping information. All genes located on BTA24 were located on human chromosome 18 and supported the previously reported regions of conserved synteny. The comparative data revealed the presence of at least six conserved regions between these chromosomes.

**D165**

**Genetic diversity of Italian, French, Spanish and Nordic cattle breeds as assessed by AFLP markers**

R. NEGRINI[1], E. MILANESI[1], M. ZANOTTI[2], K. MOAZAMI-GOUDARZI[3], C. RODELLAR[4], I. OLSAKER[5], L.-E. HOLM[6], I. J. NIJMAN[7], J. A. LENSTRA[7], P. AJMONE-MARSAN[1]
[1]*Institute of Zootechnics, Catholic University of S. Cuore, Piacenza, Italy;* [2]*Institute of Zootechnics, University of Milano, Italy;* [3]*INRA, Jouy-en-Josas, France;* [4]*Facultad Veterinaria, Zaragoza, Spain;* [5]*Division of Genetics, The Norwegian School of Veterinary Science, Oslo, Norway;* [6]*Danish Veterinary Laboratory, Copenhagen, Denmark;* [7]*Faculty of Veterinary Medicine, Utrecht Univerity, The Netherlands*

The EU-sponsored Resgen project (CT98-118) is investigating genetic diversity in European cattle by assessing molecular markers and variation in selected genes. A total of 98 AFLP markers have been assayed on 160 cattle genotypes in order to estimate genetic variability within and between 8 cattle breeds from Italy (Chianina and Grigio-Alpina), France (Limousine -Italian strain- and Normande), Spain (Betizu and Menorquina), Norway (Telemark) and Denmark (Jutland). Expected heterozygosities were relatively high in Italian Limousine, Chianina and Grigio-Alpina (Het=0.24, 0.23 and 0.23, respectively), average in Normande and Betizu (0.21), and relatively low in Jutland, Menorquina and Telemark (0.19, 0.19 and 0.17, respectively). Gst value indicates that 77 % of the AFLP variation is maintained within breeds. Da distances between breeds range from 0.04 (Telemark-Jutland) to 0.10 Chianina-Betizu and in general reflect geographic distances between areas of origin. PCOOA based on Jaccard distance between individuals groups animals according to their breed of origin. However, it also indicates the genetic originality of the Chianina, which is probably the most ancient Italian autochtonous cattle breed, and of the Spanish Betizu, which is autochtonous in the Basque and Navarra regions.

**D166**

**Analysis of polymorphism of two horse genes potentially important in genetic resistance to intracellular bacteria**

JÁN MATIAŠOVIC, LEONA KŘENKOVÁ, PETR HOŘÍN
*University of Veterinary and Pharmaceutical Sciences, Institute of Animal Breeding and Genetics, Brno, Czech Republic*

For further association and expression analysis, two genes involved in resistance to intracellular bacteria were screened for polymorphic markers. The 5' UTR, 3' UTR and a part of the CDS region were screened within the *CD14* gene, encoding the LPS receptor. No polymorphism was found using PCR-SSCP and/or sequencing. The second gene, i*NOS,* was tested using PCR-SSCP, PCR-RFLP and sequence analysis. A part of this gene comprising intron 8 down to exon 10 was analyzed. A PCR-RFLP polymorphism for MspI due to a C/T substitution was found in intron 9. Homozygotes for both alleles as well as heterozygotes were identified. Inter-breed differences between Thoroughbred and Old Kladruber in allelic frequencies were observed.

**D167**

**Integration of Genetic and Radiation Hybrid maps of the pig: The second generation IMpRH maps**

DENIS MILAN[1A], THOMAS SCHIEX[1B], MARTINE YERLE[1A], ANETTE RINK[2], CRAIG BEATTIE[2], LEE ALEXANDER[3], RACHEL HAWKEN[3], LARRY SCHOOK[3], HIROSHI YASUE[4], DANIEL POMP[5], MERETE FREDHOLM[6], GARY ROHRER[7]

*[1]Cellular Genetics Lab & Biometry and Artificial Intelligence Lab., INRA, Castanet-Tolosan, France; [2]University of Reno, USA; [3]University of Minnesota, USA; [4]NIAS & STAFF, Tsukuba, Ibaraki, Japan; [5]University of Nebraska-Lincoln, USA; [6]Royal Veterinary and Agricultural University, Frederiksberg, Denmark; [7]USDA-MARC, Clay Center, Nebraska, USA*

More than 4500 markers, ESTs and genes have been mapped on IMpRH radiation hybrid panel and submitted to IMpRH Server before 30 March 2002, whereas 757 markers only were mapped on the first generation map (Hawken et al, 1999). To take advantage of the different resolutions observed on the genetic and the RH maps, maps were constructed with Carthagene software using SIMULTANEOUSLY genetic (MARC USDA families) and RH (3800 markers mapped on IMpRH panel) data. For each chromosome, a framework map was produced using a step-wise locus adding strategy (SWLA). The resulting map was tested by various procedures (flips, simulated annealing ...) to try to find alternate better maps and to check if the difference of LOD with the second most likely map is at least of 3. Frequently, using checking procedures, we were able to identify maps demonstrating that the framework map proposed by the SWLA strategy was not a framework map. Framework maps were also manually improved to ensure the best possible coverage of each chromosome. Additional markers were mapped relatively to the framework maps, and comprehensive maps were established.

The aim is to propose soon reliable second generation maps to assign and map additional markers and genes. Data and maps will be available on IMpRH Server (http://imprh.toulouse.inra.fr). Groups which contributes with at least 100 markers share authorship of this poster. A total of 19 groups submitted data on IMpRH Server.

**D168**

**Evaluation of paternity by microsatellite analysis over two breeding seasons in American bison bulls (*Bison Bison*) held under semi-natural conditions**

CATHERINE RODEN[1,2], GUY MOMMENS[3], HILDE VERVAECKE[1,2], LINDA VAN ELSACKER[1,2]

*[1]Centre for Research and Conservation, Royal Zoological Society of Antwerp, Antwerp, Belgium; [2]University of Antwerp, Department of Biology, Wilrijk, Belgium; [3]PolyGenLab, Malle, Belgium*

Extreme sexual dimorphism -shown by bison- has been theoretically related to a high variance in male reproductive success. The assumption that only some males breed a very large part of the female herd has been raised by studies in the wild, but has never been supported by genetic data. The American bison (*Bison bison*) became a near-extinction species at the beginning of the 20th century and knew a revival mostly through bison farming. Although the species has become economically important, there is still a great lack of information on their reproductive behaviour and capacity. During the breeding season, bison herds, consisting of females and calves, are joined by several adult males in search of mating partners. This natural pattern is mimicked under semi-natural conditions. The aim of this study is to assess the assumption of extreme male reproductive variance for bison bulls on a bison farm. We present paternity data of 4 groups (yr 1: n=19, 31, 38 and 52; yr 2: n=27, 36, 41 and 43) of American bison over two years held under semi-natural conditions in Belgium, Europe. By analysing blood, hair and tissue samples using eight to ten polymorphic microsatellites, the annual reproductive 'success' of the bulls is successfully determined. We compare the annual success between years and individuals. The use of microsatellites for paternity assignment has proven to be very useful in bison in spite of their relative low genetic variance due to their historic genetic bottleneck.

**D169**

**Development of microsatellite multiplexes for use in a Swiss alpine chamois (*Rupicapra r. rupicapra*) population study using primers designed from domestic Bovidae**

JEANNETTE MUNTWYLER, GABRIELA OBEXER-RUFF, CLAUDE GAILLARD, MARIE-LOUISE GLOWATZKI-MULLIS
*University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, Switzerland*

In order to enable a rapid and low-cost method to conduct population genetic studies in Swiss alpine chamois, we developed multiplex-PCR reactions. The molecular tool of choice for the genetic analysis in this study are microsatellite DNA markers designed from domestic Bovidae that amplified successfully in a cross-species approach. We have evaluated various microsatellite primers that demonstrate polymorphism in biodiversity and parentage studies in small ruminants (M.-L.G., personal communications; Saitbekova et al., 1999 & 2001). In addition, we are taking part in the 2001/2002 ISAG comparison test of sheep and goats. From all markers tested, we have selected 30 loci rendering sufficient polymorphism in a sample of 30 Swiss alpine chamois which can be amplified in multiplex reactions. Genotyping analysis was undertaken using an ABI 3100 genetic analyser. The multiplexes described may also be of interest for other genetic epidemiologic studies focused on alpine chamois (*Rupicapra r. rupicapra*) species.

**D171**
**Genetic diversity of European cattle breeds inferred from AFLP fingerprinting**

THE EUROPEAN GENETIC CATTLE DIVERSITY CONSORTIUM (I. J. NIJMAN[1], J. A. LENSTRA[1], G. MOMMENS[2], G. DOLF[3], P. WIENER[4], D. BURTON[4], J. L. WILLIAMS[4], K. MOAZAMI-GOUDARZI[5], D. LALOË[5], S. DUNNER[6], J. CANON[6], P. ZARAGOZA[7], C. RODELLAR[7], A. SANCHEZ[8], G. ERHARDT[9], O. JANN[9], C. WEIMANN[9], P. AJMONE-MARSAN[10], R. NEGRINI[10], A. VALENTINI[11], M. ZANOTTI[12], F. PILLA[13], A. BRUZZONE[13], D. IAMARTINO[13], I. OLSAKER[14], I. MICEIKIENE[15])
*[1]Faculty of Veterinary Medicine, Utrecht Univerity, The Netherlands; [2]PolyGenLab, Malle, Belgium; [3]Institute of Animal Breeding, Nutrition and Housing, University of Berne, Switzerland; [4]Roslin Institute (Edinburgh), Roslin, UK; [5]INRA, Jouy-en-Josas, France; [6]Facultad Veterinaria, Madrid, Spain; [7]Facultad Veterinaria, Zaragoza, Spain; [8]Facultad Veterinaria, Barcelona, Spain; [9]Department of Animal Breeding and Genetics, Justus Liebig Universität, Giessen, Germany; [10]Institute of Zootechnics, Catholic University of S. Cuore, Piacenza, Italy; [11]Istituto di Zootecnica, Universitá della Tuscia, Viterbo, Italy; [12]Instituto di Zootecnica, University of Milano, Italy; [13]Facoltà di Agragria, Campobasso, Italy; [14]Division of Genetics, The Norwegian School of Veterinary Science, Oslo, Norway; [15]Gyvunu Genetikos Laboratorija, Kaunas, Lithuania*

We present a preliminary analysis of the diversity of European cattle based on Amplified Fragment Length Polymorphisms (AFLP). As part of an EU project (Resgen CT 98-118), animals (n=20) of 30 cattle breeds covering Europe were sampled with West African N'Dama and Indian zebu (Bos indicus) as outgroups. This yielded two datasets: (1) 239 AFLP fragments, of which 165 were polymorphic in 20 animals per breed and (2) 329 fragments, of which 309 were polymorphic in a subset of 5 animals per breed. Genetic distances expressed as (1- Jaccard index or band sharing) and PCO plots clearly showed a separation between the zebu and cattle breeds. Distances between zebu and taurine breeds were about twice the distances between taurine breeds, but from the taurine breeds the West African N'Dama as well as the Hungarian Grey are closer to the zebus. Distances between breeds from the same country were often relatively low, suggesting an effect of the geographical distances. Intrabreed distance values of taurine breeds are comparable, indicating a similar genetic variability within each breed. However, in some breeds we found a high spread of the individual distances, which was also apparent from PCO plots and may be an indication of stratification. Intrabreed values are about 80 % of the interbreed values. This suggests that the heavy selection on production traits or other phenotypes in some of the breeds may have homogenized only a small part of the genome, while much of the total genetic variety still has been retained.

**D172**
**Linkage mapping of 4 *calpain* genes to chicken chromosomes**

FUMIHIKO OKUMURA[1], YOKO SHINBO[1], KANAKO YOSHIZAWA[2], KOTARO KAWABE[1], TAKESHI SHIMOGIRI[1], HIDEYUKI MANNEN[2], SHIN OKAMOTO[1], HANS H. CHENG[3], YOSHIZANE MAEDA[1]
*[1]Kagoshima University, Faculty of Agriculture,*

*Kagoshima, Japan; [2]Kobe University, Faculty of Agriculture, Kobe, Japan; [3]USDA/ARS, Avian Disease and Oncology Laboratory, East Lansing, USA*

*Calpain* is a $Ca^{2+}$-requiring cysteine protease. In animals, *calpain* forms a large gene family comprising more than 15 members. In recent studies, it is found that *mu-calpain* linked to postmortem tenderization of muscle in bovine. Our studies show *mu/m-calpain* activity differentiates between quail lines selected by body weight. As mentioned above, *calpains* are variable enzymes in livestock industry. Many *calpain* genes have already mapped to mammalian chromosomes, while only *p94* gene mapped to chicken chromosome 5 (GGA5). Here, we developed PCR-RFLP of 4 *calpain* genes expressed in chicken muscle (*mu-calpain*, *mu/m-calpain*, *m-calpain* and *p94*), and located their markers on 2 backcross families (East Lansing reference population and Kobe University resource family). In human, 3 *calpain* genes (*mu-calpain*, *m-calpain* and *p94*) mapped to HSA1, HSA11, and HSA15, respectively. *mu/m-calpain* gene is not identified in human. As a result, we could develop 3 *calpain* makers and obtain their segregation data from 2 families. These results showed that *m-calpain* and *mu/m-calpain* genes mapped to GGA3, and that *p94* gene mapped to GGA5 as previously described. Map distance from *m-calpain* gene to *mu/m-calpain* gene was about 30cM. It was found that *m-calpain* gene was located on the syntenic region of HSA1 previously reported. We found that *p94* gene significantly linked to RYR3 (ryanodine receptor 3) located on HSA15. Now, *m-calpain* gene is mapping to chicken chromosome.

**D173**

**Prevalence of the Severe Combined Immunodeficiency Disease (SCID) in Arabian horses raised in Brazil**

DENISE A. A. OLIVEIRA, CLÁUDIA S. TEIXEIRA, MARCELO Y. KUABARA

*Escola de Veterinária da UFMG, denise@vet.ufmg.br, Belo Horizonte-MG, Brazil*

In the present study, 238 Arabian horses raised in Brazil, were genotyped using the PCR technique to detect the presence of the mutant gene responsible for the SCID (Severe Combined Immunodeficiency Disease). The results showed 96.2% of normal horses (229/238) and 3.8% of SCID carriers (9/238). Homozygous recessive animals were not found because all horses analyzed were older than four month, and at this age the affected animals were already dead. Checking the carriers' pedigrees it was possible to confirm the participation of one stallion identified as the possible disease disseminator. Considering the economic losses that could come from the unknowing use of carrier stallions, the results show the importance of the adoption of a control program by the Brazilian Arabian Horse Registry (ABCCA).

**D174**

**Efficacy and reliability of nine DNA microsatellite analysis in Brazilian Zebuin Cattle**

KÁTIA T. SOUZA, CRISTIANE L. OLIVEIRA, VALÉRIA P. M. CALIJORNE, MÁRCIA C. B. N. CAMPOS[1], CARLA L. P. MENEZES[1,2]

*[1]Biocod Biotecnologia Ltda.; [2]FAPEMIG*

It is important for bovine DNA testing laboratories to provide the cattle industry with accurate estimates of the efficacy and reliability of DNA tests offered. To address these issues for genealogy e registry service of the Cattle Breeders, discriminate power were obtained from breed panel data.

We have tested multiplex combinations over our Zebuin breed panels to provide an indication of the efficacies of selected markers for parentage and paternity testing. The Zebuin Breed panels consist of 80 samples from unrelated animals of Brahman, Nelore and Guzera breeds.

The nine loci are amplified in two PCR reactions: a multiplex with 05 microsatellites (TGLA 227, TGLA 126, TGLA 122, M 1824 and ETH 10) and a multiplex with 04 (TGLA 57, ETH 225, TGLA 53, BM 1818).

The PCR reactions were performed in 10 µl containing: 1 µl of STR 10x buffer, 0,5U of Taq polymerase (Promega Corp.),working solutions of primers in variable concentrations (1 a 2,5 µM/sample) and 50 ng of DNA

PCR cycles were 95ºC 1 min, 55ºC 1 min, 72ºC 2 min at 35 times. Gel electrophoresis and genotype determination were performed on the ALF express sequencer (Amersham Science Corp.).

We find out that Power of Discrimination of two combined multiplex is 0,99999999995 in Brasilian Zebuin Cattle.

**D175**

**The polymorphism in the 5'-flanking region of chicken prolactin gene**

YONG LIANG, GUANFU YANG, XIQUAN ZHANG

*College of Animal Science, South China Agricultural University, Guangzhou, 510642, China*

Prolactin is a peptide hormone secreted by pituitary glands and plays important roles in reproductive and growing processes of the individual. The signal peptide cleavage site of deduced amino acids from preprolactin cDNA of chicken prolactin (cPRL) changed from Leu-Pro-Ile-Cys in quality-type Yuehuang and Taihe Silkies to Pro-Pro-Ile-Cys in layer-type Isa Brown (Zou *et al.*, 2001). The mutation of the site made signal peptidase to have no effect on the preprolactin, which could cause Isa Brown to have no broodiness. Other nucleotide changes between the chicken cDNAs were often present in the 5'- flanking region, signal peptide, and 3'-flanking region. The polymorphism in 5'-flanking regions of cPRL was studied in quality-type Yuehuang chickens, Taihe Silkies and layer-type Leghorn chickens in the present study. Three pairs of primers were designed based on the sequence in 5'-flanking region of broiler prolactin gene (Ohkuo *et al.*, 2000). The sequences of these primers were primer pair A 5'TTACCTCCTGGCCTTTGTG3' and 5'GTTCTGGGCCTCTCACTT3', primer pair B 5'TCTCCCACTAGACTCTTTC3' and 5'CTGTGTGTTTGTCTCCAT3', and primer pair C 5'CTGTCCCTGTTTCTCAAC3' and 5'GATGACTTGCTCTACCAG3'. The polymerase chain reactions (PCR) were conducted in Mastercycler (Enppendorf Co. Ltd.) according to the following procedures: 94ºC 7 min, 30 cycles of 94ºC 50s, 59.5ºC (54ºC for primer pair B and 62ºC for primer pair C) 50s and 72ºC 2.5 min. PCR products were directly used for sequencing. The homology of the sequences from three types of chickens was analyzed with DNASIS (version 3.0). The analysis of homology showed that there were great differences between various types of chickens. A 24bp insert in 5'-flanking region of prolactin gene was found in layer-type Leghorn but not found in quality-type Yuehuang chickens or Taihe Silkies. The above result was confirmed with SSCP method.

**D176**

**Contribution to the rabbit R-banded karyotype nomenclature by FISH localization of 23 chromosome specific genes on both G- and R-banded chromosomes**

H. HAYES[1], C. ROGEL-GAILLARD[2], C. ZIJLSTRA[3], N. A. DE HAAN[3], C. URIEN[2], N. BOURGEAUX[2], M. BERTAUD[1], A. BOSMA[3]

[1]*Laboratoire de Génétique biochimique et Cytogénétique;* [2]*Laboratoire de Radiobiologie et Etude du Génome, INRA, Jouy-en-Josas, France;* [3]*Department of Cell Biology and Histology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Banding patterns of rabbit chromosomes have been described in detail using different G-, Q- and R-banding techniques, however, both international nomenclatures (1976 & 1981) are based on G-banded chromosomes. The INRA laboratory has launched a project on the rabbit genome map involving large-scale localization of type I and II markers by FISH on R-banded chromosomes. Therefore, an R-banded rabbit karyotype nomenclature in perfect agreement with the 1981 G-banded nomenclature is a prerequisite. In order to establish unambiguous correlations between G and R-banded rabbit chromosomes we have defined 23 marker genes, one per chromosome, and localized them precisely on G and R-banded chromosomes. The choice of these genes was based on reciprocal chromosome painting data between man and rabbit (Korstanje et al 1999). A 3-genome equivalent rabbit BAC library constructed by Rogel-Gaillard et al (2001) was screened by PCR with specific primers to identify and isolate clones for each gene. The BAC clones were then labelled with biotin, hybridised to rabbit chromosomes treated either for G or R bands and hybridisation sites were revealed by immunofluorescence detection. Here, we present the 23 chromosome specific genes, their localization by FISH and updated schematic representations of R-banded rabbit chromosomes.

**D177**

**Effect of *POU1F1* polymorphism on meat quality traits in Piemontese cattle**

LILIANA DI STASIO[1], STEFANO SARTORE[2], GIANLUIGI DESTEFANIS[1], ALBERTO BRUGIAPAGLIA[1]

[1]*University of Torino, Department of Animal Sciences, Torino, Italy;* [2]*University of Torino, Department of Animal Production, Torino, Italy*

The *POU1F1* gene encodes a transcription factor regulating the expression of growth hormone and therefore it has been suggested as a putative candidate gene for genetic variation of meat production traits. A preliminary investigation in Piemontese cattle suggested that *POU1F1* polymorphism could affect the variability of some qualitative characteristics of the meat. To verify the results on a larger sample, we examined 105 subjects of both sexes, slaughtered in two slaughterhouses. For each subject the *POU1F1* genotype was analysed by PCR-RFLP with *Hinf*I. In addition, sarcomer length, drip losses, cooking losses and tenderness (as Warner-Bratzler shear) at 1, 3, 7 and 11 d *post mortem* were determined on LTL muscle. The data were analysed by GLM procedure, with *POU1F1* genotype, sex and slaughterhouse as fixed effects. Two alleles were found and the frequencies of the genotypes were 3.8% for *AA*, 52.4% for *AB* and 43.8% for *BB*. Significant ($P<0.05$) differences among genotypes were observed for WBs at day 3 (6.6, 9.1, 10.8 kg for *AA*, *AB* and *BB* respectively), day 7 (5.8, 7.7, 8.8 kg) and day 11 (4.5, 6.7 and 7.6 kg). These results confirm the effect of *POU1F1* locus on meat tenderness, with the *AA* genotype associated to more favourable values. Also the sarcomer length was affected by *POU1F1* genotype (1.63, 1.75 and 1.84 μm for *AA*, *AB* and *BB*; $P<0.05$). On the contrary, the relationships with cooking losses were not confirmed. The effect of the slaughterhouse was always highly significant.

**D179**

**Independent origin of three microdeletions in RPGR exon ORF15 of canids**

BARBARA ZANGERL, QI ZHANG, JENNIFER JOHNSON, GREGORY M. ACLAND, GUSTAVO D. AGUIRRE

*Cornell University, James A. Baker Institute for Animal Health, Ithaca NY, USA*

We investigated the possible ancestral origin of mutations previously identified in the RPGR exon ORF15, two of which, a five and two base pair deletion , were causally associated with the retinal degenerations XLPRA1 and XLPRA2, respectively. The XLPRa1 deletion is shared between al least two different dog breeds. To determine phylogenetic relationship between the different mutation, we used an SNP based approach, identifying SNPs from BAC clones (RPCI81 library) associated with markers in a 30 cM interval, which includes the 500 kb XLPRA zero recombination region. 10 identified SNPs were screened in 86 dogs from breeds identified with XLPRA, and about 50 DANN samples collected from other *Canadiae* species to serve as outgroups, discriminating different alleles by either restriction digestion or using the SnaPshot ™ Multiplex Kit. Resulting haplotypes were analyzed to infer history of the observed RPGR ORF 15 mutations. Here we present data showing that each of the observed deletions in ORF 15 is linked to a distinct haplotype background in the canine XLPRA interval concluding that the three different types of mutations identified in canine RPGR ORF 15 arose independently in husky and Samoyed dogs originated in a common ancestor. Furthermore, SNP analysis of the RP3 region in several *Canadiae* species demonstrates phylogenetic evolution of this chromosomal segment.

**D180**

**Hungarian Gray Cattle Blood Group and DANN Allele Frequency in Hungary**

ALICE GYURMAN; BEÁTA BÁN

*National Institute for Agricluture Quality Control*

A Hungarian gray cattle is a native breed in Hungary. The number of adult animals is less than 5000. In the last 40 years we have been examining the parentage of each offspring yearly on the basis of the blood group determination to avoid the inbreeding as much as possible. On the basis of B blood system this breed shows big similarity, we have found altogether 16 different B alleles. The degree of homozigosity was 16% calculated on the basis of this system ( it is much higher than in other breeds in Hungary).

We started to examine the DANN microsatellites last year, using a StockMarks Paternity Typing System bovine II. version 2, and we calculated the gene frequencies of the different microsatellite loci (N=57). The most polymorphic locus was TGLA53 (we found 11 alleles) most homogenous one was the BM 1824 (only four alleles).

We summarize the gene frequencies of transferrin, haemoglobin, 8 blood group and 11

microsatellite system in a table.

**D182**
**Characterization of two SNPs (single nucleotide polymorphisms) in the porcine INSL3 gene and their exclusion as a common genetic basis of hernia inguinalis/scrotalis in pigs**

CHRISTOPH KNORR, ULRIKE PETERS, BERTRAM BRENIG
*Georg-August-University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany*
The INSL3 gene encoding Leydig cell insulin like hormone is an important candidate gene for congenital disorders of the reproductive tract in pigs. Comparative sequencing using phenotypically *hernia inguinalis/scrotalis* affected and unaffected animals showed that the porcine gene is remarkably conserved. No polymorphisms were found in the two exons or in the intron. Two SNPs were detected in the promoter region (*G-224A and A-164C)* and fast screening methods were developed for large scale studies. The A allele at position -224 is newly described and represents a rare allele with a frequency of 5% (n=375). The C allele at position -164 shows an equal distribution to the previously known A allele at that position. Screening of the two SNPs in a population of *hernia inguinalis/scrotalis* affected pigs (n=223) revealed that the SNPs can be excluded as a common genetic basis for this congenital disorder.

**D183**
**Molecular Variance In Continental Crossbill Species (Loxia)**

ANNETTE MUELLER[(1)], INA PFEIFFER[(1)] & BERTRAM BRENIG[(1)]
*[(1)]University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany*
Crossbills are birds, whose habitat ranges from the forest belt of north-east Europe and north-central Asia to North America and the Western Palaearctic. Their diet consists mainly of conifer seeds and species are defined by their bill depth and colouring of feathers. The aim of this study was to determine the genetic divergence of continental crossbill species (Loxia) and their phylogenetic relationship. Tested material included 46 blood or organ samples of five Loxia species. Our special interest was aimed to determine the genetic relationship of Loxia curvirostra rubrifasciata to other Loxia species. Ornithologists disagree whether Loxia c. rubrifasciata is a subspecies of Loxia, a hybrid of the common crossbill with the two-barred crossbill or just an aberration of the common crossbill. There are morphological indications for all three assumptions. We tested sample material of five Loxia species for allelic variations in highly polymorphic microsatellite loci and for mitochondrial DNA sequence variations

**D184**
**Species determination using mtDNA sequencing and PCR-RFLP of DNA from forensic material**

INA PFEIFFER[(1)], JOACHIM BURGER[(2)] & BERTRAM BRENIG[(1)]
*[(1)] University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany[(2)] Johannes Gutenberg University, Institute of Anthropology, Mainz, Germany*
Determination of the species origin of unknown material, e.g. dry blood drop on a leaf, is sometimes a task in forensic science. Usually, short fragments of conserved regions of mitochondrial DNA are amplified using PCR and then sequenced. In our case a detailed phylogenetic analysis of a blood drop was necessary. The required asservates normally contain particulary degraded DNA. Therefore, we have developed a PCR with mtDNA for sequencing and a less expensive PCR-RFLP method. For the interspecific sequence polymorphism we chose a part of the D-loop region for sequencing and a 200 bp fragment of the mt-cytochrome b gene. Both PCR methods allowed a reliable species identification from forensic material, e.g. a blood drop on a leaf, even those containing degraded DNA.

**D185**
**Genetic differentiation of several dog breeds by analysis of mitochondrial DNA**

INGER VOELKEL , INA PFEIFFER & BERTRAM BRENIG
*University of Goettingen, Institute of Veterinary Medicine, Goettingen, Germany*
Because of the dog`s increasing importance as "man´s best friend", there is growing interest in assigning blood, hair or saliva samples to the particular dog breed. This could not only

be a significant progress for forensic science but would also facilitate the differentiation between purebreds and crossbreds. We investigated the mtDNA of several wolves and 10 different dog breeds. For our study we chose wolf-like breeds as well as breeds with antipodal phenotypes, e.g. dachshund or boxer, including at least 30 non-related individuals. The sequence analysed was part of the mitochondrial D-loop region, with a length of about 250 bp. So far, we have sequenced mtDNA of 137 dogs belonging to different breeds. In the examined region we were able to identify at least 4 diagnostic nucleotide positions. Future studies will include the analysis of the complete D-loop region for each dog to enable a breed-screening.

**D186**
**The Polymorphism of β-Lactoglobulin Gene in Several Sheep Breeds by PCR-RFLP**

NASSIRY M.R.[1*] , MARZANOV N.S. [2]
*Animal science Dept., College of Agric., Ferdowsi Univ., P.O.Box: 91775-1163, Mashad, Iran, All-Union Research Institue of Animal Husbandary RF-142012 P.O. Dubrovitsy Podolsk District, Russia*

In the last years there has been a considerable interest in milk protein gene polymorphisms because of their potential use as genetic markers to improve the efficiency of selection for quantitative traits. The aim of this work was to analyze the genotype distribution of β-Lactoglobulin in sheep. A polymerase chain reaction assay has been used for genotyping β-Lactoglobulin A and B variants in Oparinsky, Sovite merino and Karakol sheep. DNA was extracted from blood of 58 animals. For PCR of genomic DNA , primers, wich amplified a 452 bp region of the ovine β-Lactoglobulin gene from intron II were chosen. The genetic variants A and B differ in an amino acid at position 38 (Tyr to Hys) and this base substitution gives rise to a RsaI polymorphism. The genotypes distribution in Oparinsky. Sovite merino and Karakol sheep were 20%, 30%, and 44% for AA, 40%, 45% and 56% for AB and 40%, 25% and 00% for BB respectively. The populations were in Hardy-Weinberg equilibrium.

**Key Words**. β-Lactoglobulin, Russian sheep, PCR-RFLP.

# Section E: Associations between markers and traits

## E001
## Identification of three single nucleotide polymorphisms in the chicken *IGF1* and *IGF2* genes and their association with growth traits

MARCEL AMILLS[1], NEUS JIMÉNEZ[1], DANIEL VILLALBA[2], MARC TOR[2], ESTER MOLINA[2], DOLORS CUBILÓ[2], CARINA MARCOS[1], AMADEU FRANCESCH[3], ARMAND SÁNCHEZ[1], JOAN ESTANY[2]
*[1]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Departament de Producció Animal, Universitat de Lleida, Lleida 25008, Spain; [3]Unitat de Genètica Avícola, Centre Mas Bové, IRTA, Reus 43280, Spain*

The polymorphism of the chicken *IGF1* and *IGF2* genes has been analysed in two genetically diverse chicken lines (PN and MN) of the Penedesenca breed. The PN and MN lines reach a live weight of 2.5 and 1.3 kg at 11 weeks. Live weight, average daily gain and feed efficiency were recorded at 44, 73 and 107 days. Plasmatic IGF1 concentrations were determined at 73 days by using an enzyme-linked immunoassay. We sequenced the 5'end of the *IGF1* gene and exon 2, intron 2 and exon 3 of the *IGF2* gene in three individuals from each line. Moreover, we amplified the chicken *IGF1* and *IGF2* cDNAs from reverse transcribed total RNA and sequenced them forward and reverse. We identified one replacement A→ C at the 5'end (SNP1) of the *IGF1* gene. In addition, two silent mutations were found at the *IGF2* gene. The first one was a substitution C → T at exon 3 (SNP2), whereas the second one was a G → A replacement at intron 2 (SNP3). The *IGF1* and *IGF2* polymorphisms were typed in both lines (n = 60, in each line) by using the *Hinf* I (SNP1) and *Hsp*92 II (SNP2) restriction enzymes, respectively. A primer-extension based protocol was employed to detect SNP3. Significant associations ($p < 0.05$) were found between SNP1 and average daily gain at 107 days and feed efficiency at 44 days, 73 days and 107 days. Any of these associations were simultaneously found in both lines, a feature that may indicate that they are the result of linkage disequilibrium. No association was found between the identified polymorphisms and plasma IGF-I concentration.

## E002
## Mapping susceptibility to anal atresia in the pig

PAMELA CASSINI[1], PAUL BOETTCHER[2], TETSUO HORI[3], HARUO OHKAWA[3]; BIANCA CASTIGLIONI[2], LEIF ANDERSSON[4], ELISABETTA GIUFFRA[1]
*[1]FPTP-CERSA, Centro Studi Ricerche Agroalimentari, Segrate, Italy; [2]CNR-IBBA, Instituto de Biologia e Biotecnologia Agraria, Segrate, Italy; [3]University of Tsukuba, Dept. of Pediatric Surgery, Tsukuba, Japan; [4]Swedish University of Agricultural Sciences, Dept. of Animal Breeding and Genetics, Uppsala, Sweden*

Anal atresia is a multi-factorial congenital disease, affecting approximately 1 in 5000 live births in humans. Am similar malformation naturally occurs in pigs, which can be considered a suitable model of investigation. A pedigree with an increased of the disease has previously been develped by selective breeding (Hori et al. 2001, *J. Pediatr. Surg.* 36:1370-4). Simulation was used to empirically estimate the likelihoods of different genetic models underlying the abnormality. The primary motivation of trying to establish the most likely genetic model was to aid the statistical analysis of the genome scan experiment. Each model was used in an attempt to reconstruct all of the phenotype observed in the pedigree. Ten million replicates were generated for each model and the number of correct reconstructions of the phenotypes was taken as the measure of the relative likelihood of the respective model. Genetic models differed in terms of numbers of loci, distributions of allelic effects, levels of dominance and penetrance, and importance of non-genetic effects. Preliminary results confirm indication of recessive allelic effects and suggest the presence of multiple loci, with incomplete penetrance. The existing microsatellite dataset was implemented with new markers to fully cover the genome and analysed under the new assumptions. Preliminary results from physical mapping of candidate genes belonging to the *Sonic Hedgehog* transduction system are reported.

**E003**

**A proposed model for the interaction of the scurred and polled/horned loci**

MIKA ASAI-COAKWELL[1], SHEILA M. SCHMUTZ[2]
[1]*Swiss Federal Institute of Technology, Institute of Animal Sciences, Zurich, Switzerland;* [2]*University of Saskatchewan, Department of Animal and Poultry Science, Saskatoon, Canada*

Scurs are bony growths found in the area of horn development in *Bos taurus* breeds. They are undesirable to the cow-calf and feedlot industries, which are moving towards a polled population due to economic losses caused by horns. However, scurs have been difficult to eradicate because of their complex inheritance. Scurs are sex-influenced and masked by the presence of horns. Homozygosity of the polled allele has also been suggested to mask scurs in the heterozygous condition. We were able to verify that scurs are masked using two polled male calves that were offspring of a scurred dam, an obligate homozygote for scurs. Our data suggest that homozygosity of the polled allele masks scurs in the homozygous condition as well. Eight scurred females in our study could be shown to carry the horned allele through a horned parent or horned offspring, and no scurred animals were shown to be homozygous polled. Following a genome scan using 17 embryo transfer families, the scur locus was mapped to bovine chromosome 19, whereas the polled/horned locus was mapped to the centromeric region of BTA1. The locus was further fine mapped to a location between microsatellite markers *BMS2142* (LOD =4.46) and *IDGVA46* (LOD =2.56) using 27 offspring in 5 families. We therefore postulate a receptor-ligand model to explain the interaction between the polled and scurred loci, which also accounts for the sex-influenced nature of the scurred phenotype.

Detection of QTL affecting milk production in 6 Dairy Bull DNA Repository grandsire families

**E004**

**Detection of QTL affecting milk production in 6 Diary Bull DANN Repository grandsire families**

MELISSA S. ASHWELL[1], DAVID W. HEYEN[2], YANG DA[2], TAD S. SONSTEGARD[1], CURTIS P. TASSELL[1], HARRIS A. LEWIN[2]
[1]*USDA-ARS, Gene Evaluation and Mapping Laboratory, Beltsville, Maryland, USA;* [2]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA*

Originally two research groups conducted independent genome scans in Dairy Bull DNA Repository grandsire families to identify quantitative trait loci (QTL) affecting economically important traits. Each group selected eight families for study, six that were common across both studies. We report putative QTL affecting milk production traits using the merged data from the two groups. The six common families were genotyped at 367 microsatellite markers. Genome coverage was estimated to be 2713.5cM (90%), with an average spacing of 7.4cM. QTL Express software (http://qtl.cap.ed.ac.uk) was used for regression interval mapping within each family. Phenotypic traits included daughter deviations for milk, protein and fat yields, protein and fat percentages, somatic cell score and productive life, weighted by their respective reliabilities. Permutation was used to calculate chromosome-wide $P < 0.05$ and $P < 0.01$ significance thresholds. One hundred seven putative marker effects were identified at $P < 0.05$, 36 of these at $P < 0.01$. Highly significant effects ($P << 0.01$, F-statistic > 15) were found on chromosome (BTA) 3 affecting fat percentage and protein yield, BTA6 affecting protein percentage, and BTA14 affecting fat percentage and yield. Interval analysis of the merged dataset identified putative QTL not detected in the separate studies. QTL identified in this study may be useful for marker-assisted selection to improve milk production and manipulate milk protein and fat components.

**E005**

**Construction of a physical map along the porcine chromosome 7 q .**

ANGELA BARBOSA[1], CHRISTINE RENARD[1], CELINE URIEN[1], NOËLLE BOURGEAUX[1], JEAN CLAUDE SAVE[1], CARINE GENÊT[2], DENIS MILAN[2], FRANÇOIS PIUMI[1], CLAIRE ROGEL-GAILLARD[1], PATRICK CHARDON[1]
[1]*Laboratoire de Radiobiologie et d'Etude du Génome INRA CEA Jouy en Josas, France and* [2]*Laboratoire de Génétique Cellulaire, INRA, Castanet Tolosan, France*

Several QTLs in the pig have been recently fixed between SLA and the SO102 markers on the long arm of SSC7. In order to find new markers and precise the QTL locations, a physical map of the chromosomal segment was extended from the SLA class II region toward the telomere. PCR screening of a BAC library with primer pairs designed from human genes known to map to HSA6 and with chromosome walking primers, lead to a sorting of 111 BACs distributed in 6 contigs. Several primers used for BAC screening were tested on the ImpRH panel to ensure the relative location of the clones. The closest contig from SLA encompassed 50 BACs representing about 1.3 MB. It harbors 10 loci, including the TAPASIN locus and the SW1856 and SW2019 markers. Whereas in humans the DNA segment between TAPASINE and HLA DPA is 150 kb long, the orthologous porcine DNA segment appears to be larger than 1 MB. One of the synthenic breakpoints has been assigned close to SW2019. A second 1.3 MB long contig made of 22 clones contains ZNF76 plus 8 additional loci. Alignment of BAC-end sequences on the human draft sequence revealed a straight conservation of the distance between genes for this region. Further along the chromosome, we mapped successively 4 contigs of 16, 13, 4 and 7 BACs characterized by the NDR, PIM, NFYA and GLO loci, respectively. New markers have been characterized for all contigs. Thus with BACs covering the SLA region we have now assigned about 230 BACs in the peri-centromeric region of SSC7.

**E006**

**Effects of variation in the FUT1-Gene on various Traits in Swine**

STEFAN BINDER[1], KAY-UWE GÖTZ[2], GEORG THALLER[1], RUEDI FRIES[1]

[1]*Lehrstuhl für Tierzucht, Technische Universität München, Freising-Weihenstephan, Germany*

[2]*Bayerische Landesanstalt für Tierzucht, Grub, Germany*

Oedema disease is caused by the adherence of *E. coli* strains to *ECF18R* receptors in the porcine intestinal tract. The *FUT1*-gene was identified as a candidate for the expression of these receptors. Resistant and susceptible animals can be distinguished by a variation in this gene. Because of strong linkage disequilibrium between the *FUT1*- and *MHS*-locus and the known effects of the *MHS*-genotype on production traits, we investigated the influence of the *FUT1* variation on these traits. We genotyped 813 DL (German Landrace), 576 PI (Piétrain) and 68 DE (German Large White) from 332 boars. The genotype effect on production traits were estimated with PROC GLM of the SAS software package using two different regression models. For the stress resistant DL and DE we applied a model including effects of test year, test month, sire, test station and the *FUT1*-genotype. When analysing the PI animals, the effect of *MHS*-genotype were added to the model. No significant effects were found for the growth trait ADG (average daily gain), the carcass composition traits SF (sidefat thickness), FA (fat area) and CL (carcass length) and for the meat quality trait PH24MLD ($pH_{24}$ musculus longissimus dorsi). However, the PH1MLD ($pH_1$ musculus longissimus dorsi) showed a significant *FUT1* effect ($p=0,026$) for the PI breed. As expected, we found high significant effects of the *RYR* receptor ($p<0,001$) on the meat quality traits, FA and CL, whereas there was no effect on SF and ADG.

**E007**

**Genes controlling appetite show interdependencies in allele frequency in beef cattle**

FIONA C. BUCHANAN, TRACEY D.THUE, SHEILA M. SCHMUTZ

*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada*

Previously we have identified SNPs in two genes, leptin (*LEP*) and corticotrophin-releasing hormone (*CRH*). These genes are associated with the control of appetite. They comprise only a small part of elaborate pathways. For example, CRH indirectly releases glucocorticoids which stimulates the release of leptin that in turn decreases appetite. CRH has been shown to stimulate expression of alpha melanocyte stimulating hormone (□MSH) the agonist for melanocortin-4 receptor (MC4R) which reduces appetite. We genotyped 116 steers with DNA tests for *LEP* (BTA4), *CRH* (BTA14) and *MC4R* (BTA24). We propose that genotypes at unlinked genes from pathways controlling body weight may well be coadaptive. Their allele frequencies are not independent of each other which is referred to as interchromosomal coadaptation.

Genotypic frequencies not in Hardy-Weinberg Equilibrium (HWE) would support this. Genotype combinations were analysed by chi-square to determine whether or not the observed were significantly different from expected HWE ratios. Genotype frequencies were not in HWE between *CRH* and *LEP* (P = 0.0001) or *LEP* and *MC4R* (P = 0.0001). This suggests that selection for carcass composition and/or growth has altered gene frequencies at several related genes in current North American beef cattle.

**E008**
**Feather pecking behaviour and stress response in Laying hens: a QTL-analysis**

A.J. BUITENHUIS[1], T.B. RODENBURG[2], Y.M. VAN HIERDEN[4], B. ASK[1], M. SIWEK[1], S.J.B. CORNELISSEN[1], M.G.B. NIEUWLAND[3], H. VOS[1], P. DE GROOT[1], S.M. KORTE[4], P. KOENE[2], H. BOVENHUIS[1], J.J. VAN DER POEL[1]
*[1]Animal Breeding and Genetics Group; [2]Ethology Group; [3]Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands; [4]Department of Behaviour, Stress Physiology and Management, Institute for Animal Science and Health (ID-Lelystad BV), Lelystad, The Netherlands*
The concern for animal welfare in the West European countries results in a change in housing systems for laying hens from battery cages to free-range systems. Feather pecking (FP) behaviour is a major problem in free range housing systems. In order to look for genes involved in FP behaviour a QTL experiment was designed. The F2 population originates from a cross between randomly chosen birds of a high FP line (HFP) and a low FP line (LFP). Reciprocal crosses were made between the HFP and the LFP line to generate the F1 animals. From the F1 animals 7 males were selected and mated to 28 females to produce 650 F2 hens. The birds have been tested for FP behaviour in a social FP test at 6 weeks and 30 weeks of age, respectively. The corticosterone response to manual restraint was measured at 32 weeks of age as a measure for coping strategy. To date we have finished the genotyping of 180 microsatellite markers and phenotyping of the F2 population. By combining the genotypic and phenotypic data from this experiment we should be able to identify the chromosomal regions involved in FP behaviour and corticosterone response to manual restraint.

**E010**
**Quantitative trait loci for birth weight, longissimus muscle area, and marbling on bovine chromosome 5.**

EDUARDO CASAS, JOHN W. KEELE, STEVEN D. SHACKELFORD, ROGER T. STONE, MOHAMMAD KOOHMARAIE
*USDA-ARS, U. S. Meat Animal Research Center, Clay Center, Nebraska, U.S.A.*
A study to detect quantitative trait loci (QTL) on bovine chromosome 5 (BTA5) affecting growth, carcass composition and meat quality traits was pursued. Thirteen microsatellite markers were genotyped on 547 progeny from a Brahman X Hereford sire mated to mostly composite (MARC III) dams. Traits analyzed were birth weight (kg), marbling, and longissimus muscle area ($cm^2$). Significant QTL were detected when the expected number of false positives (ENFP) was less than .05 (F-statistic greater than 16.6), and suggestive when the ENFP was less than 1 (F-statistic between 10.0 and 16.59). The effect of the QTL on the traits was measured in standard deviation units (SD). Significant QTL were detected for birth weight (ENFP= .0007) at 57 cM from the beginning of the linkage map, and for longissimus muscle area (ENFP= .049), at cM 53. Suggestive evidence for the presence of a QTL for marbling (ENFP= .2) was detected at cM 75. The effect of the QTL were 0.5 SD, 0.4 SD, and 0.36 SD, for birth weight, longissimus muscle area, and marbling, respectively. Before implementing marker-assisted selection programs, the effect of the QTL on BTA5 needs to be estimated in additional target populations.

**E011**
**Association of genetic markers in candidate genes with growth and carcass traits in Korean cattle (Hanwoo)**

E.R. CHUNG[1], Y.S. KIM[1], W.T. KIM[1], S.K. HAN[2]
*[1]Laboratory of Animal Molecular Genetics, Division of Applied Animal Science, College of Life Science and Natural Resources, Sangji University, Seoul, Korea; [2]Laboratory of Molecular Genetics, Department of Dairy Science, College of Animal and Life Science,*

*Konkuk University, Seoul, Korea*
Candidate genes are targets for genetic marker studies because of their biological significance on the quantitative traits of interest. The possible association between growth and carcass traits differences and genotype variations in growth hormone(GH), growth hormone receptor(GHR), growth hormone releasing hormone(GHRH), leptin(Lep), myogenic factor 5(Myf5), heart-fatty acid binding protein(H-FABP) and calpain(Cal) genes was examined in 430 Korean cattle from National Livestock Research Institute, R.D.A. Performance traits analyzed were growth traits(birth weight, 6, 12 and 18 month weights and average daily gain) and carcass traits(carcass weight, carcass percentage, backfat thickness, eye muscle area, marbling score and grade of meat quality). Genetic markers of candidate genes were determined by PCR-RFLP or SSCP techniques. Preliminary data analysis was performed using the GLM procedure of SAS computer program with a linear model. Allele frequencies were compared between high and low grade of meat quality using contingency to the chi-square tests. There were no significant associations between genotype frequencies of candidate genes and any of the above traits. However, significant differences($p<0.05$) in allele frequencies for the GH, Lep and Myf5 genes were observed between the two groups selected for high and low grade of meat quality. Differences in gene frequencies of A and B alleles between high and low groups were 0.88 and 0.12 and 0.76 and 0.24 for GH, 0.51 and 0.49 and 0.64 and 0.36 for Lep and 0.42 and 0.58 and 0.56 and 0.44 for Myf5, respectively. Further studies are need to confirm the significant associations seen in this study.

**E012**
**Population-wide linkage disequlibrium between a SNP and a OTL affecting milk protein production one bovine chromosome 6 in Israeli-Holsteins**

MIRI COHEN, EYAL SERROUSSI, MICHA RON, MISHA REICHENSTEIN, ALLA PLIS-FINAROV, MOSHE SHANI, JOEL I. WELLER
*Institute of Animal Sciences, ARO, The Volcani Center, Bet Daga, Israel*
A QTL affecting protein content and percentage was detected in the Israeli Holstein population by a daughter design analysis. This QTL was localized to a confidence interval of 4 cM near the center of bovine chromosome 6 by interval mapping. A total of 6,524 ESTs expressed preferentially in the mammary gland of mice were bioinformatically selected from 52,000 clones of two mammary gland cDNA libraries. Of these ESTs, only kiaa0914 was included in the genes identified from the human physical map of the region syntenic to the QTL confidence interval. A total of 388 Israeli-Holstein bulls were genotyped for a SNP located in intron 9 of this gene, in which Adenosine was replaced by Guanine. The frequency of the G allele was 44%. The effect of this polymorphism on the sire genetic evaluations was analyzed by a model that also included the effect of sire birth date to account for genetic trend. The effect allele substitution was significant ($p<0.01$) for fat concentration, and highly significant ($p<0.0001$) for protein concentration, but did not significantly affect milk, fat, or protein yield. The substitution effects were 0.045% fat, and 0.035% protein. The A allele was associated in increased fat and protein concentration. The profile of effects observed was similar to the effects found previously by the daughter-design analysis, but accounts for only half of the observed QTL effect. Thus this mutation is closely linked to the QTL polymorphism.

**E013**
**Mapping quantitative trait loci for twinning in Holstein dairy cattle**

JENIFER CRUICKSHANK[1], MARGARET DENTINE[1], P. JEFFREY BERGER[3], BRAIN W. KIRKPATRICK[2]
*[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, Wisconsin, USA; [2]Department of Animal Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA; [3]Department of Animal Science, Iowa State University, Ames, Iowa, USA*
Twinning in dairy cattle has been associated with many negative health and reproductive events that cause economic loss to the producer. Reports have suggested that twinning rates are increasing and that there may be a positive relationship between milk production and twinning frequency. Quantitative trait loci (QTL) for twinning rate on bovine chromosomes 5, 7, 19, and 23 have been previously identified. The objectives of this study were to detect and confirm the existence and effects of

these QTL in dairy cattle. This project utilized sire predicted transmitting ability (PTA) values for twinning rate estimated from North American Holstein calving data. Half-sib families of 25 sires with high twinning rate PTA comprised the population under investigation. DNA extracted from semen samples was analyzed using 45 microsatellite markers on four chromosomes. Marker heterozygosity of the patriarchs averaged 56%. Some of these families are related and will be combined into larger, multi-generation families for additional analysis. For twinning QTL identified in Holsteins, chromosomal positions will be more narrowly defined. Frequencies of haplotypes associated with twinning will be estimated in elite Holstein cow populations. The effect of these QTL on milk, fat, and protein production, productive life, and somatic cell score will also be estimated. Suggestive evidence of twinning QTL was found on chromosomes 5 and 23 in families analyzed to date.

**E015**
**Fine mapping of genes regulating heat loss in mice**

KARI ELO[1], MERLYN NIELSEN[1], DALE VAN VLECK[2], DANIEL POMP[1]
[1]*University of Nebraska-Lincoln, Department of Animal Science, Lincoln, Nebraska USA;* [2]*Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, Lincoln, Nebraska USA*

Three mapping populations have been produced from lines of mice that have undergone 16 generations of divergent selection for high and low heat loss using direct calorimetry. Two populations consisted of F2 intercrosses originating from either outbred (MH x ML, n=560) or inbred (IH x IL, n=640) high and low selection lines. The IH x IL F2 animals were further intermated to produce an advanced intercross line (AIL) that has been phenotyped at F11 (n=2,080). The main aims of this study are to identify QTL underlying energy balance and body composition and, using fine-mapping results from the AIL in conjunction with the human and mouse whole-genome sequences, identify candidate polygenes affecting these traits. Maintenance heat loss (kcal/$kg^{0.75}$/day) and feed intake (g/$kg^{0.75}$/day), body weights (3 and 6 wks and at tissue harvest) and tissue weights (brown adipose, subcutaneous and gonadal fat depots, liver and heart) were measured in all three populations. Total body fat percentage was estimated in the IH x IL F2 (using chemical extraction) and in the AIL at F11 (using dual-energy X-ray absorptiometry). Microsatellite markers have been genotyped in the MH x ML (n=149) and IH x IL (n=92) F2 populations. Summaries of phenotypic evaluation and initial QTL mapping results in the F2 intercrosses will be presented. This study will facilitate enhanced understanding of the polygenic genetic architecture of energy balance and body composition.

**E016**
**Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population**

MARINA GOLIK[1], EYAL SERROUSSI[1], JOEL I. WELLER[1], EPHRAIM EZRA[2], MICHA RON[1]
[1]*Institute of Animal Sciences, ARO, The Volcani Center, Bet Daga, Israel;* [2]*Israel Cattle Breeders Association, Caesaria Industrial Park, Israel*

Several studies have shown that a missense mutation in the bovine *DGAT1* gene located at the centromeric end of bovine chromosome 14 has a major effect on milk yield and composition. A total of 374 Israeli-Holstein sires were genotyped for this polymorphism by Fluorescent Allele-specific PCR. Of these 10 were homozygous for the mutation and 80 were heterozygous. The frequency of the mutant allele was 13.4%. The effect of this mutation on the sire genetic evaluations was analyzed by a model that also included the effect of sire birth date to account for genetic trend. The effect of *DGAT*1 allele substitution was highly significant ($p<0.0001$) for milk and fat yield, and fat and protein concentration. For all five traits, the effects associated with cows homozygous for the mutant allele was approximately twice the effects associated with cows heterozygous for the mutant allele, relative to cows without the mutant allele. Thus the effect of this QTL is approximately codominant. The allele substitution effects for the mutant allele were -308 kg milk, 7.8 kg fat, -2.9 kg protein, 0.17% fat, and 0.06% protein. A total of 1747 cows and 407 bulls were also genotyped for microsatellite ILSTS039, which is tightly linked to DGAT1, and strong population-wide linkage disequilibrium was observed. Allele 225, which was the shortest of eight alleles

observed, was closely associated with the mutant *DGAT1* allele. Frequency of this allele was 12% in cows and 16.8% in bulls.

**E017**

**Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition**

BERNARD GRISART[1], WOUTER COPPIETERS[1], FRÉDÉRIC FARNIR[1], LATIFA KARIM[1], CHRISTINE FORD[2], PAULETTE BERZI[1], NADINE CAMBISANO[1], MYRIAM MNI [1], SUZANNE REID[2], PATRICIA SIMON[1], RICHARD SPELMAN[3], MICHEL GEORGES[1], RUSSELL SNELL[2]

*[1]Department of Genetics, Faculty of Veterinary Medicine, University of Liège (B43), 4000-Liège, Belgium;[2]ViaLactia Biosciences (NZ) Ltd., University of Auckland Medical School, Auckland, New Zealand; [3]Livestock Improvement Corp.Hamilton, New Zealand*

We recently mapped a QTL with major effect on milk yield and composition to the centromeric end of bovine chromosome BTA14 within a 5 cM interval. A strong candidate gene called *DGAT1* (*Diacyl Glycerol Acyl Transferase 1*), was identified in the BAC contig covering this interval. Maximum likelihood LD analysis suggested the existence of two "young" QTL alleles (Q1 and Q2, associated with an increased milk fat content) embedded in distinct haplotypes and several q alleles. *DGAT1* was completely sequenced for individuals with known QTL phenotype. Four polymorphisms were observed : two in introns, one in 3'UTR and one in exon 8. For the latter, the Q1 and Q2 alleles were shown to be identical, and they differed from all q alleles by the substitution of a highly conserved amino acid (K232A). Surprisingly, the amino acid of the Q1 and Q2 alleles was shown to correspond to the ancestral state (phylogenetic consideration). Allele specific RT-PCR experiments allowed us to demonstrate that the K allele is characterized by an increased proportion of alternatively spliced transcripts. Investigations of the activity of the corresponding K, A and alternatively spliced allozymes are underway. Around 2,000 progeny-tested individuals were genotyped for *DGAT1* polymorphisms. An association study allowed us to evaluate that K232A accounts for 50% of the variation in breeding value for fat content.

**E018**

**Associations of beta-lactoglobulin alleles with milk productions traits in Churra sheep**

BEATRIZ GUTIÉRREZ, JUAN J. ARRANZ, MOHAMMED EL ZAREI, YOLANDA BAYÓN, FERNANDO DE LA FUENTE, FERMIN SAN PRIMITIVO

Various associations between milk protein polymorphisms and milk production trains have been described in different ruminant species. In sheep, the effect of the three alleles at the beta-lactoglobulin locus *(LGB)* on milk traits has revealed differences among dairy breeds. The objective of this study was to estimate the effects of the varians at the *LGB* locus on milk traits in Churra sheep, an indigenous Spanish breed. Beta-lactoglobulin alleles were determined using PCR-RFLP on DANN isolated from frozen blood. Samples were collected from 750 ewes belonging to the selection nucleus of Churra sheep, corresponding to 14 flocks connected via artificial insemination. Available milk traits were milk protein and fat yield as well as protein and fat percentage. Date were analysed using a mixed linear model, considering as random effect the factor "individual lactation" which includes the genetic and permanent environ mental effect associated with each animal. Allele frequencies obtained were $LGB^A = 0.31$ and $LGB^B = 0.69$. Regarding milk production effects, AA homozygous animals showed higher concentrations of milk solids (mainly fat content), indicating that milk from these ewes could be more valuable for cheese processing.

**E019**

**Genetic predisposition of Thoroughbred racehorses to exercise-induced pulmonary haemorrhage in South Africa**

CINDY HARPER[1], ALAN GUTHRIE[1], DUNCAN McDONALD[2], KOOS VAN DEN BERG[3], ENETTE VAN DYK[3], PAUL MORLEY[4], KENNETH HINCHCLIFF[5]

*[1]Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Ondertepoort, South Africa; [2]Jockey Club of Southern Africa, Turffontein, South Africa; [3]Department of Companion Animal Studies, Faculty of Veterinary Science, University of Pretoria, Ondertepoort, South Africa; [4]Department of Environmental Health, College of Veterinary Me-*

*dicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA; [5]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*

Exercise-induced pulmonary haemorrhage (EIPH) is a problem that occurs worldwide in horses that participate in strenuous exercise. EIPH does not appear to affect all horses to a similar extent, with the degree of haemorrhage varying from barely noticeable to severe. It has been speculated that EIPH may have a genetic basis but the occurrence of this condition is influenced by environmental factors. In this study EIPH was defined as epistaxis or blood at one or both nostrils following racing. South African racing authorities do not allow the use of furosemide to treat or prevent the condition, therefore, the data collected from Jockey Club records in this country is a realistic reflection of a proposed predisposition in the South African racing Thoroughbred population for the period examined. Individual horses that showed epistaxis as a result of EIPH while racing between 1990 and 2001 were examined. The incidence of haemorrhage was directly related to the following factors, age, sex, weight carried, racing district, distance run, environmental temperature and humidity, track surface and track condition and the significance of each factor established. The sire, dam and sire of the dam of each case were determined and each sire and dam sire were examined for the number of offspring that developed EIPH within the period under investigation. These data were corrected for the total number of offspring produced by each stallion during this period. Sires and dam sires with more than 50 offspring were compared and it was found that certain of these sires produced significantly more offspring with EIPH. Blood samples from these animals were collected and allele frequencies of a number of microsatellite markers were determined.

**E020**

**The Jerusalem Resource Population: A multi-generation quasi-Full-sib Intercross Population for High Power and High Resolution QTL Mapping in Poultry. Biometrical characteristics**

E. HEIFETZ[1], H. KHATIB[1], D. HELLER[2], Y. EITAN[1], M. SOLLER[1]

*[1]Department of Genetics, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel; [2]Dept. of Animal Science, Faculty of Agriculture, Rehovoth, Israel*

A full-sib intercross line (FSIL) is a mapping population constructed to provide the QTL mapping advantages of an F2 population for an outcrossing species. In the FSIL design, two parent individuals, from the same or different populations, are mated to produce a large full-sib family. The full-sibs are intercrossed at random to produce the first FSIL1 generation; the FSIL1 progeny are randomly intercrossed in turn, to produce the FSIL2 generation, etc. The high degree of linkage disequilibrium introduced by the limitation to two founder individuals gives the FSIL its statistical power. The Jerusalem Resource Population, now at FSIL15, was constructed by crossing a single White Rock heavy breed male with five semi-inbred Leghorn layer females. The population was phenotyped in each generation for growth rate, anatomical and egg production traits, and in some generations for immune response. Biometrical analysis of the first nine generations showed that inbreeding accumulated at a rate of 0.012 per generation, following the first full-sib generation. The population did not show any obvious effects of inbreeding. Time trends in trait value were not found, and phenotypic variation, heritabilities and genetic correlations were similar to those found for normal populations. Thus, the JRP appears to represent a genetically normal chicken population, within which QTL affecting a variety of traits of economic importance are segregating.

**E021**

**Fine-mapping of a QTL affecting egg white thinning in chicken**

MERVI HONKATUKIA[1], MARIA TUISKULA-HAAVISTO[1], JOHANNA VILKKI[1], NINA SCHULMAN[1], DIRK-JAN DE KONING[3], ANNELI VIRTA[2], ASKO MÄKITANILA[1]

*[1]Animal Production Research, MTT Agrifood Research Finland, 31600 Jokioinen, Finland, [2]Food Research, MTT Agrifood Research Finland, 31600 Jokioinen, Finland, [3]Roslin Institute,Roslin, EH25 9PS, U.K*

Our previous genome scan of chicken revealed three putative areas affecting egg white thinning. The $F_2$ population was derived from a cross between two genetically and phenotypically extreme egg layer lines. Egg white qual-

ity was measured as albumen height in Haugh units at ages of 40 and 60 weeks, HU40 and HU60, respectively. Because the confidence intervals for the detected QTL were over 50 cM wide, a method to improve the QTL locations after initial detection was needed. We chose the most significant QTL on chromosome 2 for further analyses. For fine-mapping a backcross design with multiple marker regression was used together with denser microsatellite marker intervals. Both poor and superior $F_2$ hens in egg white quality were selected for the female parents of backcross generation. Adding three microsatellite markers on the QTL area narrowed its position from 55 to 31 cM. Moreover, a grid search fitting two QTL was performed on the chromosome to test whether there could be more than one QTL on the chromosome affecting egg white thinning. It was tested with a standard F-test whether the best two QTL model explained significantly more variance than the best single QTL. The result suggested that instead of one QTL there are two distinct QTL areas affecting egg white thinning. In addition, the results from the backcross generation indicated that one of the QTL areas predominantly affects HU40 and the other HU60.

**E022**

**Mapping of Quantitative Trait Loci for growth and fatness in chickens**

LINA U. JACOBSSON[1], HEE-BOK PARK[1], PAUL B. SIEGEL[2], LEIF ANDERSSON[1]

*[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences Uppsala, Sweden; [2]Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg, USA*

Two chicken lines divergently selected for growth have been used to create an F2 intercross mapping population. The parental lines show a 9-fold difference in body weight at 8 weeks of age and differ markedly in traits related to appetite and deposition of fat. These two extreme chicken lines have been established by selecting for high and low body weight at 8 weeks of age for over 40 generations by P. B. Siegel. The mapping population was generated by crossing 30 individuals from each parental line reciprocally to generate 8 F1 males and 76 F1 females. A total of 974 F2 individuals were phenotyped for growth and body composition traits including body weight from 0 to 10 weeks, abdominal fat, and plasma concentrations of insulin and glucagon. A genome scan using 100 markers on 25 linkage groups in the chicken genome has been completed. The scan covers 85% of the chicken genome and an additional 50 markers are being typed to reach a coverage of 90%. Results of QTL analyses will be reported.

**E023**

**Chicken fatness QTL mapping using an Advanced Intercross Line**

DANYEL G.J. JENNEN[1], ADDIE L.J. VEREIJKEN[2], RICHARD P.M.A. CROOIJMANS [1], TINEKE VEENENDAAL[1], HENK BOVENHUIS[1], JAN J. VAN DER POEL[1], GERARD A.A. ALBERS[2], MARTIEN A.M. GROENEN[1]

*[1]Animal Breeding and Genetics, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands; [2]Nutreco, Boxmeer, The Netherlands*

We have performed a total genome scan in chikken resulting in the localisation of QTLs for several economically important traits including fat deposition. Briefly, 480 F2 animals were individually genotyped for 284 microsatellite markers, and 3 different batches of 2000 F3 animals each were phenotyped for fatness traits. Deposited abdominal fat was measured in 2000 F3 animals at 7, 9 and 10 weeks of age. QTL analysis, using a regression interval mapping approach, were performed for total fat and fat percentage for the three groups of animals. Six different regions, located on six different chromosomes, were identified that showed highly significant F-statistics for several of the fatness traits, indicating the presence of (a) gene(s) at these locations that control the amount of fat deposition in chicken. For the fine mapping of the regions containing the fatness QTLs an Advanced Intercross Line (AIL) has been produced. Over 3000 F9 animals were produced and measured for the fatness traits and typed with markers from the six identified QTL locations.

**E024**

**Frequency of *ER* and *RYR1* alleles and their association with reproductive performance in primiparous sows of synthetic line 990**

MARIAN KAMYCZEK[1], MARIAN RÓŻYCKI[2], ANDRZEJ JANIK[2], ANNA

KWACZYŃSKA[1], MONIKA SIKORA[1], ANNA RADKO[2], TOMASZ ZABEK[2]
[1]*National Research Institute of Animal Production,Experimental Station,Pawłowice, Poland;* [2]*National Research Institute of Animal Production, Kraków/Balice, Poland*

Estrogene receptor (*ER*) and ryanodine receptor (*RYR1*) genes were evaluated for their associations with litter size in primiparous sows line 990. Total of 548 sows were genotyped for both loci. Relationship between *ER*, *RYR1* genotypes and litter size were evaluated using analysis of variance. The polymorphism of the *ER* gene was determined by the PCR/RFLP method. The PCR product was digested by the restriction endonuclease *Ava*I. It was found that observed allele frequency was 0,924 for allel $ER^A$ and 0,076 for allel $ER^B$. Low frequency of $ER^B$ allele causes that in presented material only 7 homozygous $ER^{B/B}$ sows was found. In primiparous sows average number of pigs total born (8,80 and 9,20) and average number of pigs born alive ( 8,60 and 8,80 ) were respectively for $ER^{A/A}$ and $ER^{A/B}$ genotype. The polymorphism of the *RYR1* gene was also determined by using PCR/RFLP method. It was found that observed allele frequency was 0,620 for allel $RYR1^C$ and 0,380 for allel $RYR1^T$. Average number of total born pigs ( 9,81, 9,87 and 9,95 ) and average number of pigs born alive (8,71, 8,77 and 8,62) were respectively for $RYR1^{C/C}$, $RYR1^{C/T}$ and $RYR1^{T/T}$ genotype. Positive effect of $ER^B$ allele on litter size in primiparous sows line 990 was small. It was found that genotype in *RYR1* locus give no influence on litter size at first litter. Research is still continued and more data for next litters will be collected soon.

**E025**

**Construction of a BAC contig at a cattle QTL region controlling resistance to trypanosomosis on BTA7 that is homologous to the murine TIR1 region on MMU17**

SIMON KANG'A[1,2], PHILOMEEN NILSSON[1], TOM GOLDAMMER[3], JOEL MWAKAYA[1], ELIUD N.M. NJAGI[2], MANFRED SCHWERIN[3] , OLIVER HANOTTE[1]
[1]*The International Livestock Research Institute (ILRI), Genetics & Genomics, Nairobi Kenya;* [2]*Kenyatta University, Biochemistry Department, Nairobi, Kenya;* [3]*Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere (FBN), Forschungsbereich Molekularbiologie, Dummerstorf, Germany*

In an effort to improve the mapping resolution of a quantitative trait loci (QTL) controlling resistance to trypanosomosis on *Bta*7, a previously unknown region of homology between *Bta*7 and the murine *Tir*1 trypanotolerance QTL region on *Mmu*17 was identified using a combination of comparative and radiation hybrid (RH) mapping. We report here the building up of a bovine BAC contig spanning this region. Four BAC clones from the RPCI 42 bovine library were identified with two BAC clones covering the entire homologous segment between the two QTL. The size of the homologous region is estimated to be around 300kb. One of the BAC clones includes a chromosomal breakpoint of conserved synteny between *Bta*7 and mouse *Mmu*17 and *Mmu*8. All BAC clones were physically located to the *Bta*7 QTL region by fluorescent *in situ* hybridisation. The region of homology contains eight genes, which were identified using comparative mapping, and ordered in cattle using a 12,000rad RH panel (Texas A&M). PCR amplification using the four BAC clones as templates confirms this gene repertoire and order. Sequencing of the BAC clones may identify new genes and provide information on the comparative genome organisation of the region of homology.

**E026**

**Identification of QTL affecting production traits in a cross between Red Jungle fowl and White Leghorn chicken**

SUSANNE KERJE[1], ÖRJAN CARLBORG[1], KARIN SCHÜTZ[2], PER JENSEN[2], LEIF ANDERSSON[1]
[1]*Swedish University of Agricultural Sciences, Department of Animal Breeding and Geneics, Uppsala, Sweden;* [2]*Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Section of Ethology, Skara, Sweden*

Red Jungle fowl chicken are considered to be the wild ancestor to our modern chicken. We have crossed one Red Jungle fowl male with four White Leghorn females to generate a three generation mapping population. 41 F1 offspring were intercrossed and 853 F2 individuals were hatched. A genome scan including 105 genetic markers distributed over 25 chromosomes/linkage groups was carried out and

the resulting linkage map were used for QTL analysis. Data were collected for weight at 1, 8, 46, 112 and 200 days and growth were calculated between the different recordings. Total and average egg weight were also recorded. A total of 9 traits were used for the QTL analysis and resulted in 15 chromosomal regions significant at the 5% genome wide level. When searching for suggestive QTLs two more regions were discovered. Six of the detected QTL had an effect on several traits. For body weight at 200 days we found six significant QTL and all of them showed an additive inheritance with no significant dominance effects. The observed QTL explains a large portion of the two-fold difference in growth between the two parental lines.

**E027**

**Investigation of candidate genes for the growth and fatness QTL on pig chromosomes 1, 7, and 13 in a Berkshire x Yorkshire family and commercial populations**

KWAN-SUK KIM[1], HAUKE THOMSEN[1], JOHN BASTIAANSEN[2], YUANDAN ZHANG[1], JACK C.M. DEKKERS[1], GRAHAM S. PLASTOW[2], MAX F. ROTHSCHILD[1]

*[1]Department of Animal Science, Iowa State University, Ames, IA, USA; [2]Sygen International, Berkeley, CA, USA*

Quantitative trait loci (QTL) analyses using molecular markers have detected several important genomic regions for growth and fatness traits in pigs. The improved comparative map between human and pig chromosomes and knowledge of the biological mechanisms of these traits suggest several candidate genes in the identified QTL regions. To further investigate the identified growth and backfat QTL, we have studied three positional and biological candidate genes, *MC4R*, *HMGA1* and *GHRL,* which mapped to pig chromosomes 1, 7 and 13 respectively. We have identified single nucleotide polymorphisms (SNPs) within these genes and used them for QTL and association analyses. All three genes were mapped to positions within the backfat and growth QTL regions that were identified in a Berkshire x Yorkshire family. The identified SNPs were significantly associated with observed variation in F2 animals for backfat and growth traits using single marker analyses. Some interactions were also detected from combined analyses of these SNPs. Phenotypic associations of these polymorphisms were also found to be present in several commercial populations. These combined results suggest that these genes are good candidates for the growth and fatness QTLs reported on pig chromosomes 1, 7, and 13. The results obtained to date suggest that polymorphisms identified in these genes will be useful for marker-assisted selection for growth and fatness traits in the pig.

**E028**

**Detection of QTL for birth weight in Charolais within the SEGFAM resource population**

CHRISTA KÜHN[1], FRANK BECKER[2], HANS-JÜRGEN PAPSTEIN[3], JOCHEN WEGNER[3], OLAF BELLMANN[3], JÜRGEN VOIGT[4], VOLKER GUIARD[5], MANFRED SCHWERIN[1]

*[1]Res. Unit Molecular Biology; [2]Res. Unit Reproductive Biology; [3]Res. Unit Muscle Biology and Growth; [4]Res. Unit Nutritional Physiology "Oskar Kellner", [5]Res. Unit Genetics and Biometry, Research Institute for the Biology of Farm Animals, Dummerstorf, Germany*

To investigate the genetic and physiological background of divergent nutrient transformation for growth and lactation in dairy and meat type cattle a resource population (SEGFAM) is established in the Research Institute for the Biology of Farm Animals. In SEGFAM the Charolais and the German Holstein breed serve as representatives of the accretion and secretion metabolic type, respectively. Substantial differences between those breeds exist for a variety of physiological traits. However, there was indication, that unlike the situation in inbred lines of model organisms QTL with effect on these traits still segregate within selected cattle breeds. This hypothesis of segregating QTL within a phenotypically extreme breed was tested for the trait birth weight by a whole genome scan in a half sib design originating from the Charolais founder sires of the SEGFAM population. Five families each consisting of a Charolais sire, which was mated to Charolais and German Holstein cows, as well as dams and offspring were genotyped with 198 microsatellite markers distributed across all chromosomes. Variance analysis indicated putative QTL for birth weight on chromosomes

4, 5, 6, 14, 15, 16, 19, und 23. For the QTL positions on BTA4, 5, 6, 19, and 23 corresponding results for birth weight or related traits have been found in other populations. Our results indicate, that in spite of extreme phenotype segregating QTL for the respective traits can be detected within high selection cattle breeds.

**E029**

## Associations between polymorphisms in 5' flanking regions of milk protein genes and traits of individual milk proteins in cattle

ANDREAS W. KUSS, JOCHEN GOGOL, HEINZ BARTENSCHLAGER, HERMANN GELDERMANN
*University of Hohenheim, Institute of Animal Husbandry and Breeding, Department of Animal Breeding and Biotechnology, Stuttgart, Germany*

Bovine milk protein yield as well as the relative composition of protein fractions were found to be associated with variants of the milk protein encoding genes (Ehrmann et al. 1994a & b). The aim of the study presented here was to quantify milk proteins coded by specific loci and investigate their individual association with polymorphic gene variants. The study was based on material from the two breeds German Holstein Friesian and Simmental. A number of milk samples per cow and lactation was analysed. Isoelectric focussing was used for allelic identification of polymorphic milk proteins and alkaline Urea-PAGE in combination with densitometry was applied for quantification of milk protein fractions (α-s1-Casein, β-Casein, κ-Casein, α- Lactalbumin, β-Lactoglobulin). DNA variants were analysed in the 5'-flanking regions of the β-Lactoglobulin and κ-Casein genes by PCR-RFLPs. Experimental data, together with milk performance and pedigree data were submitted to analysis of variance. The results reveal associations between specific DNA variants and yield as well as the relative portion of the individual milk proteins encoded by the respective loci. These findings are interpreted as distingt influence of the variable gene positions on the investigated milk-parameters and are of relevance for further genetic analysis of milk protein yield and composition.

*References; Ehrmann,S., H.Bartenschlager and H.Geldermann: J.Arnim.Breed.Genet. 144, 49-53 1997a. and 114 121-132, 1997b.*

**E030**

## QTL population to investigate the genetics of the pale, soft and exudative (PSE) meat in chickens

MÔNICA C. LEDUR[1], JORGE A.F. LARA[2], KERLI NINOV[1], CRISTINA A. BONASSI[1], MASSAMI SHIMOKOMAKI[2], ERALDO L. ZANELLA[1], GIOVANNI R. BERTANI[1], ALEXANDRE L. NEPOMUCENA[3]
*[1]Embrapa Swine and Poultry, Concórdia, Brazil; [2]Londrina State University, Department of Food and Drugs Technology, Londrina, Brazil; [3]Embrapa Soybean, Londrina, Brazil*

An F3 resource population was developed to identify quantitative trait loci (QTL) affecting the functional properties of chicken meat, including PSE. This population was originated by crossing two divergent lines, a broiler (TT) and a layer (CC) line, developed at Embrapa Swine and Poultry Research Center. 1497 F3 animals were raised as broilers up to 42 days, when were slaughtered and the following phenotypic traits were measured: body weight, abdominal fat, breast fillet, parts and carcass weight, and pH 15 min after slaughter. The color (L* value), water-holding capacity, and pH were measured 24 hours postmortem in triplicate breast fillet samples. Blood samples from parental, F1, F2 and F3 populations were collected for DNA analyses. After the slaughter, thigh muscle samples were removed and stored in liquid nitrogen for RNA analyses. Genome scan and candidate gene analyses will be performed to identify QTL regions related to these meat quality traits. We evaluated the incidence of pale, soft and exudative meat in our population. PSE meat results in low yield of industrial products and unacceptable appearance by consumers. PSE meat was considered when the pH 24 h postmortem was below 5.8 and the L* value (color measurement) was above 52.0. The incidence of PSE was 532/1497 animals (35,5%). We are now investigating two candidate genes for PSE, the *RYR1* and *RYR3*, both related with mechanisms regulating myoplasmatic $Ca^{2+}$ concentration and excitation-contraction coupling.

**E031**

**Associations of bovine leptin polymorphisms with circulating leptin concentrations**

SILVIA C. LIEFERS[1,2], MARINUS F.W. TE PAS[1], ROEL F. VEERKAMP[1], CAROLE DELAVAUD[3], YVES CHILLIARD[3], TETTE VAN DER LENDE[2]

*[1]ID-Lelystad, Division of Animal Sciences, Lelystad, The Netherlands; [2]Wageningen University, Animal Breeding and Genetics Group, Wageningen, The Netherlands; [3]INRA-Theix, Herbivores Research Group, St-Genes-Champanelle, France*

Leptin is a protein involved in the regulation of feed intake, fertility and immune functions. To investigate the association of leptin polymorphisms with circulating leptin levels, 323 Holstein heifers were typed for three polymorphisms at the bovine leptin gene locus. The *Sau*3AI RFLP is located in the intron, the *Hph*I RFLP at the beginning of the second exon and the BM1500 microsatellite is located 3,6 kb downstream of the leptin gene. The *Hph*I polymorphism causes an Ala to Val substitution in the coding region of the gene. From late pregnancy until 80 days after calving blood samples were taken every two weeks. Leptin concentrations were determined using a RIA. During pregnancy the *Hph*I-BB genotype showed higher ($P<0.05$) leptin levels compared to the AA and AB genotypes, and the BM1500 A-allele showed lower ($P<0.05$) leptin levels compared to the presence of the BM1500 B- or C-allele. During pregnancy and lactation the presence of the BM1500 B-allele caused higher ($P<0.05$) leptin levels than the presence of the other two alleles. The two exons including the exon/intron boundaries of the leptin gene of 90 animals were sequenced to investigate linkage of the polymorphisms to mutations at the coding region. Several mutations were found and it seems that the BM1500 A-allele is linked to an Arg to Cys mutation in exon 1. Furthermore it seems that the recombination frequency is high for this region.

**E032**

**Using information on segration of dam marker alleles within a daughter design; for mapping QTL affecting milk production traits in Israel Holstein dairy cattle**

EHUD LIPKIN, GILI GRUZMAN, ADAM FRIEDMANN, MOSHE SOLLER

*Department of Genetics, Hebrew University of Jerusalem, Jerusalem 91904, Israel*

The daughter design for mapping QTL uses only the information that is provided by the alleles of the sire of the family. However, a relatively small number of maternal grandsires (MGS) or great grandsires (MGGS) are active in any given years, and these provide the bulk of the dams; in addition, the dams are chosen so that they are not in close relationship with the sire, limiting the selection of MGS. Thus, there is much opportunity for linkage disequilibrium among the dams of the daughters. Including the dam alleles in QTL mapping will: (i) increase statistical significant of the results, (ii) expand the genetic map, due to additional generations of recombination between marker allele and QTL among the dam chromosomes, allowing more accurate determination of QTL location, and, (iii) provide information in regions where the sires are not informative, due to homozygosity of the sire at the QTL or at the markers. To test this possibility, densitometric data on dam allele frequency in pooled milk samples were obtained during a daughter design analysis of milk production traits by selective DNA pooling. Within each sire-marker-trait combination, alleles originating from the dam only (e.g., all except the sire alleles), were tested for significance of the allele frequency difference between high and low pools. There was a good agreement with the results obtained by analysis of sire alleles. In addition, as expected if the genetic map has expanded, markers showing statistical significance and peaks of significance for three different milk production traits (protein percent, protein yield and milk yield) were more tightly clustered than found for the sire marker alleles.

**E033**

**QTL analysis for milk production traits and SCS on chromosome 23 in the Spanish Holstein-Friesian population**

SAGRARIO MARCOS[1], DELFINO HERNÁNDEZ[2], MALENA SERRANO[1], MARÍA J. CARABAÑO[1]

*[1]INIA, Departamento de Mejora Genética Animal, Madrid, España; [2]CONAFE, Madrid,España*

As a way to improve the validity of previously detected QTL's on BTA 23, we present an analysis of that chromosome for milk produc-

tion traits and somatic cell score (SCS) in the Spanish Holstein-Friesian cattle to compare results between studies from different populations. This study is being performed in a grand daughter desing (GDD) comprising 742 sons distributed over twenty six paternal half sib families.The number of sons per sire ranges from 10 to 59 and is on average 28,5.The phenotypic units of measurement for statistical analisys are daughter yield deviation (DYD) based on the national database and provided by the National Association of Spanish Holstein-Friesian (CONAFE) for milk yield (Kg), fat yield (Kg), protein yield (Kg), fat percentage, protein percentage and SCS. Eleven microsatellites markers covering 61 cM of BTA 23 are being genotyped. Multiple-marker interval mapping with both regression and maximum-likelihood methods will be applied.

**E035**

**QTL for fatty acid composition in pigs using either the backfat thickness or the carcass weight as a covariate in a regession method**

ALEX CLOP[1], ANNA MERCADE[1], CRISTINA ÓVILO[2], MIGUEL PEREZ-ENCISO[3], ALBERT CERCOS[1], ANNA TOMAS[1], ANA FERNANDEZ[2], AGUSTINA COLL[1], JOSEP M. FOLCH[1], CARMEN BARRAGAN[2], MARIA A. OLIVER[4], ISABEL DIAZ[4], LUIS VARONA[5], LUIS SILIO[2], JOSE R. NOGUERA[5], ARMAND SANCHEZ[1]

*[1]Department de Ciènca Animal i dels Aliments, Facultat de Veterinàra, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; [2]Departamento de Mejora Genética y Biotecnologia, INIA, Madrid 28040, Spain; [3]SAGA-INRA, Castanet-Tolosan 31326, France; [4] IRTA-CTC, Monells 17121, Spain; [5]Area de Producció Animal, Centre UdL-IRTA, Lleida 25198, Spain*

We have analized a $F_2$ pedigree obtained by mating 3 Iberian boars to 31 Landrace sows in order to detect OTL for fatty acid composition in backfat. We recorded the percentages of the most relevant fatty acids by gas chromatography. These fatty acids were Myristic (MYR), Palmitic (PA), Palmitoleic (PAL), Stearic (STE), Vaccenic (VAC), Oleic (OLE), Linoleic (LIN), Linolenic (LINL), Gadoleic (GAD) and Eicosadienoic (EIC). We also calculated their average chain length (ACL), double bond index (DBI), unsaturated index (UI) and peroxidability index (PI). We constructed a linkage map of the whole genome by using the CRI-MAP version 2.4 software. The QTL analysis was performed by a linear regression method using either the carcass weight (model 1) or the backfat thickness (model 2) as a covariate. Model 1 showed significant QTL in chromosome 4 (LIN, DBI and PI) 6 (DBI and UI), 8 (PA, PAL and ACL), 10 (MYR) and 12 (LINL and GAD). Model 2 showed significant effects on chromosomes 8 (PA, PAL and ACL), 10 (MYR) and 12 (LINL, GAD, ACL, PI). QTL on chromosomes 8 (PA, PAL and ACL), 10 (MYR) and 12 (LINL and GAD) showed significant F-value in both models at genomewide level of significance. The results of this study suggest that some QTL have effect for both fat deposition and fatty acid composition, and other QTL only affect fatty acid composition, independently of the fat deposition traits. An adequate choice of the covariate used in the model of analysis plays a crucial role.

**E036**

**Segregation of resistance to nematode infection in F2 lambs of Suffolk x Gulf Coast Native sheep and associated QTL**

JAMES E. MILLER[1], NOELLE E. COCKET[2], GRANT A. WALLING[3], TRACY L. SHAY[2], ROYAL A. McGRAW[4], STEVEN C. BISHOP[3], CHRIS A. HALEY[3]

*[1]Louisiana State University, Department of Pathobiological Sciences, School of Veterinary Medicine, Baton Rouge, Louisiana USA, [2]Utah State University, Biotechnology Center, Logan Utah USA, [3]Roslin Institute, Edinburgh, Scotland, [4]University of Georgia, Department of Physiology and Pharmacology, College of Veterinary Medicine, Athens, Georgia USA*

Sixty-two, 86 and 84 $F_2$ lambs from Suffolk x Gulf Cost Native (Native) F1 ewes were born in 1998, 1999 and 2000, respectively. Lambs grazed until weaned, at which time they were dewormed and maintained on concrete for 6 weeks. Subsequently, they grazed for six weeks and nematode infection level was evaluated by fecal egg count (FEC) and blood packed cell volume (PCV). For 1998 lambs, mean FEC and PCV ranged from 933-19,433 eggs per gram (EPG) and 14.7-30.7%, respectively. For 1999 lambs, mean FEC and PCV ranged from 633-31,267 EPG and 7.3-27.0%, respectively. For 2000 lambs, mean FEC and PCV ranged from 50-21,650 EPG and

15.0-33.0%, respectively. Typically, under similar challenge, the mean FEC of the parent Suffolk and Native breeds is in the 7-15,000 EPG and 500-3,000 EPG range, respectively. Similarly, mean PCV is in the 15-19% and 24-28% range, respectively. The range of FEC and PCV indicated that resistance to infection segregated. A selective genotyping strategy was used in which 50 microsatellite markers were screened across 40% of the F2 lambs (20% highest FEC and 20% lowest FEC) and their parents. In total, 80 lambs (including 26 lambs of Sire 1, 25 lambs of Sire 2 and 29 lambs of Sire 3), 3 sires and 56 dams were genotyped. Within-family linkage analysis for marker effects suggest QTL for FEC on chromosomes 1, 3 and 19. Associations with these markers will be further explored using interval mapping.

**E037**

**Multiple QTL mapping with epistatic interactions for non-insulin-dependent diabetes mellitus in rat**

TAKESHI MIYAKE[1,2], AKIRA NARITA[2], TAKAHISA YAMADA[2], GAUDENZ DOLF[1], CLAUDE GAILLARD[1], KOZO MATSUMOTO[3], YOSHOYUKI SASAKI[2]

[1]*Institute of Animal Genetics, Nutrition and Housing, University of Berne, 3012 Berne, Switzerland;*

[2]*Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan;* [3]*University of Tokushima School of Medicine, Tokushima 770, Japan*

The Otsuka Long-Evans Tokushima Fatty rat is an animal model for obese-type non-insulin-dependent diabetes mellitus (NIDDM) in humans. The newly developed multiple QTL mapping method, Monte Carlo Interaction Mapping (MCIM), was applied to map QTLs and epistatic interactions between two QTLs for NIDDM in 160 $F_2$ crossbred rats with 213 informative markers. As indicators of NIDDM susceptibility, the plasma glucose levels at 0, 30, 60, 90 and 120 min (mg/dl x min) were determined by using the oral glucose tolerance test. For each trait, the optimal model was determined by forward model-selection procedure. Threshold values for significance tests were determined by permutation tests, and they were severer than those obtained by Chi-square values with Bonferroni correction. Seven QTLs with main effects only, four QTLs with main and epistatic effects and four QTLs with epistatic effects only, were significantly identified among all analyzed traits. The QTL on Chr. 7 (*Nidd1/of* ) had a main effect ($p < 0.05$) on six traits and an epistatic effect ($p < 0.10$) on one trait. For the plasma glucose level at 0 and 120 min, adjusted for body weight, the optimal models were able to account for a total of 75% and 59% of the phenotypic variance, respectively. Because both multiple QTLs and epistases are included in the model simultaneously, MCIM allows not only for the accurate identification of QTLs but also the correct partition of the phenotypic variance into the various genetic components.

**E038**

**Distribution of Haplotypes in BTA 6, 14, 19, and 21 Within One Commercial Line of *Bos taurus* and Their Associations With Growth Traits**

JENIFER L. KNEELAND[1], CHANGXI LI[1], BRENDA MURDOCH[1] , JOHN BASARAB[2], WARREN SNELLING[3], BERNHARD BENKEL[4], STEPHEN S. MOORE[1]

[1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada;* [2]*Alberta Agriculture, Food and Rural Development, Lacombe Research Center, Lacombe, Alberta, Canada;* [3]*USDA, ARS, US Meat Animal Research Center, Clay Center, Nebraska, USA;* [4]*Agriculture and Agri-Food Canada, Lethbridge Research Center, Lethbridge, Alberta, Canada*

The objective of this study was to verify and fine map quantitative trait loci (QTL), for growth in industry beef cattle herds, that have been previously identified in experimental reference herds. By verifying and fine mapping QTLs that affect growth one can identify positional candidate genes and gain a greater understanding of their biology and function. Co-segregation between a particular genetic marker and a QTL in a population is critical for successfully mapping QTLs. Common haplotypes are expected to carry on and segregate among individuals of commercial breeding lines. Genes underlying QTL of interest may be present in these common haplotypes, thus making it possible to map them to a particular chromosomal location. We report here the identification and mapping of QTLs for birth weight, pre-weaning average daily gain, and average daily gain on feed in a commercial line

of *Bos taurus* using the identical-by-descent haplotype sharing method. One hundred and seventy-six calves of twelve bulls were typed using forty-nine microsatellite markers chosen from BTA6, 14, 19 & 21 (8 – 18 markers from each chromosome). For each calf the alleles inherited from each parent were identified. Haplotypes of each calf can then be identified along the length of each chromosome. Statistical analysis using the General Linear Model (GLM) procedure in SAS (version 8) identified twenty-one chromosomal regions that have association with growth traits at a significance threshold of $p<0.05$.

**E039**

**High-resolution mapping of trypanotolerance QTL Tir2 and 3 using F12 advanced intercross lines**

JOSEPH K. NGANGA[1,2], JOHN P. GIBSON[1], STEPHEN J. KEMP[3], FUAD A. IRAQI[1]
*[1]International Livestock Research Institute, P. O. Box 30709, Nairobi, Kenya; [2]Jomo Kenyatta University of Agriculture and Technology P. O. Box 62000, Nairobi, Kenya; [3]School of Biological Sciences, Donnan Laboratories,University of Liverpool UK*

Different mouse strains show variation in response to trypanosoma congolense infection (Trypanosomosis). In previous studies, we have mapped quantitative trait loci (QTL) for trypanosomosis resistance (trypanotolerance) in two F2 populations to chromosomes 17, 5 and 1 and designated as *Tir1*, *Tir2* and *Tir3* respectively. Using advanced intercross lines (AIL) approach, only *Tir1* has been fine mapped to a confidence interval less than 1cM. In order to fine map *Tir2* and *3*, $F_{12}$ C57BL/6J x A/J AIL fixed for the susceptible and resistance alleles at *Tir1* locus were generated. The two AIL populations and the parental controls were then challenged with *T. congolense* at 12 weeks of age and followed for survival times over 180 days. Mice from the two survival extremes of the population fixed for the susceptible alleles were genotyped with a panel of microsatellite markers across the previously mapped regions. The data was analyzed with maximum likelihood and least square interval-mapping methods. The mean survival time of AIL fixed for the susceptible and resistant QTL on chromosome 17 was 95 and 125 days respectively whereas that of AJ and C57BL parental lines was 60 and 80 days respectively. Results of QTL analysis showed that chromosome 1 comprise of three significant loci, which 95% confidence interval (CI) between 3-6cM, while chromosome 5 has 2 loci with a 95% CI of 1cM and 2cM. The confidence range obtained can now facilitate positional cloning of genes underlying the QTL.

**E041**

**Association of GH and IGF-1 polymorphisms to growth traits in a synthetic beef cattle breed**

ANDRÉA P. PEREIRA[1], MAURÍCIO M. ALENCAR[2], HENRIQUE N. OLIVEIRA[3], LUCIANA C.A. REGITANO[2]
*[1]Federal University of São Carlos, Genetics and Evolution Department, São Carlos, Brazil; [2]Embrapa Southeast Cattle Research Center, São Carlos, Brazi;, [3]São Paulo State University, Animal Breeding and Nutrition Department, Botucatu, Brazil. This work was supported by FAPESP and CNPq*

The Canchim beef cattle (5/8 Charolais + 3/8 Zebu), has been selected for meat production in Brazil since 1953.In the present work the effect of candidate genes polymorphisms was investigated in 688 animals born between 1998 and 2000. From these 307 belonged to the original Canchim population (GG1) that was formed in 1953 and the remaining belong to a Canchim population formed by recent crosses (GG2). DNA extracted from blood samples using a salting out procedure was amplified for a 223 bp fragment, spanning intron IV and exon V of the growth hormone (*GH*) gene, and for type 1 insuline like growth factor (*IGF1*) microsatellite in 25 µl reactions. *GH* products were digested with *Alu*I and fragments were resolved in 3% agarosis electrophoresis. *IGF1* products were separated by electrophoresis in 8 % non denaturing polyacrylamide gels and silver stained to identify the alleles. Genotype effects on breeding values for birth weight (BW), weaning weight (WW) and yearling weight (YW) were investigated by the SAS GLM procedure. The statistical model included the effects of genetic group and *GH* and *IGF-1* genotypes. Significant effects were found for *GH* genotype on YW ($P \leq 0.05$), with positive effects associated to the V allele, and for *IGF1* on BW ($P \leq 0.01$) and YW ($P \leq 0.01$).

**E042**

**Combined approaches at INRA to fine map QTL influencing growth, fatness and carcass composition traits in pig.**

JULIETTE RIQUET[1], MARIE-PIERRE SANCHEZ[2], OLIVIER DEMEURE[1], NATHALIE IANNUCCELLI[1], KATIA FEVE[1], HÉLÈNE GILBERT[2], CARINE GENÊT[1], MICHÈLE BONNET[1], CHRISTOPHE PERY[2], YVON BILLON[2], JEAN GOGUÉ[2], JEAN-CLAUDE CARITEZ[2], PASCALE LE ROY[2], CHRISTINE RENARD[3], JEAN-PIERRE BIDANEL[2], DENIS MILAN[1]

*INRA, France: [1]Laboratoire de Génétique Cellulaire, Castanet-Tolosan; [2]Station de Génétique Quantitative et Appliquée, Jouy-en-Josas; [3]Laboratoire de Radiobiologie et d'Etude du Génome, Jouy-en-Josas*

A whole genome QTL analysis of growth, fatness and carcass composition data from a F2 experimental cross between 6 Meishan (MS) sows and 6 Large White (LW) males has been performed at INRA. Highly significant QTL effects were detected in several chromosomal regions: the telomeric regions of SSC 1q (growth, fatness, lean cuts weights) and SSC 2p (lean and fat cuts weights), SSC 4 (growth, fatness) and SSC 7 (growth, fatness, lean and fat cuts weights) were selected for further analyses. Unlike other studies, our analysis did not reveal any imprinting effect for the SSC2 QTL. Further investigations are underway to dissect chromosomal regions containing QTLs: (1) backcross animals produced from F1 original animals are being studied; (2) synthetic commercial lines related to the INRA Meishan population are being analysed to identify recombinant chromosomes interesting for fine mapping of the QTL by progeny testing, and to study the effects of selection on haplotype frequencies in QTL regions; (3) the effects of QTL regions are also being investigated in other experimental populations (pure Large White, LW x Piétrain). In parallel we are developing in QTL regions (1) a physical map based on radiation hybrid maps and BAC contigs, and its comparison with human genome map; (2) a denser genetic map with new microsatellites and SNP. A study of the transcriptome of animals with various QTL genotypes is also planned to better define QTL effects.

**E043**

**QTL research on duration of tonic immobility in quail**

ODILE ROUSSOT[1], KATIA FÈVE[1], FLORENCE PLISSON-PETIT[1], FRÉDÉRIQUE PITEL[1], ALAIN VIGNAL[1], JEAN-MICHEL FAURE[2], ANDREW D. MILLS[2], DANIEL GUÉMENÉ[2], CHRISTINE LETERRIER[2], SANDRINE MIGNON-GRASTEAU[2], PASCALE LE ROY[3], MIGUEL PEREZ-ENCISO[4], CATHERINE BEAUMONT[2]

*[1]Laboratoire de Génétique Cellulaire, INRA, Castanet-Tolosan, France; [2]Station de Recherches Avicoles, INRA, Nouzilly, France; [3]Station de Génétique Quantitative et Appliquée, INRA, Jouy-en-Josas, France; [4]Station d'Amélioration Génétique des Animaux, INRA, Castanet-Tolosan, France*

The study of behavioural traits is essential to the assessment of animals stress and welfare. A QTL detection study was initiated to identify the genome regions involved in the control of fearfulness, an important component of animals capacity of adaptation. An F2 cross was carried out between two quail lines that had been divergently selected for more than 25 generations for or against duration of tonic immobility (DTI), a catatonic-like state of reduced responsiveness to external stressful stimulation. A total of 1048 animals were obtained and measured for several traits including DTI and NI, the number of inductions necessary to induce immobility reaction. The difference (DIFF) between DTI and NI (both centred and reduced) was analyzed. Segregation analysis led to expect the existence of a gene explaining more than one standard deviation of DIFF. As no genetic map of quail was available yet, a set of 432 AFLP™ markers was developed. Using variance analysis, significant effects were observed for alleles of several markers belonging to one linkage group. A QTL analysis tends to confirm these results so that the existence of a QTL within this group is highly suspected.

**E044**

**Association of bovine butyrophilin encoding gene polymorphism with milk fat percentage in Polish Black-and-White and German Holstein-Friesian cattle**

CHANDRA SHEKHAR PAREEK[1], RAVI SHEKHAR PAREEK[1], KRZYSZTOF

WALAWSKI[1], HANS-MARTIN SEYFERT[2]
[1]*Department of Animal Genetics, University of Warmia and Mazury, Olsztyn, Poland;* [2]*Division of Molecular Biology, Research Institute for the Biology of Farm Animals, Dummerstrof, Germany*

Butyrophilin is a type I membrane protein of immunoglobulin super-family that is secreted in association with the milk fat globule membrane from mammary epithelial cells. A protein sequence polymorphism concerning aa position 35 (*Pro* vs *Glu*) was identified and verified in the active Polish Black-and-White and German HF breeding population. The observed allele A encodes for aa *Pro* at position 35 in contrast to *Glu* encoded by allele B. The association of these allelic variants with milk production traits among Polish Black-and-White and German HF population was investigated. A total of 88 and 298 breeding bulls from Polish Black-and-White and German HF was genotyped for A and B alleles by PCR-RFLP method. In addition, a half-sib pedigree from Polish-Black-and-White bull was genotyped for A and B alleles, since animals revealed significant differences in the parameters, *viz.*, milk, fat, protein yields and their percentages. The frequencies of alleles A and B were observed as 25.3%, 74.7% in 83 half-sib progenies from Polish-Black-and White cattle and 57.9%, 42.1% in German HF bulls, respectively. A significant association was found between this protein sequence polymorphism and milk fat percentage. Allele B correlates with a significantly increased milk fat percentage without affecting the milk yield.

**E045**
**Mapping of an immotile short tail sperm defect in Finnish Yorkshire on porcine chromosome 16**

ANU I. SIRONEN[1], MAGNUS ANDERSSON[2], PEKKA UIMARI[3], JOHANNA VILKKI[1]
[1]*MTT Agrifood Research Finland, Animal Production Research, Animal Breeding, FIN-31600 Jokioinen, Finland;* [2]*University of Helsinki, Department of Clinical Veterinary Sciences, Helsinki, Finland;* [3]*University of Helsinki, Department of Biosciences, Helsinki, Finland*

An immotile short tail sperm defect (ISTS) has recently been identified as a hereditary disorder within the Finnish Yorkshire pig population. The syndrome is inherited as an autosomal recessive disease exclusively expressed in male individuals as shorter sperm tail length and immotile spermatozoa. Currently 61 boars are known to have the syndrome. Based on the assumption of a recent common origin of the disease-causing mutation, a genome-wide search was performed with 228 evenly spaced microsatellites by homozygosity mapping of affected and unaffected DNA pools. One locus, SW2411 on Chr 16, demonstrated a significantly skewed allele distribution between the two pools. Linkage analysis of five markers in this region mapped the disease-causing gene within a 6-cM confidence interval region with a highest LOD score of 7.7 at marker SW419. Based on haplotype data, the mutation lies between markers SW2411 and SW419, and therefore the confidence interval determined with linkage analysis can be reduced to a 3 cM region proximal to SW419. It appears that three-marker haplotypes can be used for marker-assisted selection within analyzed pedigrees, but pedigree data is still needed for MAS. Furthermore, current fine mapping may reveal a more precise population-wide associated haplotype and facilitate identification of a new gene affecting sperm tail development.

**E046**
**QTL mapping experiment in F2 cross of chickens divergently selected for antibody response to SRBC**

M. SIWEK[1], S.J.B. CORNELISSEN[1], M.G.B. NIEUWLAND[2], A.J. BUITENHUIS[1], H. BOVENHUIS[1], J.J. VAN DER POEL[1]; H.K. PARMENTIER[2]
[1]*Animal Breeding and Genetics Group,* [2]*Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, NL – 6700 AH Wageningen, The Netherlands*

The aim of this paper is to present a first data of experiment of mapping QTL associated with immune response. The experimental F2 population originates from a cross (ISA Warren) between two divergently selected lines for either high (H line) or low (L line) primary antibody response to sheep red blood cells (SRBC) at 5 days after primary intramuscular immunisation with SRBC at 37 days of age. For the QTL experiment reciprocal crosses have been made to produce 1240 F2 animals. The phenotypic data for the immunological

responses have been determined. Blood samples were collected from birds in the day 0 and the test day for relevant antigens i.e.: primary antibody response against SRBC at 35 days, *E.coli* at 55 days, KLH-DNP (keyhole limpet haemocyanin – dinitrophenyl) at 82 days, *Mycobacterium butyricum* at 103 days, secondary antibody response against SRBC at 127 days and cellular response against ConA (ConcanavalinA) at 132 days of age. For the genotypic analysis the Whole Genome Scan has been performed, 174 microsatellite markers were chosen, distributed over the chicken genome, approximately 20 centiMorgan (cM) apart, to type in total 722 animals. In QTL analysis the half sib model will be used for each of 24 half sib families (12 per reciprocal cross).

**E047**

**A genome scan for gastrointestinal nematode resistance in cattle**

TAD S. SONSTEGARD[1], LOUIS C. GASBARRE[1], CURTIS P. VAN TASSELL[1], R. MARK THALLMAN[2], TEREZINHA PADILHA[1,3]

[1]*United States Dept. of Agriculture, Agricultural Research Service, Beltsville, MD, USA;* [2]*United States Dept. of Agriculture, Agricultural Research Service, Clay Center, NE, USA;* [3]*EMBRAPA-ARS Labex Program, Beltsville, MD, USA*

Breeding for host resistance offers an alternative method to control disease caused by gastrointestinal (GI) parasites. However, the genetic factors affecting host resistance and transmission in cattle have yet to be identified. Using a population of linebred Angus cattle that was divergently selected for resistance to GI parasites, we performed a genome-wide scan to identify QTL for traits related to infection. Genotypes for 196 microsatellite markers were generated from ~300 progeny with phenotypic records for parasite challenge, parents, and over 70 sires from the historic pedigree. Traits analyzed were mean, peak, and final numbers of nematode eggs/gram (EPG) of feces and mean and final serum pepsinogen levels. Marker segregations were determined using Genoprob. The analysis model included sex of calf, age of dam at calving, age of calf at parasite challenge, calving season, sire, and within-sire regression on the probability of inheriting one of the two QTL alleles of the sire. The number of progeny with both marker data and phenotypes ranged from 2 to 14 in each of the 43 sire families. A total of 175 of 37,539 tested effects were significant within family ($P<0.001$), and 17 marker by trait combinations on seven chromosomes out of 905 tested were significant ($P<0.01$) in across-family F-tests. In both analyses, adjacent markers were found to be significant. A permutation test is under development to account for non-normality of the EPG data and multiple testing.

**E048**

**A deletion of HSPA1B causes hereditary myopathy of diaphragmatic muscles in Holstein-Friesian cattle**

MAYUMI SUGIMOTO[1], HIDEFUMI FURUOKA[2], YOSHIKAZU SUGIMOTO[3]

[1]*National Livestock Breeding Center, Nishigo, Japan,* [2]*Obihiro University of Agriculture and Veterinary Medicine, Department of Veterinary Pathology, Obihiro, Japan;* [3]*Shirakawa Institute of Animal Genetics, Nishigo, Japan*

Hereditary myopathy in Holstein-Friesian cattle is an autosomal recessive disease characterized by late onset, ruminal tympany, and central core-like structures in the diaphragmatic muscles. A whole-genome scan of 26 family members, including 12 affected, showed significant linkage of a hereditary myopathy locus to a 0.6-cM interval in BTA23q21, equivalent to HSA6p21.3. The maximum LOD score, 7.67, was obtained for marker CYP21, near the genes that encodes heat-shock 70-kd protein 1A (HSPA1A) and 1B (HSPA1B). HSPA1A and 1B are duplicated heat-shock protein, 70-kd, (HSP70) loci within the bovine major histocompatibility complex on 23q21, 8 kb apart from each other, known to be the major mammalian chaperones. Sequencing of affected samples revealed a complete deletion of HSPA1B. Molecular chaperones prevent aggregation and refold unfolded proteins. Since central core-like structures in affected diaphragmatic muscles shows strong immunoreactivity for actin and ubiquitin, impaired protein clearance by incomplete HSP70 may underlie the pathogenesis of myopathy.

**E049**

**Genetic mapping of quantitative trait loci for fatness in chickens**

KEN TATSUDA, KUNINORI FUJINAKA, YUMIKO KURODA
*Hyogo Prefectural Agricultural Institute, Kasai, Japan*

To identify the quantitative trait loci (QTL) affecting abdominal fat deposition and lipid content in chickens, QTL analysis was carried out using a resource population of 222 $F_2$ progeny from a cross between a Satsumadori (a Japanese native breed showing little fat deposition) male and a White Plymouth Rock (a broiler breed showing significant fat deposition) female. We chose 80 microsatellite loci from 575 loci publicly available from 16 linkage groups, based on their utility and location. One QTL affecting the ratio of abdominal fat weight to live body weight at 16 weeks of age was mapped at 149 cM on chromosome 7, with a LOD score of 8.9, and accounted for 33.3% of the variance. The closest loci to the QTL were MCW0316 and ADL0169. Two QTLs affecting the lipid content in thigh meat at 16 weeks of age were mapped at 111 cM on chromosome 3 with a LOD score of 6.0, accounting for 75.1% of the variance and at 194 cM on chromosome 5 with a LOD score of 5.7, accounting for 69.5% of the variance. The closest loci to the QTLs were MCW0212 on chromosome 3 and ADL0298 on chromosome 5. The difference in these two traits in $F_2$ was associated with the allele types of each marker, which suggests the possibility of using marker-assisted selection for fatness in chickens.

**E050**

**Comparative sequence analysis of the *Insulin-IGF2* region harbouring a major QTL for muscle development in the pig**

ANNE-SOPHIE VAN LAERE, VALERIE AMARGER, LEIF ANDERSSON
*Swedish University of Agricultural Sciences (SLU), Department of Animal Breeding and Genetics, Uppsala, Sweden*

We and others have identified a paternally expressed Quantitative Trait Locus (QTL) primarily affecting muscle development at the distal tip of pig chromosome 2p. *IGF2* (*Insulin-like Growth Factor 2*) was identified as the major candidate gene due to its perfect colocalisation with the QTL, its paternal imprinting, and its important role in prenatal development in mammals. This QTL is segregating in our Wild Boar/Large White intercross and previous studies did not reveal any sequence difference in the *IGF2* coding sequence. Thus, we assume that the QTL may be due to one or more regulatory mutations. We have now determined 32 kb contiguous genomic sequence containing the *Insulin-IGF2* region. This region contains a very high GC content, many CpG islands and very few interspersed repeats. A comparative sequence analysis with the homologous region in humans and mouse revealed that all coding sequences are well conserved between species but also that a large proportion of the non-coding sequence in this region is surprisingly well conserved between species. The results will facilitate the further characterization of this important QTL in the pig and we are currently doing comparative sequence analysis of chromosomes with "known" QTL status in the search for causative mutation(s).

**E051**

**Mapping economic trait loci for exterior traits in a commercial pig population**

XIAOLING GUO[1], CHRISTIAN LOOFT[1], ESTELA FABUEL PEÑALVER[1], NORBERT REINSCH[1], HOLGER LOOFT[2], ERNST KALM[1]
*[1]Christian-Albrechts-University of Kiel, Institute of Animal Breeding and Husbandry, Kiel, Germany; [2]PIC Germany Ltd, Schleswig, Germany*

The aim of our project was to map economic trait loci for porcine exterior traits. The design of the project is based on a five line cross. 19 hybrid boars (Piétrain$^+$ x (Hampshire x Piétrain)) were mated to 52 hybrid sows (Leicoma x (Large White x Landrace). Their 333 progenies were fattened on our experimental farm and slaughtered at 115 kg live weight. For these animals 23 exterior traits as for example leg scores were scored by a trained person following a subjective linear scale. A genome scan covering all chromosomes with 159 microsatellite markers and 3 class-I-markers (*Ryr1*, *RN, Pit1)* was performed. Marker linkage maps were computed with CriMap and used for interval mapping using a least-squares regression model in parental half-sib families. Effects for five exterior traits, i.e. the fore legs angle, ear shape, the fore feet position, the leg

status and the hind legs step, were detected on chromosomes 4 ($P_{exp.} < .01$), 6 ($P_{exp.} < .01$), 6 ($P_{exp.} < .10$), 7 ($P_{exp.} < .01$) and 13 ($P_{exp.} < .10$), respectively.

**E052**

**Gene mapping progress in cattle and updated comparative map with man, mouse, rat and pig**

H. HAYES, C. ELDUQUE, M. GAUTIER, L. SCHIBLER, E. CRIBIU, A. EGGEN
*Laboratoire de Génétique biochimique et Cytogénétique, INRA, Jouy-en-Josas, France*

Our on-going goal is to improve and update the comparative genome organization between cattle and man but also among the most detailed mammalian species genomes ie cattle, mouse, rat and pig. In the past years, we have localized by FISH over 250 genes on the bovine chromosomes using bovine BAC and YAC probes and caprine BAC probes. In this work, we have compiled all the genes mapped in cattle, goat, sheep and pig (linkage mapping, somatic cell hybrid and radiation hybrid mapping, FISH) and for which the human ortholog is known and mapped (1646 genes). When possible the corresponding data in mouse and rat were included. For each human chromosome the genes were ordered precisely from telomere to telomere using the human genome sequence data (UCSC GoldenPath). This comparison of conserved syntenies among the human, bovine, mouse, rat and pig genomes provides an overall and detailed picture of the organization of the bovine genome relatively to the human genome, confirms that the degree of synteny conservation is higher between man and cattle or pig than between man, cattle or pig and mouse or rat and delineates 99 conserved chromosomal segments between man and cattle with a size ranging from 1 to 120 Mb. It also shows that the limits of the conserved chromosomal segments between man and cattle are mainly located in G bands or at the G/R bands junctions and that most of the regions for which no comparative mapping data exists is located in G bands known to be gene poor. This compilation contributes to the construction of comparative maps focusing on the order of genes within segments of conserved synteny and constitutes a powerful tool for positional candidate gene cloning approaches.

**E053**

**Detection of QTL for resistance to gastro-intestinal nematode infection in mice**

FUAD A. IRAQI[1], DAVID M. MENGE[1,2], JERZY M. BEHNKE[3], ANN LOWE[3], ALAN J. TEALE[1,4], JOHN P. GIBSON[1], LEYDEN R. BAKER[1], DAREK WAKELIN[3]
*[1]International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya; [2]Jomo Kenyatta University of Agriculture and Technology P. O. Box 62000, Nairobi Kenya, [3]School of Biological Sciences, University of Nottingham, NG7 2RD, U.K.; [4]Present address, Institute of Aquaculture, University of Sterling, FK9 4LA, U.K.*

Gastro-intestinal (GI) nematodes are arguably the most important disease in domestic ruminants. Current control mainly relies on chemotherapy, but resistance to all major anthelmintics is now widespread. There is a considerable interest in understanding the naturally occurring genetic resistance to GI nematodes in sheep. This can be done more rapidly in well-defined genetic mouse model. Different mouse strains show variation in response to GI nematode infection. Here we report results of a genome-wide search for QTL in an $F_2$ cross between the resistance, SWR and the susceptible CBA parental mouse strains that was challenged by trickle infection with *Heligmosomoides polygyrus*. Eight traits associated with resistance to GI nematodes infection were measured-i.e total worm count, three time points of faecal egg count post infection, immunoglobulin G1 and E titers, granuloma score and mucosal mast cell protease. Interval mapping analysis after selective genotyping detected 30 significant QTL affecting at least one of the resistance traits. Twenty-five QTL were associated with more than one resistance trait suggesting either pleiotropy or tight linkage as major mechanism of the QTL for gastro-intestinal resistance. Candidate genes within these QTL were identified. Some of the genes appear to have mechanisms controlling the intestinal immune and inflammatory responses known to protect against gut parasites. High resolution mapping of these QTL is ongoing using the advanced intercross lines (AIL) approach.

**E054**

**Chromosome-wide scanning of SSC3 for QTL associated with prolificacy in a Meishan x Large White population using a comparative mapping approach**

J.S. MELVILLE[1], Z. JIANG[1], A.M. VERRINDER GIBBINS[1], J.A.B. ROBINSON[1], J.P. GIBSON[2]

*[1]CGIL, Department of Animal and Poultry Science, University of Guelph, Guelph, Canada; [2]International Livestock Research Institute, Nairobi, Kenya*

Previous genomic scans have identified porcine chromosome 3 as harboring a suggestive QTL associated with ovulation rate. Based on the published linkage, cytogenetic and radiation hybrid (RH) maps, we have identified 41 genes and gene family clusters that have been placed on SSC3. Based on the location of orthologues of these genes on the human sequence map, we found that SSC3 is homologous to the regions 0 - 155 Mb on HSA2, 0 - 12 Mb and 45 - 130 Mb on HSA7, and 0 - 50 Mb on HSA16. Eighty-seven orthologous genes were selected as landmarks at approximately 3 Mb intervals in order to cover these homologous human segments. All primers for these orthologous genes were designed based on porcine gene or EST sequences and their orthologous status was confirmed by direct sequencing of PCR products amplified from Meishan and Large White genomic DNA pools. The sequences from the two pools were also compared to reveal any polymorphisms in these genes. All of these markers were typed on a pig/hamster RH panel in order to construct a high-resolution comparative map between SSC3 and the human genome. A Meishan x Large White population is being used to scan SSC3 for QTLs associated with sow productivity traits using high and low pools based on phenotypic performance.

**E055**

**Association studies using random and "candidate" microsatellite loci in infectious goat diseases**

GABRIELA OBEXER-RUFF[1], URSULA SATTLER[1], DOMINIQUE MARTINEZ[2], JEAN-CHARLES MAILLARD[2], CHRISTOPHE CHARTIER[3], NASIKAT SAITBEKOVA[1], MARIE-LOUISE GLOWATZKI[1], CLAUDE GAILLARD[1]

*[1]University of Berne, Institute of Animal Genetics, Nutrition and Housing, Berne, Switzerland; [2]CIRAD-EMVT, Petit-Bourg, Guadeloupe, FWI, & Montpellier, France; [3]AFFSA-Niort Laboratoire de Recherche Caprines, Niort, France*

We have established a set of 30 microsatellites of Bovidae origin for use in a biodiversity study in Swiss and Creole goats (Saitbekova et al. 1999, Glowatzki-Mullis, personal communications). Additiona microsatellites located next to "candidate" genes of interest, such as cytokine genes (IL4, IL12, INFgamma) and MHC class II genes (DRB, DYA) were tested in the caprine species in order to detect possible associations with two infectious caprine diseases. Microsatellite analysis was undertaken using an ABI373. In the first study, a total of 82 unrelated Creole goats, 37 resistant and 45 susceptible to Heartwater disease (Camus et al., 1995) were analysed. In this study, the two loci DRBP1 (MHCII) and BOBT24 (IL4) were positively associated with disease susceptibility, demonstrating a corrected p Value of 0.002 and 0.005, respectively. In a second investigation, we tested 36 goats, experimentally infected with the nematode parasite Trichostrongylus colubriformis (Chartier & Hoste, 1997). These animals were devided in a "low"- and "high"-excreting group on the basis of two independently recorded fecal egg counts. For this parasite resistance study, we detected a significant association of one of the alleles of the bovine microsatellite locus SPS113 with "low"-excretion (resistance). The MHC class II locus DYA (P19), was weakly associated with susceptibility in both diseases ($p_c$=0.05). In future experiments, we will extend the sample size in order to verify the described associations.

**E056**

**Positional Cloning of a Novel Gene, *LIMBIN,* Responsible for a Bovine Chondrodysplastic Dwarfism**

HARUKO TAKEDA[1], MARIKA TAKAMI[2], TOMOKO OGUNI[3], TAKEHITO TSUIJ[4], KAZUHIRO YONEDA[2], YUJI MISHINA[5], HIROAKI SATO[3], NAOYA IHARA[1], TOMOHITO ITOH[1], YASUO MORITOMO[6], YOSHIKAZU SUGIMOTO[1], TETSUO KUNIEDA[2]

*[1]Shirakawa Institute of Animal Genetics, Nishi-shirakawa, Fukushima 961-8061, Japan;*

*[2]Graduate school of Natural Science and Technology, Okayama University, Tsushima-naka, Okayama 700-8530, Japan; [3]Animal Husbandry Research Institute, Kumamoto Prefectural Agricultural Research Center, Kikuchi-gun, Kumamoto 861-1113, Japan; [4]Department of Oral Morphology, Okayama University Graduate School of Medicine and Dentistry, Shikata-cho, Okayama 700-8525, Japan; [5]National Institute of Environmental Health Science, Research Triangle Park, North Carolina, USA; [6]Faculty of Agriculture, Kyusyu Tokai University, Aso, Kumamoto 869-1404, Japan*

Chondrodysplastic dwarfism in Japanese brown cattle breed is an autosomal recessive disorder characterized by short limbs. We have previously mapped the locus responsible for the disease on a distal end of bovine chromosome 6. Here we narrowed the critical region for approximately 2 cM by linkage analysis, constructed a BAC and YAC contig covering this region and identified a novel gene, *LIMBIN (LBN)*, which possessed disease specific mutations in the affected calves. One mutation was single nucleotide substitution resulting in a creation of a cryptic splicing donor site and the other was one-base deletion resulting in a frameshift mutation. The *LBN* gene consists of 22 exons with 3,627-base open reading frame encoding 1,209 amino acids. Strong expression of *Lbn* gene was observed in limb buds of developing mouse embryos and in proliferating chondrocytes of epiphyseal growth plate. These findings indicate that *LBN* is responsible for bovine chondrodysplastic dwarfism and plays a role in skeletal development.

**E057**

**Comparative genome analysis between pig chromosome 4 and human chromosome 1 and 8**

FRIDA BERG[1], ALAN ARCHIBALD[2], SUSAN ANDERSON[2], LEIF ANDERSSON[1], MARIA MOLLER[1]

*[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Science, Uppsala, Sweden; [2]Department of Genomics and Bioinformatics, Roslin Institute, Scotland, UK*

The published draft human genome sequence facilitates comparative genome analyses of less analysed genomes such as the pig. Previously a major QTL (*FAT1*) that has large effects on fatness and growth was detected on pig chromosome 4q (SSC4q) using a Wild Boar intercross. We are pursuing both genetic and comparative mapping approaches in order to narrow the QTL interval. We have defined the QTL more precisely by tracking the inheritance of the Wild Boar QTL allele through seven generations of backcross pigs. Pig chromosome 4 is homologous to parts of human chromosome 1 and 8 (HSA1 and 8). We now have evidence that the QTL is located in a region homologous to HSA1q. Just outside the QTL boundary the breakpoint to HSA8 is defined by the gene *VATPase*. The homologues of the two markers flanking the QTL interval are both located in the interval HSA1q23-q24. New genetic markers for eight genes in that region have been developed and genotyped in a three generation intercross between the European Wild Boar and the domestic Large White comprising 236 animals. Single Nucleotide Polymorphisms (SNPs) were developed by amplifying gene sequences located in the QTL interval, using homologous human and/or mouse sequences for primer design and microsatellites were isolated from BACs corresponding to coding sequences within the same QTL region. A pig gene map was constructed and shows a high degree of conserved synteny with the human sequence map of HSA1q. The result supports the utilisation of the human genome sequence draft in our search for potential candidate genes for the QTL.

**E058**

**Mapping QTL affecting milk composition traits in dairy cattle using a complex pedigree**

AMANDA J. CHAMBERLAIN[1], HELEN C. McPARTLAN[2], THAMY BALASINGHAM[2], MICHAEL J. CARRIK[2], PHILIP J. BOWMAN[2], NICHOLAS A. ROBINSON[2], MICHAEL E. GODDARD[1,2]

*[1]Institute of Land and Food Resources, University of Melbourne, Australia; [2]Victorian Institute of Animal Science, Melbourne, Australia*

The families of most progeny tested males are too small for a grand-daughter design, but there are many relationships between families that could be used in mapping QTL. The aims of this experiment were 1) to detect QTL in a complex real-life pedigree, and 2) to estimate

QTL allele effects for MAS. Variance components estimates and QTL allele effects were calculated using restricted maximum likelihood, after Gibbs sampling was used to estimate the probability that QTL alleles are identical by descent. A QTL was detected on chromosome 14, accounting for approximately 47% of the genetic variance for fat% , and on chromosome 20, accounting for approximately 45% of the genetic variance for protein%. The standard deviation of predicted QTL transmitting ability for chromosome 14 was 67L for milk, 0.08% for fat% and 0.02% for protein% and 67L, 0.05% fat% and 0.03% protein% for the QTL on chromosome 20. The correlations between the effect of QTL alleles on milk, fat% and protein% were also calculated and were found to be not significantly different from +1 or –1 which is consistent with the presence of only 2 alleles at each QTL.

**E059**

**Identification of quantitative trait loci (QTL) in sheep for live body composition using x-ray computed tomography**

COLIN R. CAVANAGH, MARILYN JONES, GINA ATTARD, IMKE TAMMEN, HERMANN W. RAADSMA

*ReproGen, Centre for Advanced Technologies in Reproduction and Genetic, The University of Sydney, Camden NSW 2570, Australia*

A whole genome scan was implemented to detect quantitative trait loci (QTL) for body composition using an experimental back-cross between Awassi and Merino sheep. Computer Tomography (CT) was used to predict yield for body composition in 164 live wethers. The genome scan involved 117 informative microsatellite markers covering 26 autosomes and provided around 70% coverage of the genome. Preliminary results indicate the existence of thirty-one QTL identified (un-adjusted for body weight at the time of scanning) for carcass lean, carcass fat, internal fat, carcass weight, and bone quantity ($P<0.05$). Of these, seven reached chromosome wide significance ($P<0.01$) for predicted carcass lean, and bone content. Adjustment for body weight as a covariate, resulted in a different subset of thirteen QTL of which four were in common with unadjusted yield traits described above and seven reached chromosome wide significance ($P<0.01$) for internal fat, carcass fat, carcass lean and bone content. The procedure to use CT information in analysis of body composition for QTL detection without the need for slaughter of animals opens the possibility to screen for QTL in growth rate and tissue yield at different stages of the growth phase. The presence of QTL for body composition is the first stage in gene identification and paves the way for marker assisted selection or introgression to be utilised in sheep breeding programs to improve body composition.

**E060**

**Progress in QTL mapping for growth and fatness traits on porcine chr 7**

O. DEMEURE[1], N. IANNUCCELLI[1], G. LAVAL[1], C. RENARD[2], J. RIQUET[1], C. GENÊT[1], C. GASNIER[3], J.P. BIDANEL[4], D. MILAN[1]

*[1]Laboratoire de Génétique Cellulaire, INRA, Castanet-Tolosan, France; [2]Laboratoire de Radiobiologie et d'Etude du Génome, INRA, Jouy-en-Josas, France ; [3]Gene+, Erin, France; [4]Station de Génétique Quantitative et Appliquée, INRA, Jouy-en-Josas, France*

The porcine chromosome 7 contains QTLs influencing growth, back fat thickness and intramuscular fat content. To precise the QTLs position, we followed various approaches. Among them we performed comparative mapping analysis in the chromosomal region containing QTLs, we also analysed additional animal populations. 23 genes selected in a human region covering 40 Mb were mapped on IMpRH panel. We observed a high conservation of gene order and distances between our framework map and the UCSC human genome assembly, excepting for three genes, found 20 Mb upper than their human position. In parallel, to precise effects of QTL, we studied a synthetic LW x MS population under selection. Considering that if QTL have a significant effect on trait, the frequency of favourable alleles should increase in selected animals, we studied evolution of allele frequency for markers covering the genome on animals from F0 and F2 to F5 generations. Simulations have been done for each marker, to verify that evolution of frequencies could not be explained by genetic drift only. On chr7, one Meishan haplotype was highly selected. Results also suggest that all Meishan alleles do not have the same effect. Comparing these results with results obtained on an other synthetic line, we are trying to identify an IBD segment contain-

ing QTL(s). These combined approaches should allow us to better map genes involved, and to identify markers of interest for Marker Assisted Selection in synthetic lines.

**E063**

**Use of radiation hybrid mapping to locate candidate genes for female reproductive traits on porcine chromosome 8**

ANNEMARIE H. KING[1], ZHIHUA H. JIANG[2], GARY A. ROHRER[3], JOHN P. GIBSON[2], DAVE WADDINGTON[1], ALAN L. ARCHIBALD[1]

*[1]Roslin Institute, Roslin, Midlothian, UK; [2]University of Guelph, Guelph, Canada; [3]USDA-MARC, Clay Center, Nebraska, USA*

Improvement of porcine reproductive performance by traditional selective breeding programs has been limited by the low heritabilities of traits such as ovulation rate, prenatal survival and litter size. Therefore, the identification of candidate genes for use in marker-assisted selection programmes would be particularly desirable. Quantitative trait loci (QTL) for prenatal survival, litter size, teat number and ovulation rate have been mapped to chromosome 8 in earlier studies. The number of genes mapped to pig chromosome 8 and thus positional candidate genes is limited. We have developed a radiation hybrid map of pig chromosome 8, in order to determine whether specific genes map within the QTL. A skeleton RH map based on 44 markers (including 15 genes) has been established using the Cambridge-Roslin pig-hamster whole genome RH panel (http://www.ri.bbsrc.ac.uk/radhyb/) and the Carthagene software (http://www.inra.fr/bia/T/CarthaGene/). The mapped genes include the pig homologues of the Booroola (*BMPRIB*) and SPARC-like 1 (*SPARCL1)* genes. The resulting gene map confirms the earlier observations of extensive conservation of gene order between human chromosome 4 and pig chromosome 8, but with at least one major rearrangement. AHK is supported by a BBSRC industrial CASE studentship with Sygen as the industrial sponsors.

**E064**

**The estimation of the candidate genes polymorphism effect on the reproductive traits in line 990 sows**

AGNIESZKA KORWIN-KOSSAKOWSKA[1], MARIAN KAMYCZEK[2], DANUTA CIEŚLAK[1], MARIUSZ PIERZCHAŁA[1], JOLANTA KURYŁ[1]

*[1]Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzębiec,05-552 Wólka Kosowska,Poland; [2]National Research Institute of Animal Production, Pig Hybridization Centre, 64-122 Pawłowice,Poland*

The following genes: *FSHB*, *OPN*, *PRL* and *PRLR, LEP* and *LEPR*, were investigated as a candidate genes for reproduction traits. The material consisted of 519 sows from Polish Synthetic 990 line with differentiated level of reproduction indicators. Several performance traits were recorded: total number born (ILUR), number born alive (ILURZ) number piglets at age of 21 days (IL21), number weaning piglets (ILODS), litter weight at age of 21 days (WAG21), litter weight at weaning (WAGODS). The relationship between each gene genotypes and reproductive traits was evaluated according to the last squares method. In *PRL* l*ocus* no significant effect was observed in first parity for litter size but in later parities the 2/3 animals produce more piglets than 1/1, 3/3 and 1/2 sows. For traits ILUR, ILURZ, and IL21 this effects are significant at $P<0.01$. In *locus PRLR*, amplified according to (Vincent 1998), significant effect for number born alive (ILURZ) of the BB genotype was observed but only in first parity. In the current study the *LEP* and *LEPR* regions were amplified according to Stratil et al 1997 (*LEP*) and 1998 (*LEPR*). In first parity they were no differences between genotypes for reproduction traits but in later parities the significant effect was observed for the traits ILUR and ILURZ (*LEP*) also WAG21 and WAGODS (*LEPR*). In *OPN locus,* in later parities significant effect was observed for the traits IL21, ILODS, and WAG21, in all cases value of the traits was highest for homozygote AA. No significant associations of the *FSHB* locus with litter size and weight of the piglets were observed.

**E065**

**Genetic correlations among libido, scrotal circumference and reproductive female traits in Nelore breed**

C.R.QUIRINO[1], J.A.G. BERGMANN[2], M.Y. KUABARA[2] , C.G. FONSECA[2], V.R.VALE FILHO[2], V.J. ANDRADE[2], S.R. REIS[2], R. MENDONÇA[2]

*[1]State University of North Fluminense-RJ,*

*Brazil; [2]Federal University of Minas Gerais State, Veterinary Faculty, Belo Horizonte-MG, Brazil*

The objective of this study was to estimate genetic correlation between libido and scrotal circumference of yearling Nelore bulls and age at first calving and days to first calving of their half sib sisters. Days to first calving were calculated as the number of days between the beginning of the breeding season and the date of the first calving. Least squares means and standard errors were 2.4±.5 and 32.9±2.1 cm for libido and scrotal circumference, respectively. Least squares means and standard errors for age at first calving and days to first calving were, respectively, 37.8±3.9 months and 298.3±20.9 days. Genetic correlation between libido and age at first calving and days to first calving were -.21±.32 and -.18±.11 and, -.66±.58 and -.42±.25 for sister and daughter, respectively. Genetic correlation between scrotal circumference and age at first calving and days to first calving were -.09 ± .30 and -.10±.23, respectively. Results suggested that selection for libido would be more effective than selection for scrotal circumference when trying to improve female reproduction.

## E066

**Association analysis between the deletion mutant allele of Claudin-16 deficiency and carcass traits in Japanese Black cattle**

NAOHIKO KOBAYASHI[1], TAKASHI HIRANO[2], TAKAYUKI IBI[3], TUYOSHI OHTANI[1], YOSHIYUKI SASAKI[3], YOSHIKAZU SUGIMOTO[2]

*[1]Gifu Prefectural Livestock Research Institute Hida Beef Cattle Department, Gifu, Japan;[2]Shirakawa Institute of Animal Genetics , Fukushima, Japan; [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan*

We have reported that Claudin-16 (CL-16) deficiency, an autosomal recessive disease in Japanese Black cattle, is caused by a deletion mutation of 37-kb region including exon 1 to 4 of CL-16, resulting in lack of the CL-16 expression. The CL-16 DNA-based diagnostic tests revealed that the mutant allele has widely been distributed among sires highly evaluated. Whether the mutant allele is tightly linked to economically important trait loci became an important issue. We thus analyzed the association of the mutant allele with carcass traits available. Normal allele (D) and mutant allele (d) were diagnosed in 464 fattened offspring of three carrier sires (Sire 1-Sire 3: Dd), and 1,207 reproduction cows of Sire 1. The fattened offspring were 230 normal (DD) and 234 carrier (Dd), and the reproduction cows were 577 DD and 630 Dd. Sire, sex, farm and age (linear and quadratic) were considered as the source of variance in order to evaluate factors affecting carcass traits.

The significant association of the CL-16 genotypes was not seen in the carcass traits when the effects of environmental factors were eliminated ($p > 0.05$). Moreover, a significant association of the genotypes was not seen with the predicted breeding value for carcass traits in reproduction cows of Sire 1. Furthermore, the carcass traits had no significant linkage with the approximately 30 cM region including CL-16 locus in a half-sib family of Sire 1 comprising 136 offspring ($p > 0.05$). Therefore, exclusion of the mutant allele d from Japanese Black cattle population would not deteriorate the carcass traits.

## E067

**The relationship between polymorphisms of the porcine *myogenin*, *MYF3* and *MYF5* genes and microstructural characteristics of *Longissimus* muscle.**

JOLANTA KURYL[1], DANUTA KLOSOWSKA[2], DANUTA CIESLAK[1], GABRIELA ELMINOWSKA-WENDA[2], WOJCIECH KAPELANSKI[3], MARIUSZ PIERZCHALA[1], KONRAD WALASIK[2]

*[1]Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzebiec, Poland; [2]University of Technology and Agriculture, Department of Histology, Bydgoszcz, Poland; [3]University of Technology and Agriculture, Department of Pig Breeding, Bydgoszcz, Poland*

The effect of polymorphisms in the *MyoD* family genes on microstructural characteristics of porcine *Longissimus* muscle was investigated in 115 crossbreds [Pietrain x (Large White x Landrace)]. The porkers were slaughtered at about 105 kg live body weight. Muscle samples taken from M. *longissimus lumborum* for histological examinations were cut in cryostat and subjected to double reaction for activity of NADH-TR oxidoreductase and myofibrillar ATPase to identify muscle fiber types (STO, FTO, FTG). Ten muscle bundless were

randomly selected to evaluate the proportion of muscle fibers and a content of pathological fibers. The diameters of fibers were measured using a Leica Q 500 MC image analysis system. A polymorphism in the myogenin gene (*MYOG*) was identified at its 3' end (Soumillion et al., 1997) and that in the *MYF3* gene in intron 1 (Knoll et al., 1997). Two polymorphisms in the *MYF5* gene were identified with *Hinf*I and *Dde*I according to te Pas et al. (1999) and Stratil & Cepica (1999), respectively. The diameter of FTO fibers and content of both STO and FTO fibers in the bundle were affected with the *MYF5/Hinf*I genotype. The content of giant fibers in the bundle was significantly dependent on *MYOG* genotype ($P<0.05$) and *MYF5/Hinf*I genotype ($P<0.01$). Significantly ($P<0.05$) lowest content of pathological fibers in the bundle was observed in *Longissimus* muscle of porkers of AA genotype at the *MYF3*/*Dde*I *locus*. We concluded that the typed polymorphisms are probably linked to causal mutations influencing microstructural characteristics of porcine *Longissimu*s muscle.

**E068**

**Selective DNA pooling as a rapid screen for marker allele frequency differences between two groups: An approach for improved sensitivity**

MAXY MARIASEGARAM[1], NICHOLAS A. ROBINSON[2], MICHAEL E. GOODARD[1,2]
[1]*Institute of Land and Food Resources, University of Melbourne, Australia;* [2]*Victorian Institute of Animal Science, Melbourne, Australia*

DNA pooling is a strategy where individual DNA samples from each of two test groups are pooled and amplified in a PCR reaction. This enables rapid screening of a large number of markers for linkage with polygenic traits. The technique is combined with selective genotyping, whereby subjects will only be added to the pool if their scores for the trait under study fall at the extremes of the phenotypic distribution. DNA from the "high" and "low" groups respectively can then be pooled separately. We have utilized such a method to identify genes affecting milk yield and composition in Australian dairy cattle using a genome wide scan. This poster focuses on techniques to make selective DNA pooling reproducible and sensitive to small changes in allele frequency differences between groups. Of critical importance is the accurate quantification of DNA. It is proposed that quantitative competitive PCR (QC-PCR) offers the best prospects in this regard. By using a suitable correction procedure, we have also shown that accurate estimates of allele frequencies can be obtained from a pool.

**E071**

**Major genes for high resistance to *Haemonchus contortus* and *Fasciola gigantica* in Indonesian Thin Tail (ITT) sheep**

H.W. RAADSMA[1], E.T. MARGAWATI[2], D. PIEDRAFITA[3], E. ESTUNINGSIH[4], S. WIDJAJANTI[4], BERIAJAYA[4], SUBANDRIYO[5], P. THOMSON[1], T.S. SPITHILL[3]
[1]*Reprogen, The University of Sydney, Camden, Australia;* [2]*Research and Development Centre for Biotechnology-LIPI, Cibinong, Indonesia;* [3]*Department of Biochemistry and Molecular Biology, The University of Monash, Australia;* [4] *Veterinary Research Institute Balitnak, Bogor Indonesia and* [5]*Central Research Institute for Animal Science, Bogor, Indonesia*

We have undertaken a molecular characterisation of a putative major gene for resistance *Fasciola gigantica* and *Haemonchus contortus* in Indonesian Thin Tail (ITT). Our gene mapping approach uses 10 resource families (900 progeny), which are a backcross between resistant (ITT) and susceptible (Merino) genotypes in a susceptible (Merino) background. All progeny are being phenotyped for expression of resistance by challenge with immature parasites. Preliminary segregation analysis in 400 progeny from 7 sires, confirmed the presence of a gene with large effect for resistance to both *F gigantica* ($FG^R$) and *H contortus* ($HC^R$)in this population. A full genome scan using a panel of 85 highly polymorphic microsatellite markers provided support for 3 QTL and a chromosomal location of putative major resistance genes $FG^R$ and $HC^R$ for a positional candidate cloning approach. Preliminary evidence of the puative QTL for $FG^R$ and $HC^R$ mapped to different chromosomes, suggesting that resistance to these two parasitic diseases is not under control of the same gene.

**E072**

## Fine mapping of quantitative trait loci (QTL) affecting susceptibility to ascites in chicken

TARIK RABIE[1], RICHARD CROOIJMANS[1], ADDIE VEREIJKEN[2], GERARD ALBERS[2],TINEKE VEENENDAAL[1], ROSILDE DIJKHOF[1], JAN VAN DER POEL[1], HENK BOVENHUIS[1], MARTIEN GROENEN[1]
*[1]Wageningen University, Animal Breeding and Genetics Group,Wageningen Institute of Animal Sciences, Wageningen, The Netherlands; [2]Nutreco, Boxmeer, The Netherlands*

An experimental population was created for mapping of loci that are involved in the resistance/susceptibility of broilers to develop pulmonary hypertension syndrome (PHS) also known as ascites. A total genome scan with microsatellite markers was performed on the F1 and F2 generations (10 full-sib families with a total of 476 individuals),whereas an F3 generation (4202 animals) was phenotyped. A regression analysis identified three genomewise significant QTL on chromosomes 2, 4 and 6 (genomewise p values of 0.01, 0.04 and 0.02 respectively) and suggestive QTL on chromosomes 1, 2, 3, 5, 8, 10, 13 and 28 (p values between 0.11 and 0.77). To validate and to narrow down the QTL regions, the original cross was used for breeding up to the F8 generation. Approximately 4000 F8 animals were measured for the ascites related traits, and are currently being analysed with microsatellite and SNP markers on chromosomes 2, 4, 8, 10 and 28. Furthermore, extensive physical mapping (BAC contig building) is used for the targeted development of SNP markers and for the construction of detailed gene maps for these QTL regions.

**E073**

## Characterization of QTL associated with resistance to nematode infection in sheep

TRACY L. SHAY[(1)], JAMES E. MILLER[(2)], ROYAL A. McGRAW[(3)], GRANT A. WALLING[(4)], STEVEN C. BISHOP[(4)], CHRIS S. HALEY[(4)] & NOELLE E. COCKETT[(1)]
*[(1)]Utah State University, Department of Animal, Dairy and Veterinary Sciences, Logan, Utah USA, [(2)] Louisiana State University, Department of Pathobiological Sciences, Baton Rouge, Louisiana USA, [(3)] University of Georgia, Department of Physiology and Pharmacology, Athens, Georgia USA and [(4)] Roslin Institute, Edinburgh, Scotland*

Gastrointestinal parasites have a profound effect on sheep production. In this study, 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) was constructed at Louisiana State University and included F2 offspring of F1 parents produced from Gulf Coast Native (resistant) and Suffolk (susceptible) crosses. A selected group of these F2 lambs and their parents were genotyped for 50 microsatellite markers. Preliminary QTLs for parasite resistance were identified on ovine chromosomes 1 and 3. Interval mapping was then applied using all markers genotyped on each of these chromosomes. The most significant results were detected on chromosome 1, with a putative QTL localized to the center of this chromosome.

**E074**

## QTL for birth weight and daily gain at weaning on chromosome 23 in German Angus cattle

CHRISTINA WEIMANN, HORST BRANDT, MATTHIAS GAULY, GEORG ERHARDT
*Justus-Liebig-University of Giessen, Department of Animal Breeding and Genetics, Giessen, Germany*

Five paternal half sib families of German Angus (n = 428) were typed (including dams) with 9 microsatellites (*INRA132*, *RM033*, *BM1815*, *BM1258*, *BOLA-DRB1*, *BM1818*, *BM1905*, *CSSM5*, *DYMS1*) located on bovine chromosome 23. All dams were in the same parities for both years. The family size varied from 52 to 57 progenies for which individual birth weight and daily gain at weaning were measured. Animals were weaned at an age of 9 to 10 months. The average birth weight was 37.4 kg (± 5.5 kg) while average daily gain at weaning was 954 g (± 117 g). The number of alleles per marker ranged from 5 to 15 while heterozygosity ranged from 0.54 to 0.88. A sex averaged linkage map was constructed with the BUILD option of the CRIMAP package (v. 2.4) covering a distance of 88.0 cM. The marker distances varied from 5.1 to 18.0 cM. A QTL-analysis for birth weight and daily gain was done using the half sib analysis of the

QTL-express programme (http://qtl.cap.ed.ac.uk). Sex and year of birth (1999 and 2000) were included as fixed effects in the model. Permutation test (1000 iterations) was done to set chromosome wide significance thresholds. Within German Angus breed a QTL for birth weight could be detected at position 54 cM (p = 0.05). In addition a QTL for daily gain at weaning was detected at position 16 cM (p = 0.05) in one family.

**E075**

**A total genome scan for porcine hernia inguinalis and scrotalis**

K.BORNEMANN-KOLATZKI[1], C. KNORR[1], U. PETERS[1], B. HARLIZIUS[2], B. BRENIG[1]

*[1]University of Goettingen, Institute of Veterinary Medicine, Goettingen, German; [2]current address: IPG Institute for Pig Genetics BV, Beuningen, Netherlands*

There are numerous references that genetic factors are involved in the development of hernia inguinalis and scrotalis. Hernias result from an abnormal wide inguinal canal, whereby parts of the net and intestine descent into the inguinal canal (hernia inguinalis) or into the scrotum (hernia scrotalis). About 3% of all litters in pigs are affected. Neither in pigs nor in man the mode of inheritance could be clarified so far. The goal of this project was the identification of genes, which increase the susceptibility to develop hernia. For a genome scan, 71 full-sib pairs and their parents were genotyped with 110 polymorphic markers. Linkage analyses have been carried out with the Genehunter package combining parametric and model free allele sharing methods. The results showed that genetic heterogeneity is present, i.e. in different families different genome regions with influence on susceptibility for the defect have been identified.

**E076**

**Superdominance and dominance as reasons of heterosis in litter size in mice.**

C. BRUNSCH, I. STERNSTEIN

*Institute of Animal Sciences, Agricultural and Horticultural Faculty, Department of Breeding Biology and Molecular Animal Breeding, Humboldt-University of Berlin, Germany.*

The genetic reasons of heterosis in litter size with regard to superdominance and dominance effects were explained in two reciprocal mice inbred line cross experiments C57Bl/6J x NMRI/DKFZ and C57Bl/6J x Balb/cJ. 2014 $F_2$ animals could be used as base for the formation of performance groups after $F_1$ intercross. This concerned the mice with extreme high (≥ 13 offspring) and extreme low litter size (≤ 5 offspring) in both experiments. Altogether 112 microsatellites were analysed with an average distance of 29 cM. On base of estimations of dominance degree the evaluation of relations between extent of heterozygosity of microsatellites and litter size within the groups was done by chi square test according to Pearson's table of two times two contingence. In the case of superdominance theory of heterosis associations to heterosis in litter size exist on chromosomes 2, 10, 11 ,17, 18 and 19. Dominance as reason of heterosis could be described on chromosomes 5, 6 and 13. These genome regions are interesting for the development of strategies in heterosis breeding.

**E077**

**A significant QTL for the heel depth in Holstein cattle**

BERNHARD G BAUMGARTNER[(1)], BERND KRIEGESMANN[(2)], STEPHAN JANSEN[(2)], HERMANN H. SWALVE[(3)], BERTRAM BRENIG[(2)]

*[(1)] University of Barcelona, Department of Biochemistry and Molecular Biology, Barcelona, Spain, [(2)] University of Göttingen, Department of Veterinary Medicine, Göttingen, Germany, [(3)] Martin Luther University, Institute for Animal Breeding and Husbandry, Halle-Wittenberg, Germany*

Feet and leg problems are a major cause for an involuntary culling of cows in dairy herds. Attempts to improve upon this situation commonly are limited to the use of selection decisions based on scores for these traits within type score systems. Due to relatively low heritabilities for feet and leg traits the chances for a genetic improvement are very limited if conventional breeding strategies are used. Marker assisted selection should offer greater benefits. Up to now, several QTLs have been identified with granddaughter-designs and whole genome scans using microsatellites. However, none of these have been proven highly significant, nor linked to important genes.

In contrast, we chose a candidate gene approach. We isolated and characterized the bovine bovine sulfate transporter Slc26a2gene

and identified a polymorphism in the coding region. Differences in the sulfate transport efficiencies of the 2 alleles were demonstrated in cell culture.
Leg scores were obtained from 3 independent AI studs in Germany and the polymorphism was typed in 300 bulls. Using the PEST software contrasts between genotypes AA vs AB and AA vs BB were estimated. Among other findings, the B-allele appeared to be associated with higher heels in Holstein cattle and hardness criteria show favorable, although insignificant differences for the BB genotypes. Breeders desire higher heels and hence this polymorphism may be very valuable to improve efficiency in breeding programs.

**E078**
**Localisation and regional mapping of a major gene controlling ovulation rate in L*a-caune* sheep.**

FRÉDÉRIC LECERF[(1)], LOYS BODIN[(2)], JEAN-MICHEL ELSEN[(2)] & PHILIPPE MULSANT[(1).]
[(1)] INRA, Laboratoire de Génétique Cellulaire, Castanet, France and [(2)] Station d'Amélioration Génétique des Animaux, Castanet, France.
A new major gene increasing ovulation rate (OR) has been evidenced in the *Lacaune* population. Using non-prolific dairy ewes as support, we generated half sib back-cross (BC) families issued of 7 F1 sons (heterozygous) of 3 sires. After excluding the *Booroola* and *Inverdale* regions, a full genome scan was performed on these BC ewes. A first linkage of the *Lacaune* prolificacy gene was first obtained with markers IDVGA46 and BM17132 on the ovine chromosome 11 (OAR11). Ovine markers from OAR11 were added to the map around the *Lacaune* locus and the closest flanking markers are CSAP and CSSM15 at about 2 cM from the *Lacaune* locus. The localisation was confirmed by adding other animals (F1 and F1xBC). In order to define more precisely the human orthologous region and the existing breakpoints between HSA17 and BTA19 / OAR11, we have performed comparative mapping between these 3 species using a bovine radiation hybrid panel. This permitted us to define precisely the synteny between these species. The orthologous regions of bovine chromosome 19 and human chromosome 17 are screened for additional polymorphic markers to improve the precision of the localisation of the *Lacaune* gene. Human genes localised in this region and considered as possible positional and functional candidates will be tested.

**E079**
**Development of a resource population to detect QTL affecting resistance to gastrointestinal parasites of cattle.**

LOUIS C. GASBARRE [(1)], TAD SONSTEGARD [(2)], CURTIS P. VAN TASSELL[(2,3)] & TEREZINHA PADILHA[(4)]
*[(1)] Immunology and Disease Resistance Laboratory, [(2)] Gene Evaluation and Mapping Laboratory, [(3)] Animal Improvement Program Laboratory and [(4)] EMBRAPA-ARS LABEX Program, Animal and Natural Resources Institute, Beltsville Agricultural Research Center, Beltsville, USA*
Gastrointestinal nematodes severely reduce the efficiency of raising cattle on pasture. Genetic management of 15-25% of cattle can considerably reduce parasite transmission in cattle herds. A selection program was initiated using parental stock from Wye Angus of the U of MD. To date ≈350 progeny covering 4 generations have been phenotyped. Pedigree records trace back to the founding animals of the herd, and pedigree analysis reveals that >90% of the tested animals are paternally descended from a Wye bull born in 1944. DNA for genetic analysis has been acquired from all tested animals and from over 70 sires in the historic pedigree. Phenotyping is accomplished by placing weaned calves on pastures infected with the 2 most common nematode parasites of US cattle. Calves are monitored weekly for 19 physiologic measures. After at least 120 days on test, calves are selected for replacement breeders, re-challenge, or immediate kill. At kill an additional 23 immunologic or parasitologic measures are recorded. Calves are assigned as: 1) Type I- always low parasite eggs per gram of feces (EPG) values, 2) Type II - increasing EPG values followed by a drop to levels of Type I calves, and 3) Type III - consistent high EPG levels. Reinfected calves show secondary EPG values consistent with those observed during the primary exposure. The selection has increased the percentage of Type I and Type III calves, and the range of EPD values has been reduced to 0.5 of the mean EPG value.

**E080**

**Linkage and comparative mapping of the gene responsible for susceptibility towards *E. coli* F4ab/ac diarrhoea in pigs.**

CLAUS B. JØRGENSEN[(1)], SUSANNA CIRERA[(1)], SUSAN ANDERSON[(2)], ALAN ARCHIBALD[(2)], TERJE RAUDSEPP[(3)], BHANU CHOWDHARY[(1,3)] LEIF ANDERSSON[(4)] & MERETE FREDHOLM[(1)]

*[(1)] Royal Veterinary and Agricultutral University, Department of Animal Science and Animal Health, Frederiksberg, Denmark, [(2)]Roslin Institute, Department of Genomics and Bioinformatics, Midlothian, Scotland, United Kingdom [(3)]Texas A&M University; Department of Veterinary Anatomy and Public Health, College Station, Texas, USA [(4)] Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala, Sweden.*

In 1995, Edfors-Lilja and coworkers localised the locus for the *E. coli* K88ab (F4ab) and K88ac (F4ac) intestinal receptor to pig chromosome 13. Using the same family material we have refined the map position to a region between the microsatellites markers Sw207 and Sw225. Primers from these markers were used to screen a pig BAC library and the positive clones we used for FISH analysis. The results of the FISH analysis helped us to propose a new candidate gene region in the Sscr 13q41-q44 interval. Shot-gun sequencing of the BAC clones showed that the candidate region contains an evolutionary break point between human and pig. In order to further characterise the rearrangements between Sscr13 and Hsap3 detailed gene mapping of Sscr13 was carried out. Consequently, a detailed comparative map between Sscr13 and Hsap3 was constructed and two candidate regions on human chromosome 3 were identified for the gene responsible for susceptibility towards *E. coli* F4ab/ac diarrhoea in pigs.

***Nomenclature: E. coli* F4ab/ac diarrhoea in pigs, linkage mapping, comparative mapping**

**E081**

**Age-dependent quantitative trait loci affecting body weight in a cross between the selected mouse line NMR18 and DBA/2**

ERSIN KARATAYLI[(1)], ULLA RENNE[(2)], STEFANIE KARLE[(1)], OSWALD ROTTMANN[(3)] & GUDRUN A. BROCKMANN[(1)]

*[(1)]Research Institute for the Biology of Farm Animals, Department of Molecular Biology, Dummerstorf, Germany, [(2)]Research Institute for the Biology of Farm Animals, Department of Genetics and Biometry, Dummerstorf, Germany, [(3)]Technical University München-Weihenstephan, Institute of Animal Breeding, Freising, Germany*

Quantitative trait loci (QTL) influencing body weight at the age of 2, 3, 4, 5, and 6 weeks were mapped by linkage analysis in a F2-population of a cross between the high growth selected mouse line NMR18 and the control line DBA/2. In addition to body weight, QTLs influencing abdominal fat weight and muscle weight were mapped at the age of 6 weeks. The two mouse lines descent from different genomic origin. They differ in body weight by 115% at 42 days. Genome-wide significant QTL effects were mapped for body weight in at least one of the selected ages on chromosomes 1, 2, 3, 4, 7, 9, and 14. A shift in the effects of QTLs was observed depending on the age of the animals. The QTLs affecting body weight coincide with QTLs for abdominal fat weight on chromosomes 1, 2, 7, 14, and with QTLs for muscle weight on chromosomes 1, 7, 14. The effects found for all QTLs affecting body weight on different ages were mostly additive, accounting together for 35-45 % of the phenotypic variance of body weight within the corresponding F2-population. The information on the age-dependent QTL effects is useful for the elucidation of the underlying genes.

# Section F: Bioinformatics

**F002**

**A Danish – Chinese collaborative project: towards sequencing of the porcine genome**

THE DANISH PORCINE GENOME CONSORTIUM
*The Royal Veterinary and Agricultutral University, Department of Animal Science and Animal Health, Division of Animal Genetics, Frederiksberg, Denmark & Danish Institute of Agricultural Sciences, Department of Breeding and Genetics, Tjele, Denmark.*

An international consortium has been established between The Royal Veterinary and Agricultural University, The Danish Institute of Agricultural Science and The National Committee of Pig Breeding, Health and Production, on the Danish side, and Chinese Academy of Science (CAS Human Genome Centre/Beijing Genomics Institute) on the Chinese side. The long-term goals of the consortium are to obtain sequence information from the full complement of pig genes and work towards determining the major part of the DNA sequence of the porcine genome. This work will greatly facilitate the elucidation of the function of genes of phenotypic importance for traits that are of interest to the pig industry. In the initial phase of the project we have contributed by establishing 94 cDNA libraries representing mRNA isolated from different tissues/different developmental stages/different animals. From each of the libraries approximately 10.000 ESTs will be generated by sequencing from the 5' end. The status of the sequencing project will be presented.

**F003**

**Development of Human-Livestock Comparative Database for Gene Discovery**

SUE DeNISE, DAVID ROSENFELD, KIM MARSHALL, PHILIP CHEVALIER, TIM RULE, CRAIG KENT-BASHAM, DOBORAH SILER, LAURIE MOORE, KAREN BLOCK, BONNIE FERRIE, MICHELLE HUTTON, MICHAEL SPENCER, MICHAEL TORRES
*MMI Genomics, Davis, California, USA*

A comparative human-livestock database has been developed from public information and proprietary sequence derived from whole-genome shotgun sequencing of livestock. Contigs of assembled livestock genomic sequences were blasted to the human draft genomic sequence and their locations, based on human orientation, were stored in the comparative database, thus providing a human backbone for the livestock genomes. Gene indices were developed from public EST data from bovine, porcine and gallus species and consensus sequences of contig assemblies were stored in the database. The gene indices were linked to the genomic sequences by blast homology. These gene indices were also compared to NCBI Reference Sequence data (**http://www.ncbi.nlm.nih.gov/LocusLink/refseq. html**) and RefSeq accession numbers were stored as an attribute. The database contains public information from genetic linkage maps and physical maps from radiation hybrid panel data and BAC sequencing. In addition, locations of quantitative trait loci, identified by flanking sequences from the scientific literature, have been uploaded into the database. Visualization tools are being developed to allow multiple across-species and across-map comparisons. This unique resource will be used for comparative mapping of syntenic regions among all species for discovering economically important genes and understanding regulatory regions.

**F004**

**SNP allele frequency estimation based on the analysis of sequencing traces**

GREGOR DURSTEWITZ, ANDREAS WINTER, RUEDI FRIES
*Technische Universität München, Lehrstuhl für Tierzucht, Freising-Weihenstephan, Germany*

The detection of single nucleotide variation is most efficiently achieved by comparative fluorescence-based sequencing of PCR-products. We evaluated the possibility of estimating allele frequencies during the process of SNP discovery through the analysis of sequencing traces using the software suite *phred*, *phrap*, *polyphred* and the visualization tool *consed* for semi-automated polymorphism detection. The frequency estimates are based on the comparison of normalized amplitude values of the two alternative bases resulting from pooled DNA with normalized amplitude values of the corresponding bases resulting from DNA of homozygous and heterozygous individuals. Ampli-

tude values are extracted from the output generated by the base calling program *phred*. Best normalization is achieved through optimised binning of amplitude values. This is done by varying the range of bases used for calculation of the average amplitude value for a given base as well as by varying the number of adjacent bases excluded from average calculation. The approach was automated using *python* scripts and tested with several DNA pools with known frequencies. Predicted frequencies were usually within +/- 0.05 of the actual frequency. For some polymorphisms it was necessary to add DMSO to the PCR to ensure equal amplification of both alleles. The described approach is particularly useful for simultaneous SNP searching and preliminary allele frequency estimation in the context of association studies.

**F005**

**Mouse Genome Informatics (http://www.informatics.jax.org): The Comprehensive Integrated Online Mouse Information Resource**

DAVID GARIPPA, JUDITH A. BLAKE, CAROL J. BULT, JOEL E. RICHARDSON, JIM A. KADIN, MARTIN RINGWALD, JANAN T. EPPIG AND THE MOUSE GENOME INFORMATICS GROUP
*The Jackson Laboratory, Bar Harbor, ME 04609 USA*

The Mouse Genome Informatics (MGI) web site provides access to a comprehensive integrated public information resource for mouse genetics, genomics and biology. The MGI database integrates information on over 30,000 mouse genes and experimental data from over 71,000 references. The database provides extensive information on genes, including nomenclature and function; genetic, physical, and comparative maps; allelic variation; mouse mutant and strain phenotypes; and molecular segments and sequence clusters. Curated mouse orthologies with the human, rat and 17 other mammalian species are available. The Gene Ontology, Phenotype Classification Terms and Mouse Anatomical Dictionary provide controlled vocabularies for annotation and assistance in database searches. Many different types of gene expression information are integrated into the database. Gene expression for mouse development with over 23,000 references can be accessed. Emerging mouse genomic sequence data are integrated with genetic and biological data. The MouseBLAST server provides a sequence-level entry point into MGI gene data. Cancer-related genetic and phenotypic data, including images for different strains of the laboratory mouse, also are provided. MGI collaboratively curates data with SWISS-PROT and NCBI/LocusLink and provides extensive links with these and other online resources such as GenBank, PubMed and OMIM. MGI is supported by NIH grants HG00330, HG02273, HD33745, CA89713 and DOE-FG02-ER62850.

**F006**

**A bioinformatics pipeline and integrated genomics database for beef cattle**

C.A. GILL, S.R. XAVIER, C.A. ABBEY, D.L. ADELSON
*Department of Animal Science, Texas A&M University, College Station, TX, USA*

A data management system has been developed to maintain pre-existing genotypic and phenotypic data, as well as newly generated DNA sequences. Data from the Texas A&M Angleton project, resulting from the generation of reciprocal backcross families between Angus and Brahman cattle, have been assembled in a relational database. The database contains entries for 712 individuals, 84 phenotypes and genotypes for 406 markers. Web-based queries provide input for various statistical and mapping packages. We have also implemented a local BLAST and FASTA pipeline on a 48CPU SGI Origin 3800 supercomputer. A collection of PERL scripts ports ABI traces from a LINUX machine through the Phred/Phrap suite of programs to assign quality scores and remove any vector or *E. coli* sequences. Cleaned sequences are sent to Repeat Masker and then submitted to the supercomputer for similarity searches against appropriate databases. Our continuing efforts to positionally clone genes affecting carcass and growth traits have resulted in the generation of several large sequence data sets (>5000 traces). Many of our sequences come from BACs and similarity searches against the assembled human genome thereby provide comparative positional information on these BACs. Parsed BLAST output is then added to our relational database. Since we are constantly generating new sequence data and the public databases continue to expand, our BLAST results are

refreshed on a monthly basis.

**F007**
**Annotation and classification of porcine ESTs**

NORIYUKI HAMASIMA[1], KOHEI SUZUKI[1], HIDEAKI SUZUKI[1], TAKASHI AWATA[2]
[1]*STAFF-Institute, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan;* [2]*National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki 305-8602, Japan*
In order to integrate porcine ESTs into a radiation hybrid map presendet at another poster session (Suzuki et al.), we have analyzed porcine ESTs from cDNA libraries from the back fat tissue. On the other hand, as of the end of October 2001, 98,969 ESTs appeared in the GenBank database. After removing porcine mitochondral genome, repetitive sequences and endogenous virus sequences with RepeatMaster program, we clustered these sequences with d2_cluster with stackPACK v 2.1 (Electric Genetics PTY Ltd.) and obtained 71,628 sequences. These sequences were annotated with BLAST search against non-redundant GenBank database. We have selected 4,380 sequences with the homology score value >500. According to the human gene name, wie have localized these sequences to the human High-Throughput Genomic Sequences database and classified them according to the Gene Ontology Categories.

**F008**
**Intelligent software agents for managing distributed genomics data**

JOHN W. KEELE[1], JIM E. WRAY[1], MICHAEL N. HUHNS[2], LARRY M. STEPHENS[2], WARREN M: SNELLING[1], GREGORY P. HARHAY[1], RANDAL R. BRADLEY[1]
[1]*U. S. Meat Animal Research Center, State Spur 18D, Clay Center, Nebraska 68933, USA;* [2] *University of South Carolina, Department of Computer Science and Engineering, Swearingen Engineering Center, Columbia, South Carolina 29208, USA*
Maintaining a comparative genomics information system is challenging because data resources are distributed, heterogeneous and dynamic. Data originates from multiple resources distributed around the globe, and formats vary among resources. Data changes daily, so the information system needs to frequently download and integrate information from external resources to remain current. The system needs to reconcile differences in data format. Multi-agent software systems have the ability to integrate information from many external resources and reconcile differences in format. We are developing an autonomous multi-agent system to download sequence, functional annotation, and mapping information from external sites and integrate it into comparative maps. A data manager agent starts multiple pairs of data checker - downloader agents in response to messages from users or after querying instructions from a database at initialization. Each data checker agent detects changes in a file date for an external resource. When a change in date is detected, the data checker agent sends a message to its corresponding data downloader agent to download the file. Once a download is complete, messages are sent to agents controlling downstream processes indicating that new data is in the pipeline to be integrated into the information system. Downstream processes may include gunzip, formatdb, cross_match, blast and perl data parsers. Agents can be distributed over multiple platforms.

**F009**
**A rapid screening method for SNP discovery by MALDI-TOF mass spectrometry of RNase T1 digests**

STEFAN KREBS, IVICA MEĐUGORAC, MARTIN FÖRSTER
*University of Munich, Institute for Animal Breeding, Munich, Germany*
MALDI mass spectrometry is an established platform for high-throughput genotyping of single nucleotide polymorphisms (SNPs). For broad application of SNPs in animal genetics, however, the number of SNPs is far from sufficient. We present a method for SNP discovery that can use existing MALDI genotyping platforms and is automation-compatible. The method is based on *in vitro* RNA transcripts from PCR products, that can be used to obtain highly informative sequence fingerprints by digestion with the guanosine-specific ribonuclease T1. In these fingerprints a mutation can be detected as a mass shift or absence of a wildtype peak or appearance of an additional peak. Due to mass degeneracy of larger frag-

ments and multiple presence of shorter fragments in a given sequence a certain fraction of possible mutations will remain undetected with this method. Screening of both strands from one PCR product is possible by using both T3- and T7-tailed primers and the respective RNA polymerases and markedly decreases the probability of missing a SNP. We have used a simulation of RNase digests from all possible mutations of a set of randomly generated sequences that provides estimates for the general detection probability in dependence of PCR product length. A software package is provided that helps to design PCR primers by plotting out regions with a high SNP discovery score and calculates expected mass fingerprints and peaklists from the target sequence selected for screening. The method was tested on known polymorphisms and de novo sequenced genomic BAC clones.

**F010**
**SWISS-PROT, TrEMBL, InterPro & CluSTr and animal genetics**

MINNA H. LEHVASLAIHO; ROLF APWEILER
*EMBL Outstation – European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK*

SWISS-PROT Protein Knowledgebase is an annotated protein sequence database. It strives to provide entries with high-quality annotation, a high level of integration with other databases and a minimal level of redundancy. SWISS-PROT is accompanied by TrEMBL, a computer-annotated protein sequence database that can be considered as a preliminary section of SWISS-PROT. TrEMBL contains the translations of all coding sequences present in the EMBL Nucleotide Sequence Database, that have not yet been released as manually annotated, finished entry into SWISS-PROT. Together SWISS-PROT and TrEMBL provide the user with a tool for various aspects of animal genetics. The highest represented domestic animals in the databases are Gallus gallus (1027 entries in SWISS-PROT/1776 entries in TrEMBL), Bos taurus (1332/1529), and Sus scrofa (784/1479). InterPro is an integrated documentation resource for protein families, domains and sites. It is a powerful diagnostic tool formed by a collaboration between SWISS-PROT + TrEMBL, Pfam, PRINTS, PROSITE, ProDom, SMART and TIGRFAMs. CluSTr is a database of clusters of SWISS-PROT + TrEMBL proteins, based on pairwise sequence similarity. It is a useful resource for whole genome analysis.

**F011**
**SNP detection and characterisation from overlapping Bos taurus ESTs**

JOHN C. McEWAN, ANNA SANTURE, AMONIDA ZADISSA, MARK J. SCHREIBER
*AgResearch, Invermay Agricultural Centre, PB 50034 Mosgiel, New Zealand*

Assembly of 367,811 public and private *Bos taurus* EST and mRNA sequences resulted in 39,129 contiguous sequences (contigs) containing 2 or more ESTs and 62,232 singletons. Putative SNPs were identified from the 18,508 contigs containing four or more ESTs. The following screening criteria were used: a minor allele frequency greater than 15%, a minimum of 2 sequences with the minor allele, no variants with more than 5% frequency in the surrounding 10 bases, and less than 1 SNP per 200 bp of contig length. A total of 4493 putative SNPs were identified in 2262 contigs at a frequency of one SNP per 728bp. The average EST depth at the SNP site was 12.5 sequences. Using protein homology alignment, 1768 of these SNPs were identified as being in the protein coding regions of 1158 contigs. These represented 1051 unique SwissProt/TrEMBL hits. Based on the derived protein coding frame, 1127 of the putative SNPs were identified as coding for a change in amino acid. A number of criteria were used to assess the impact of the amino acid on the function of the protein. SNPs were ranked using a weighted index value, derived from amino acid similarity and substitution scores. This method was used as an initial automated screen to identify likely candidates for further study. This resource markedly increases the number of potential type 1 markers available for *Bos taurus* and also identifies candidates that may have a functional impact on phenotypic traits.

**F012**
**Analysis of genetic marker linkage without pedigree information**

ARDESHIR NEJATI-JAVAREMI[1], GHODRAT RAHIMI[2]
*[1]Anim.Sci.Res.Inst., Karaj, Iran; [2]Univ. Ma-*

*zandaran, Dept.Anim.Sci., Sari, Iran*
Increasing numbers of genetic markers are available in animals. Analysis of linkages between marker loci requires that their distance be known. Estimating map distance between marker loci is achieved by using genotypes of individuals at marker loci plus pedigree information. Pedigree information is required to determine linkage phase and to infer any meiotic recombination which takes place. Linkage measure is then based on some functions of the number of recombination. Although collecting pedigree information in most animals is relatively cheaper than obtaining marker genotypes, there are many situations that the pedigree may not be known effectively. Populations of fish reared in ponds or honeybees which mate out of human control are just a few examples. However, in many of the species where obtaining pedigree information is difficult it is quite possible to keep separate lines and their crosses. When two marker loci are linked it is possible to introduce gametic phase disequilibrium (GPD) between them through crossing two lines with differing allelic frequencies at those loci. GPD across generations is a function of recombination rate between loci and allelic frequency difference in two lines. In this study a simulation program has been developed to estimate recombination rates by monitoring change in GPD across generations. The program does not require individual pedigree information and shows performance of this simple methodology across a range of population structures.

**F013**
**Analysis pipeline for large-scale SNP dectetion in EST sequences**

FRANK PANITZ, LONE B. MADSEN, HENRIK STENGAARD, CHRISTIAN BENDIXEN
*Danish Institute of Agricultural Sciences, Research Centre Foulum, Dept. of Animal Breeding and Genetics, Tjele, Denmark*
As part of our participation in a number of EST projects various tissue-specific cDNA libraries from different species were analyzed for polymorphic mutations that could modify gene function or gene expression. Focussing on identifying missense SNPs in coding regions of genes, we devised an integrated approach that detects candidate sites in tens of thousands of EST sequences in a few hours. The SNP discovery pipeline is implemented on a Linux cluster (12 processors on 6 nodes) which allows parallel processing of multiple sets of EST sequences. After sequencing on a 96-well capillary instrument (MegaBace) sequence traces are run through a pipeline of programs that assign sequence quality values (Phred), mask contaminating vector sequence (Crossmatch) and mask repeated elements (RepeatMasker). Subsequently, clustering is performed on a DeCypher Supercomputer based on the BlastN algorithm (Tera-BlastN) and resulting lists of binned sequences are submitted to the contig assembly program Phrap. Each contig is scanned for polymorphisms using PolyBayes. Resulting output files containing SNP information (e.g. position, variation, template depth) are extracted into a database by in-house parsing tools. Together with Blast information of the contig consensi and/or individual sequences, lists of candidate SNPs are generated that are used to design assays for verification of polymorphisms and mendilian segregation in appropriate family material.

**F014**
**A bootstrap approach to reduce the bias of genetic distances estimated from population samples of different size**

HENNER SIMIANER
*University of Göttingen, Institute of Animal Breeding and Genetics, Göttingen, Germany*
Estimation of genetic distances between populations is based on estimated allele frequencies derived from samples of the respective populations. It is generally recommended, that equal sample sizes are used in all populations. If sample sizes are different, some popular distance measures, like Nei's (1972) standard genetic distance or Rogers' (1972) distance show a severe bias in form of an overestimation of the distance of populations represented by small samples. This phenomenon is demonstrated both with simulation results and with a real data set of 13 red cattle breeds, where sample size was on average 64 animals but one breed was only represented with 12 animals. This bias can be reduced by estimating distances from bootstrap samples (Efron and Tibshirani, 1993) of uniform size. Using this approach in the red cattle data reduced the average distance of the breed with the smallest sample to the other breeds by 54 per cent. The

effectiveness of the suggested resampling approach is compared with other correction methods suggested in the literature (Nei, 1987).
*References:* Efron, B., Tibshirani, R.J. 1993. An Introduction to the Bootstrap. Chapman and Hall, New York; Nei, M. 1972. American Naturalist 106: 283 – 292; Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York; Rogers, J. 1972. Measure of genetic similarities and genetic distances. Studies in Genetics VII, U. of Texas Pub

**F015**

**Multilocus iterative allelic peeling of marker genotypes generated from a linebred Angus population divergently selected for resistance to gastrointestinal nematodes.**

CURTIS P. VAN TASSELL[1], R. MARK THALLMAN[2], TAD S. SONSTEGARD[1], LOUIS C. GASBARRE[1], TEREZINHA, PADILHA[1,3]
[1]*United States Dept. of Agriculture, Agricultural Research Service, Beltsville, MD, USA;* [2]*United States Dept. of Agriculture, Agricultural Research Service, Clay Center, NE, USA,* [3]*EMBRAPA-ARS Labex Program, Beltsville, MD, USA*

Gastrointestinal nematodes can dramatically affect efficiency of cattle raised on pasture, and therefore, a selection program was initiated to investigate the role of host genetics on disease and parasite transmission. The objective of this study was to test the performance of GenoProb software for QTL analyses on this population. The population consists of over 300 progeny representing four generations of divergent selection for resistance to parasite infection. The parental animals were derived from a linebred Angus population descended from a single bull born in 1944. This historic portion of the pedigree spans another five generations. The complete pedigree included information for 931 animals, and 383 of those had genotypic data recorded for 196 microsatellite markers. GenoProb provided a solution for this complex pedigree by implementing multilocus iterative allelic peeling and was used to calculate genotype probabilities for each individual represented in the pedigree. GenoProb uses an incomplete penetrance model for marker data and generates probabilities of scoring errors. These probabilities identified animals with likely pedigree errors and problem markers that needed additional analysis to generate correct marker data. Finally, inheritance probabilities calculated were used for a quantitative trait locus analyses.

**F016**

**The Animal-Trust-Center approach in DNA-based traceability systems.**

DAVID LÓPEZ HERRÁEZ[1], HOLGER SCHÄFER[1], EBERHARD MANZ[2], VOLKER WAGNER[2], MICHAEL WINK[1]
[1]*University of Heidelberg, Institut für Pharmazeutische Biologie;* [2]*Generatio GmbH, Heidelberg, Germany*

Traceability of animals and meat along the entire production chain becomes a major application of DNA-fingerprinting. ISAG provides support for laboratory setup by recommending markers and organising comparison tests. Communicating identity data (DNA derived or other) across an open network requires technology specified for digital identity serving. For that purpose we introduce the Animal-Trust-Center (ATC) as the core element of the Animal-Trust-Infrastructure project, ATI* (www.vernet-info.de). ATC employs technology known from digital signatures and generates true digital identities out of biological identity data like DNA-fingerprints. This approach guarantees uniqueness, integrity and authenticity to every participant in an open network communication. A prerequisite for any comprehensive traceability and documentation system. Furthermore, only parental genotypes need to be established since DNA-fingerprinting has been optimised for informative power in paternity exclusion. With laboratory costs limited to reproductive animals the ATC might be useful in traceability systems for pigs as well. Data on selected markers, laboratory standardization achievements and the ATC interface for data submission will be presented.
*ATI is funded by the Bundesministerium für Wirtschaft und Technologie [01MS102] Generatio GmbH and Institut für Pharmazeutische Biologie are members of the ATI consortium. Further participants are: SECUDE GmbH, Darmstadt and Eurohandelsinstitut GmbH, Köln.

## Index

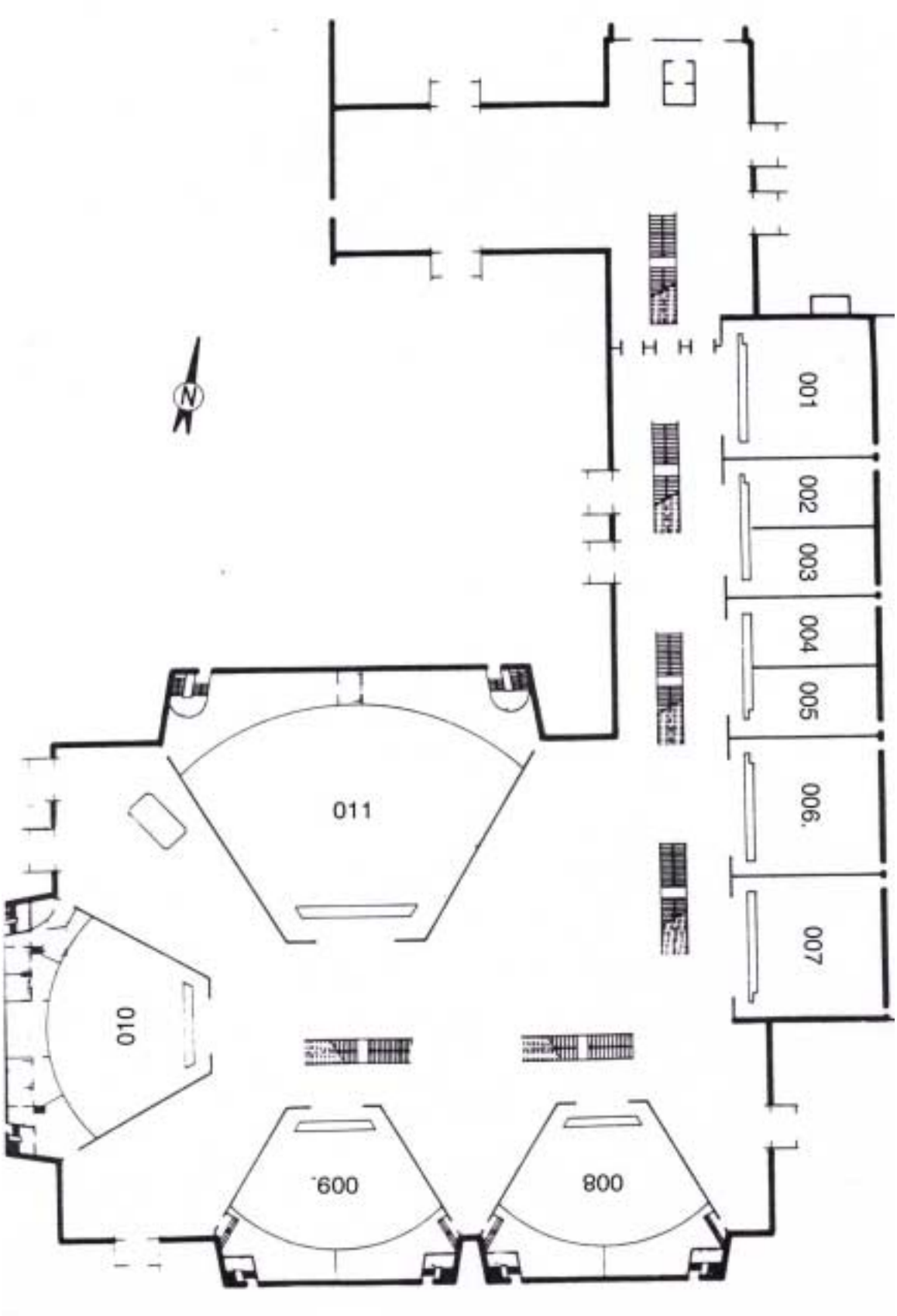
## Index

## Index

## Index

# 28th ISAG Conference 2002

## *Lecture Halls in the Blue Tower*




LI-COR
Biosciences


PYXIS
GENOMICS


Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE


Kompetenz für das Labor
Privatunternehmen seit 1879
ROTH


MWG
BIOTECH


schütt labortechnik
SCHÜTT


Niedersachsen


Sparkasse Göttingen
SEIT 1801


PYROSEQUENCING


STRATAGENE
EUROPE