How to Search

**A001**

## Analysis of genetic variation among five Chinese indigenous goat breeds by using bovine and ovine microsatellites

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Genetic variability has to be taken into account to guide genetic conservation programs. This study was conducted to determine the genetic variation between five Chinese indigenous goat breeds by using microsatellites from cattle and sheep. Blood samples from five Chinese indigenous goat breeds, Liaoning cashmere (33), Inner Mongolian cashmere (35), Tibetan cashmere (37), Wuan cashmere (49), and Matou goats for meat production (39) from Hubei province, were collected from random individuals. Fifteen pairs of microsatellite primers from cattle (BM1842, BM4621, BM6444, BM6506, BM757) and sheep (OarCP20, OarCP34, OarCP49, OarFCB11, OarFCB11, OarFCB128, OarFCB20, OarFCB266, OarFCB304, OarFCB48, MAF33, McM218) were tested to detect polymorphisms among those breeds. More than four alleles were found at six loci (BM4621, OarCP34, OarFCB11,OarFCB20, OarFCB304, OarFCB48) and generated a total of 57 alleles from the 5 breeds and 193 individuals analyzed. The other nine microsatellites were not used to assess genetic diversity because they were monomorphism or produced non-specific bands. Within-breed variation was analyzed. Heterozygosity, polymorphism information content and effective allele number had similar tendencies among breeds; Taihang had the highest values, followed by Neimonggol, Liaoning, Matou, and Tibetan breeds, in that order. Total gene diversity, average heterozygosity within each population, and coefficients of gene differentiation between breeds were calculated. The mean values of the three parameters are 0.831, 0.800, 0.038, respectively. The results show that variation among the breeds is low.

**A002**

**Comparison between serological and molecular genetic tests for parentage control in pigs in Germany.**

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For more than 40 years, blood typing for parentage control and blood type cards have been offered to pig breeders by the Goettingen Institute. The test sera were self produced and controlled in ISAG Comparison Tests. For blood group testing we used the following systems, depending on the available test sera: *A-S, D, E, F, G, H, K, L* and 3 electrophoretic systems: *EsD, GPI, PGD*. We calculated a cumulative exclusion probability (CEP) of 97.5% in 1980 over all breeds. After 20 years of intensive selection, we now decreased to a *CEP* of about 90%, which means that within the breeds gene frequencies have changed and thereby biodiversity was lost, particularly in the smaller German Large White and Hampshire breeds. (*CEP*: 94,52% in German Landrace, 83,31% German Large White, 91,93% Pietrain and 85,44% in Hampshire). After introduction of PCR techniques mainly the microsatellites raised in importance for identification of individuals and parentage control. In collaboration with the Institute in Vienna, we prepared and tested a multiplex PCR with 10 microsatellites . They were tested on the same pigs which were blood typed and CEP was 98,84% in German Landrace, 98,80% in German Large White, 99,57% in Pietrain, but only 94,51% in Hampshire. In all breeds, except the German Large White, the polymorphism information content (PIC) of the microsatellites SW240, SW857, S0155 and SW24 were the highest with more than 0.60. S0227 had the lowest *EP* in all breeds and was even fixed in Hampshire and should be replaced. The aim is to reach a CEP of more than 99.5% in all breeds.

**A003**

## Potential for breed assignment of horses

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Degree of population demarcation was investigated in eight European horse breeds, of which four represent Norwegian breeds. Altogether, 306 individuals were genotyped for 26 microsatellite loci. Two approaches were applied to assess potential for correct breed assignment: simple allele sharing statistics, and genotype simulation based on breed-specific allele frequency distribution. A clear breed differentiation was detected in the phylogenetic analysis based on allele sharing, and 95% of the individuals clustered together with animals of the same breed. Even breeds with a short period of breed divergence formed distinct clades. Moreover, the majority of the simulated individuals were assigned to their source population, but with this method potential of correct assignment was negatively correlated with genetic variation within a breed. In conclusion, the study demonstrated obvious distinction among horse breeds, and simple allele sharing statistics provided the most reliable information for testing the breed of individual animals.

**A004**

## Microphylogenesis of antigens in blood of nine strains of Kazakstan bulls

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The progress in human and animal microevolution and phylogenesis study allows scientists to use serology blood testing in the systematization of molecular taxonomy, or the immunogenetic and biochemical classification of cattle strains. As a result of antigen funds, alleles and genotypes studying unique scientific data were obtained on genetic structure peculiarities and their evolution in bulls of basic cattle breeds (alatauskaja, auleatinskaja, kazakh whitehead, black and white, golshtinofrizskaya, brown latvian, simmental, red stepee, anglerskaja), which are used at the Kazakstan artificial insemination stations, taking into account fact that they influence significantly the allelofund lines and related groups of animals of corresponding strains of cattle. Indices of immunogenetical distance (Ds and Dn) between populations were then determined. Distance varied from 0.1107 to 0.2260, or by 11.53%. This means that the differences between breeds are almost the same as within the breeds. So the reserve of immunogenetical variability within the breeds is wide. In order to receive more intergrated and obvious picture of immunogenetical relations between investigated populations the dendroprogram and the pattern of these populations position in nonlinear coordinates plane were obtained. So studying cattle allelofund of blood genetical markers will help to preserve, use rationally and enrich the genetical resources of Kazakstan animals.

**A005**

## Cytogenetical investigation and teratological screening of some Kazakhstan sheep breeds

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In 260 sheep of Karakul, Kazakh semifine wool and Edilbai breeds of different age and sex, the frequency of spontaneous chromosome aberrations was estimated. These sheep populations had different phenotypes, productivity level and were bred in different environment regions of Kazakhstan. To estimate qualitatively and quantitatively the mutation process dynamics induced by environmental mutagens and teratogens the frequency of lamb births with developmental anomalies was also studied. In Karakul sheep populations lamb anomalies were 1.5-2 times more frequent than in the Kazakh finewool sheep population (0.08±0.01% and 0.04±0.01%, respectively). In the course of teratological screening of the dam population, 474 ewes with different organ and tissue defects were found as well as 13 fetuses with developmental anomalies. Sixty-three types of inborn anomalies were detected, the descriptions of 18 types of inborn anomalies and 24 teratology variants were not observed in the available publications on sheep genetics. The specific feature of the research undertaken was the screening of the same sheep flocks for as long time as 2-3 generations changed one another. So the bone marrow cells chromosomes were studied not only for normal animals but also of 414 lambs with inborn anomalies, 50 spontaneous abortuses and fetuses of different age, 23 deadborn lambs, 14 infertile dams and 24 animals with different abnormalities. The extensive cytogenetical and teratological data allowed us to characterize the spontaneous and induced mutagenic process dynamics expressed at the chromosome, genome and morphologic levels in the breeds and animal populations bred in different environmental conditions. The unfavorable ecological situation on the vast territories of Kazakhstan includes the influence of many years long nuclear tests on the Semipalatinsk test territory and declined Aral sea level.

**A006**

**Genetic markers at goats**

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The knowledge of genetic markers, in particular of blood groups of goat until now is very much limited. Taking into account, that there is no systematization of blood groups of goat, because of generalities of antigenes sheep and goat while 4 systems of blood groups *A*-system (*Aa*- antigenes), *B* - system (*Bb*-antigenes), with *C*- system (*Ca* -antigenes), *R*-system (*R*- antigenes) are chosen. Alongside with these common antigenes the analysis of various breeds and crosses of goat on 41 alloreagents was conducted. Three breeds of goats (Altai Mountain, Orenburg, Saanen), and also two crosses 1/2 Saanen x Altai Mountain and 3/4 Saanen x Altai Mountain were investigated. The frequency of each antigene is calculated. On the basis of computer program of a method "Manhattan distances" their appropriate clasterisation was conducted. Dendrogramma which consists of 4-th dem is constructed: I - crosses 1/2 Saanen x Altai Mountain and 3/4 Saanen x Altai Mountain; II - actuates dem I and Altai Mountain. All of them form a separate cluster; III - to the given cluster adjoins Saanen goats; IV - actuates a Orenburg breed of goat. At investigated animals the frequency alleles *HBA, TFA* prevail. It is established, that the milk from Saanen goats with $_{S1}$*-Cn* by a genotype has best cheesemaking by properties, than with $_{S1}$*-Cn* and $_{S1}$*-Cn* by genotypes. Milk from an animal with a genotype $as_1$*-Cn* had mean speed of curtailing, the obtained bunch was of a dense, elastic consistence and appeared to be the most desirable for production of cheeses and cottage cheese. Milk from an animal with $_{S1}$*-Cn* genotype was fast contracted. The obtained bunch was fragile and fragile, that appeared to be not suitable for cheesemaking. On the contrary, milk from an animal with $_{S1}$*-Cn* genotype under rennet effect was contracted slowly. The bunch was received flabby on the consistence.

**A007**

**Genomic constitution of the wild gynogenetic triploid crucian carp (*Carassius auratus langsdorfi*)**

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The so-called ginbuna (*Carassius auratus langsdorfi*, Japanese silver crucian carp) is widely distributed in Japan and occurs in the triploid form as well as the diploid form. Remarkably, the triploid ginbuna reproduces gynogenetically in nature, giving rise to clonal offspring. However, little is known about the genetic background or origin of the triploid due to a lack of diagnostic morphological features. Our goal is to elucidate it through DNA analyses. We have so far been focusing on characteristic repetitive DNA sequences and mitochondrial DNA of the ginbuna, and have proposed that the triploid ginbuna might have arisen from hybrids to which the diploid ginbuna, the ancestor of the goldfish, and unknown (sub)species collectively contributed. To foster a better understanding of the triploid genomic constitution, we isolated novel genetic markers using the genomic subtraction method Representational Difference Analysis (RDA). Three series of RDA (restriction enzymes, *Bgl*II, *Hin*dIII or *Bam*HI; subtraction of triploid ginbuna amplicon from diploid ginbuna or goldfish amplicon) yielded three valuable markers; two probes detected restriction fragments existing only in most of the triploid ginbuna and all goldfish examined, whereas a probe detected a fragment in a particular clonal line of the triploid ginbuna and several individuals of the diploid ginbuna. This study provides additional evidence for the genomic contribution by the ancestor of the goldfish and the diploid ginbuna to the triploid hybrid. Further isolation of genetic markers is ongoing.

**A008**

**SWISS-PROT/TrEMBL/InterPro/CluSTr and animal genetics**

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SWISS-PROT is a manually curated protein sequence database that strives to provide a high level of annotation, a minimal level of redundancy, and a high level of integration with other databases. SWISS-PROT and its computer-annotated supplement TrEMBL provide the user with a tool for various aspects of animal genetics. The highest represented domestic animals are cow (2556 entries), chicken (2409 entries) and pig (1969 entries). InterPro, an integrated resource for protein families, domains and functional sites, and CluSTr, a database of sequence clusters, are new tools for proteome analysis with pointers to SWISS-PROT and TrEMBL.

**A009**

**Mouse Genome Database: A resource for comparative genomics**

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The entire sequence of the human and mouse genomes will be available within the next few years. Genomics initiatives on agriculturally important animal species are underway to provide cross-reference to the mouse and human maps. As data from these projects are integrated, functional genomics will use animal models to identify candidate genes for analogous functions, determine gene interactions in different contexts and finally assess their contribution to human phenotypes. Ultimately, comparative genomics will be used to address some of the most fundamental issues in evolutionary and basic biology. The Mouse Genome Database (MGD) is the community database for the laboratory mouse dedicated towards providing an integrated representation of mouse genomic and biological information. MGD currently provides a scientifically curated homology dataset primarily extracted from literature for a select group of mammalian species. The coming flood of genomic sequence data from human and mouse will shift the emphasis of data curation from literature-driven to curator-driven annotation. MGD has developed a set of criteria that support homology assertions to aid in this annotation. Oxford grid displays and whole-genome map views can also be used to generate chromosome-wide and genome-wide graphical representations of homology. A feature of gene annotation in MGD is the use of controlled vocabularies for the description of the molecular function, biological process and cellular component of gene products as part of the Gene Ontology (GO) project. These terms can be used as attributes of gene products across species aiding in the development of comprehensive comparative maps and facilitating queries across multiple databases. This will aid in the transfer by inference of biological information to non-model organisms.

**A010**

**The use of a genetic algorithm in mapping of multiple interacting QTL**

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Here we describe a general method for improving computational efficiency in simultaneous mapping of multiple interacting quantitative trait loci (QTL). The method uses a genetic algorithm to search for QTL in the genome instead of an exhaustive enumerative (step by step) search. It can be used together with any method of QTL mapping based on a genomic search, since it only provides a more efficient way to search the genome for QTL. The computational demand decreases by a factor of about 130 when using genetic algorithm-based mapping, instead of an exhaustive enumerative search for two QTL in a genome size of 2,000 cM using a resolution of 1 cM, for example. The advantage of using a genetic algorithm increases further for larger genomes, higher resolutions and searches for more QTL. We show that a genetic algorithm-based search has efficiency higher than, or equal to, a search method conditioned on previously identified QTL for all epistatic models tested, and that this efficiency is comparable to that of an exhaustive search for multiple QTL. The genetic algorithm is thus a powerful and computationally tractable alternative to the exhaustive enumerative search for simultaneous mapping of multiple interacting QTL. The use of genetic algorithms for simultaneous mapping of more than two QTL, and for determining empirical significance thresholds using permutation tests, are also discussed.

**A011**

**Genetic analysis of X-linked anhidrotic ectodermal dysplasia (EDA) in Cattle**

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In a family of German Holstein Cattle three male, maternal half-sibs with congenital hypotrichosis and abnormal sweat glands were observed. Furthermore, the affected calves almost completely lacked teeth. They had no incisors and only very few and deformed molars. The pedigree of the investigated Holstein family suggested X-linked inheritance of the genetic defect as only male animals were affected. In order to further characterize the genetic defect responsible for the observed phenotype, 20 microsatellite loci on the X-chromosome were genotyped in 10 members of the family. Haplotype analysis showed that the mutation is located on the chromosomal segment flanked by the proximal marker BMS513 and the distal marker BMS2798. Syntenic regions on the human and mouse X-chromosomes were derived from the respective comparative maps and analyzed for the presence of candidate genes, which might be causative for skin and tooth abnormalities in cattle. Interestingly, a similar phenotype was already described for human patients with anhidrotic ectodermal dysplasia (EDA) as well as the mouse mutant Tabby. In human and mouse it was shown that defects in the X-chromosomal ectodysplasin A gene (*EDA*) are causative for this disease. Therefore, the bovine homolog of the human *EDA* gene seems to be a strong candidate for the affected gene in this hereditary disease.

**A012**

**Detection and parameter estimation for dominance effects of quantitative trait loci**

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Detection of dominance effect is important for the detection of a quantitative trait locus (QTL), because methods to detect additive effects would fail to detect the QTL when dominance is the primary QTL effect. Detection of dominance effect is also important to understand the QTL functions. Dominance effect of a QTL can be detected using a marker contrast between the heterozygous and the homozygous marker genotypes. Once a QTL is determined to be present, its location can be determined by estimating the marker-QTL recombination frequency. The estimation of the QTL location needs to consider three cases: (1) the chromosome contains only one QTL; (2) additional linked QTLs exist to one side of the target QTL, and (3) additional QTLs exist on both sides of the target QTL. Formulations to estimate the marker-QTL recombination frequency for these three cases are as follows

$$\theta_{Aq} = \frac{1}{2}\{1-[(1-2\theta_{AB})\ w_1]^{1/4}\}$$

for case (1)

$$\theta_{Aq} = \frac{1}{2}\{1-\{1-\frac{(1-2\theta_{AB})^2}{(1-2\theta_{AB})^2+[1-(1-2\theta_{AB})^4]w_2}\}^{1/4}\}$$

for case (2)

$$\theta_{Aq} = \frac{1}{2}\{1-[\frac{w_3(1-2\theta_{AB})^2+(1-2\theta_{AB})^4}{1+w_3(1-2\theta_{AB})^4}]^{1/4}\}$$

for case (3)

where $\theta_{Aq}$ = recombination frequency between flanking marker *A* and the target QTL, $\theta_{AB}$ = recombination frequency between flanking markers *A* and *B*, and $w_1$, $w_2$ and $w_3$ are ratios of partial regression coefficients for cases (1-3) respectively. Dominance effect of each QTL is then estimated based on the estimated marker-QTL recombination frequency and the marker contrast that was used to detect the dominance effect.

**A013**

## Development and characterization of Japanese quail microsatellite markers and their utility in chicken

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Japanese quail (*Coturnix japonica*) is a member of the family Phasianidae. It is valued for its egg and meat and also used widely as a laboratory research animal. In order to promote the construction of a quail genetic map and the construction of a comparative genetic map in Phasianidae, this study was conducted with the aim of isolating original microsatellite markers in Japanese quail and determining their utility as cross-reactive markers between chicken and quail. A Japanese quail genomic library enriched for (CA/GT)n simple sequence repeats was screened, and then positive clones were sequenced. PCR primer-pairs complementary to unique DNA sequences flanking microsatellite repeats were designed so as to amplify DNA fragments. Optimal conditions for PCR were determined for quail and the markers were also tested on chicken using White Leghorn and Fayoumi DNA as templates. Out of a total of 368 positive clones that were sequenced and characterized, 50 original microsatellite sequences were isolated. Amplification products were obtained in 14 (28%) of the markers tested on chicken DNA at the annealing temperature optimized for quail. Mapping of markers by quail and chicken reference families is underway for the development of a genetic map for Japanese quail and the eventual construction of a comparative genetic map in Phasianidae.

**A014**

**Microsatellite-based population structures within and between five Finnish dog breeds**

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Variabilities within and between Finnish populations of Golden Retrievers, German Shepherds, Wirehaired Daschunds, Pembroke Welsh Corgis, and Bedlington Terriers were quantified with ten microsatellite loci and a sample of 50 individuals from each breed. Highest genetic diversity was exhibited in the Wirehaired Daschunds (mean allele number = 8.0; mean $H_E$ = 0.72) and lowest in the Bedlington Terriers (mean allele number = 5.2; mean $H_E$ = 0.56). Although statistically significant deviations from H–W equilibrium were observed, they occurred at an unexpectedly low frequency. Interestingly, the extremely small Bedlington Terrier population displayed genotypes in H–W proportions in all investigated loci. Genetic differentiation between the breeds was very large ($F_{ST}$ = 0.182-0.266; $D_A$ = 0.365-0.466). These estimates markedly exceed those estimated between some livestock populations. Exemplifying the level of differentiation, the highest $D_A$ distances were only slightly lower than the lowest values inferred between humans and chimpanzees. The present data imply severe bottlenecks, genetic isolation and intense artificial selection in the history of these breeds of dogs.

**A015**

## Mitochondrial diversity and the origins of North East Asian cattle

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The origins of North East Asian domesticated cattle are unclear. The earliest domestic cattle in the region were *Bos taurus*, which may have been domesticated from local aurochsen or perhaps had an origin in migrants from the early domestic center of the Near East. In this study, complete mitochondrial DNA displacement loop sequences from 30 Korean and 44 Mongolian native cattle were sequenced. Korean native cattle revealed altogether 32 sites of base substitution and 23 haplotypes, and all were taurine (*Bos taurus*) mitochondrial haplotypes. Mongolian native cattle showed two subspecies mitochondrial haplotypes, i.e. taurine and zebu types. Nine Mongolian animals showed five sites of base substitution and four zebu haplotypes, and the rest of the animals exhibited 40 sites of base substitution and 32 taurine haplotypes. The data of taurine haplotypes were analyzed with published sequences of taurine mtDNA from African, European and Japanese animals. In phylogenetic analysis with 76 haplotypes, taurine sequences form at least five clusters. The average sequence divergences among them were from 0.5 % to 0.9%. These clusters may represent different strains of ancestral aurochs, adopted at geographically and temporally separate stages of domestication in the old world.

**A016**

**Association between blood group markers and first milk yield in the GIR breed**

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Blood typing results of 138 cows from a Gir breed herd (a zebu breed), closed by approximately 30 years and submitted to controlled matings, were studied to verify association among the B, F, J, L and Z blood factors or phenogroups and the first milk yield. Significant results were found in first lactation between the animals presenting the Z blood factor (3634.43 kg, $P<0.001$), compared with the ones lacking factor Z (3074.62 kg, $P<0.05$). For the B blood group system, through means contrast, significant results were observed comparing the animals homozygous for the I1O1Y2A'B'E'3(J'K')P'Q' (4202.86 kg, $P<0.05$) and heterozygous B(P)QTE'3G'P'/ I1O1Y2A'B'E'3(J'K')P'Q' (3493.33 kg, $P<0.05$) and BQTA'B'I'(P')/ I1O1Y2A'B'E'3(J'K')P'Q' (3630.36 kg, $P<0.05$).

**A017**

**Development of a Bovine Whole Genome Radiation Hybrid Map for Comparative Mapping Across Species and the Identification of Positional Candidate Genes for Genetically Mapped Traits.**

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Positional candidate gene cloning is currently the most successful method of identifying trait genes. This project is establishing a whole genome radiation hybrid (WGRH) map in cattle. A framework map is being constructed from large number of microsatellite markers, and this will tie the WGRH map to existing linkage maps. High-resolution comparative links to maps in other species will be achieved by placing a large number of genes and ESTs on the map and will provide positional candidates genes for QTL. Two WGRH panels have been created from a primary bovine fibroblast line fused to hamster fibroblast lines deficient in either HPRT of TK (Wg3H and A23 respectively). For one panel, bovine fibroblasts were irradiated at 3000 rad dose and for the other a 10,000 rad dose was used. The 3,000 rad dose used in Cambridge has already produced RH panels in other species with resolutions ranging from 1Mb to 145Kbp.

The 3,000 rad Bovine panel used the Wg3H recipient and was initially comprised of 224 hybrid cells. These were characterised by PCR and FISH and 94 cells were selected to give the final panel (designated Tm112). On primary characterisation with 33 markers average retention of the Tm112 panel was 28% (15-30%). The Tm112 panel has been subjected to large scale culture and DNA extraction and will be available to the research community from Research Genetics Inc. Collaborators will have access to RH-mapping data via a bovine RH-database hosted at Roslin and INRA.

**A018**

**EST mapping in the pig – a new dimension to comparative mapping**

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Radiation hybrid (RH) mapping has come of age in the pig. This is evident from the mapping of over 1300 markers using this approach. Among the various markers hitherto mapped in the INRA-Minnesota RH panel (IMpRH), expressed sequence tagged sites (ESTs) are of specific significance. These markers align the porcine gene map to the human gene map, thus adding to the comparative status between the two species. A total of over 120 ESTs generated from a porcine large intestine cDNA library were mapped to different pig chromosomes using the somatic cell hybrid (SCH) and IMpRH panel. Prior to the mapping efforts, *in silico* analysis of the 5'sequences from the ESTs indicated that they represent porcine genes that are orthologous to genes already mapped in humans. The findings together with the available cytogenetic mapping data helped locating the markers to specific chromosomal regions in the pig. The mapping information was used to verify and strengthen correspondence between the pig and human genomes. Further, the data were also related to the available Zoo-FISH results between the two species. A genome-wide overview of the mapped ESTs will be presented along with comparative status with the humans.

**A019**

**A complete exon is missing in the L-gulono-gamma-lactone oxidase gene (*GULO*) causing vitamin C deficiency in the pig : a DNA-based test for the diagnosis of the deficient allele**

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Some years ago, a mutant strain of Danish pigs lacking the ability to produce L-ascorbic acid (L-AscA) was discovered, and clinical cases with scurvy were described in swine for the first time. This trait was shown to be controlled by a single autosomal recessive allele designated *od* (osteogenic disorder). In a previous study we demonstrated a close linkage of *od* to microsatellites on pig chromosome 14. In addition, the L-gulono-gamma-lactone oxidase gene (*GULO*), a candidate gene for the defect, was mapped to the same chromosome region by *in situ* hybridization (FISH). *GULO* is the critical enzyme which catalyses the terminal step of the biosynthesis of L-AscA in the liver of a variety of mammals. The objective of this study is to find a molecular defect in *GULO* that is associated with vitamin C deficiency. A cDNA clone for this enzyme was isolated by screening a pig liver cDNA library using a 545-base pairs (bp) pig *GULO*-specific probe obtained by cross-species PCR from rat and guinea pig sequence information. The cDNA clone contained 1838 bp with an open reading frame of 1320 bp similar to the rat sequence. We found that at the cDNA level of the *GULO* gene vitamin C-deficient animals were devoid of exon VIII. At the genomic level, sequencing analysis indicated that the last 382 bp of intron VII, the complete exon VIII and the first 182 bp of intron VIII were missing, as a result of an insertion of a porcine nucleotide sequence of about 2.5 kbp preceded by a 61-bp SINE sequence. These findings allowed us to perform a PCR-based test to discriminate normal *OD*/*OD*, *OD*/*od* and deficient *od*/*od* pigs.

**A020**

**Comparative mapping of a cattle trypanotolerance QTL region on *Bta* 7**

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Quantitative trait loci (QTL) influencing trypanotolerance have recently been mapped in a broad region on bovine chromosome 7 (*Bta* 7). Given the limited resolution of available livestock maps, data from the marker-rich human or mouse genomes was exploited to improve the mapping resolution of the identified QTLs. Two whole genome (WG) radiation hybrid (RH) panels were used for high-resolution comparative mapping. A total of 37 DNA markers, comprising 17 genes (6 previously unmapped), 17 microsatellites and 3 STS (generated by microdissection) were mapped by PCR against the 5000rad RH panel. In addition, a higher resolution radiation map is presented for 22 of these markers using the 12000rad RH panel. These maps are compared to published linkage maps of *Bta* 7. Comparative mapping confirms the regions of conserved synteny between cattle, human and mouse in the QTL region. However, the linear order of genes appears to differ; gene orders between cattle and human being more conserved than between cattle and mouse. Also, a previously unknown small region of conserved synteny between *Bta* 7 and a major trypanotolerance QTL region on *Mmu* 17 is revealed. The linkage maps and RH maps are generally in good agreement. The refined comparative map should allow more accurate selection of candidate genes and the narrowing down of the *Bta* 7 trypanotolerance QTL region to a region small enough to facilitate marker assisted selection or introgression of trypanotolerance.

**A021**

**Improvement of the comparative map of Chicken linkage group E48C28W13W27 to Human chromosome segment 5q23-q35**

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Six genes mapped on chicken linkage group E48C28W13W27 (74 cM) are homologous to genes on the human chromosome (HSA) 5q23-q35 region. To improve the chicken-human comparative map of linkage group E48C28W13W27, the Wageningen BAC library has been screened for BAC clones of these 6 genes (*MSX2, SPARC, POU4F3, SPOCK, CDX1* and *CAML*). In addition, 14 microsatellite markers located on E48C28W13W27 were used for screening the BAC library. So far, 54 different BAC clones have been isolated and are used to build a contig. Two genes (*SPARC* and *SPOCK*) are located on the same BAC clone. Eleven BAC clones have been sub-cloned and will be used for shutgun sequencing in order to perform sequence comparison to other species. In addition, a BLAST search was performed with genes from HSA 5q31 to find homologous chicken sequences. Primers of these sequences have been designed and were used to screen the chicken BAC library. BAC clones have been isolated for three chicken genes (*FGF1, DTR* and *UBE2*). Our results improve the chicken-human comparative map of linkage group E48C28W13W27 and HSA 5.

**A022**

## Milk protein genetic variation in Southwestern European cattle breeds

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The first milk protein genetic variants in cattle were detected in the 1960's, and since then many new variants have been detected in local cattle breeds. Presently, many of the world local cattle breeds are threatened with extinction and thus the study of their genetic diversity is an urgent conservation priority. As a result of many years of artificial selection, Iberian cattle breeds became well adapted to the constraints of the Mediterranean environment. However, changing commercial demands have driven the majority of these breeds to the verge of extinction. We have analysed the genetic variation at milk protein loci from several breeds of western Iberian cattle and compared our data with those observed in other breeds. Genetic variation at the $\alpha S_1$-Casein (CSN1S1), β-casein (CSN2), αS2-casein (CSN1S2), k-casein (CSN3, α-lactoalbumin (ALA) and β-lactoglobulin (BLG) *loci* was identified by isoelectric focusing techniques. The observed gene frequencies were statiscally analysed with Principal Component Analysis and Reynold's genetic distances were used to construct Neighbour Joining and UPGMA unrooted trees. We observed i) an inversion of allele frequencies in certain breeds, ii) a small genetic differentiation between beef cattle and dairy cattle breeds, and iii) a similarity between African breeds from *B. indicus* and *B. taurus* and Southern Iberian cattle. It is suggested that genetic drift was the main factor shaping the genetic structure of these breeds, whereas the crossing between neighbouring breeds contributes to their genetic homogenisation. The genetic similarity between Southern Iberian and African breeds, needs to be clarified by further analysis.

**A023**

**PCR-RFLPs of four genes (*OTC*, *TBG*, *ANT2* and *FMR1*) and their linkage mapping on porcine chromosome X**

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Positional candidate gene cloning of genes accounting for significant genetic variation of a QTL ultimately requires alignment of the human and swine maps for which linkage mapping of comparative markers is essential. PCR fragments were amplified and sequenced, and RFLPs found within the porcine *OTC* (ornithine carbamoyltransferase), *TBG* (thyroxine-binding globulin), *ANT2* (adenine nucleotide translocator 2, fibroblast), and *FMR1* (fragile X mental retardation syndrome protein 1) genes. Multi-point linkage analyses were performed in the USDA-MARC backcross pedigree (Rohrer *et al.* 1994 Genetics 136, 231) using CRI-MAP, version 2.4. Gene location on the USDA-MARC linkage map (Rohrer *et al.* 1996 Genome Res. 6, 371) of SSCX follows: *OTC* at position 44 cM, final order *SW2126* - 8.4 cM - *OTC* - 2.3 cM - *SW2470*; *TBG* at position 76 cM, final order *SW1346* - 1 .2 cM - *TBG* - 2.3 cM - SW2476; *ANT2* at position 90 cM, final order *SW1943* - 3.0 cM - *ANT2* - 8.0 cM -S0501; and *FMR1* at position 124 cM, final order *SW2059* - 5.0 cM - *FMR1* - 4.0 cM -*SW2588*. Even after the addition of these data there is no evidence of rearrangements in gene order between porcine and human X chromosomes.

**A024**

**Genetic differentiation within a new Polish horse breed**

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During the 1970s and 1980s, horse breeders in Poland used Trakehner stallions from East Germany crossed to Polish mares to produce sport horses. The success of this program lead to other breeding efforts involving crosses of stallions from western countries with Wielkopolska mares. This lead to the establishment of the Polish Warm Blood Horse (PWBH). In this study we examine genetic variation based upon seven blood group and ten biochemical genetic loci in the PWBH. We also examine genetic differentiation within the breeds based upon differences in the breed of stallion used to establish lines within the PWBH. Within the breed, genetic variation as estimated by expected heterozygosity was somewhat below the mean for domestic horses within each different stallion breed line. This was unexpected as crosses between different breeds would be predicted to produce higher variation. The lowest variation was within the line derived from Sella Francais stallions with *He* = 0.29. For the other six stallion breed lines *He* ranged from 0.32 to 0.36. The greatest degree of differentiation (as shown by genetic distance and *Fst)* was among individuals derived from Sella Francais stallions as compared to all other stallion breed lines. Overall, there was little differentiation seen among stallion breed lines, probably due to the use of the same mare types in the creation of this breed.

**A025**

**A mixed model method for QTL detection and marker assisted selection**

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A mixed model method is developed for QTL detection and marker assisted selection. In this mixed model method, marker effects are treated as uncorrelated random effects, providing a convenient framework for marker assisted selection using BLUP (best linear unbiased prediction) procedures. This mixed model approach is computationally efficient because it generates only a small number of equations to include marker effects in the model. The total genetic merit of QTL and polygenes is obtained as the sum of the predicted marker effects and the polygene effects. Each marker variance component required by the BLUP procedure is estimated using a maximum likelihood approach. Numerical results show that estimates of the variance components were close to the true parameters. Once marker variance components are obtained, marker-QTL recombination frequencies, QTL variances and effects can be obtained based on marker variance components.

**A026**

**Genetic variation in seven native horse breeds from Greece**

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Genetic variation at 29 loci (7 blood group, 10 biochemical and 12 microsatellite) was examined in seven indigenous Greek horse breeds. The breeds were the Andravidas (n=12), Crete Horse (n=40), Pindos (n=15), Pinias (n=30), Skyros Pony (n=126), Thessalias (n=4), and Zakynthos (n=5). Sample size for the latter two were too small for meaningful analysis. Variability values ranged from extremely low for a domestic horse breed for the Pinias to relatively high in the Skyros Pony. The high variation in the Skyros Pony was unexpected, as this is an island population with a small population size. There was no close correlation of variability measures of different types of loci within breeds. In general, the Greek horse breeds showed closest resemblance to Oriental type horse breeds although some of the breeds showed clear influence of Anglo-Norman type breeds.

**A027**

**The genetic variability of six Merino populations determined by microsatellites**

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Fine wool Merino sheep arose in the South of Spain in prehistoric times. Protected by the kingdom for centuries the breed did not leave the Iberian Peninsula until 18th Century, spreading through Europe and beyond, and giving origin to different breeds in the host countries. Microsatellite markers have been widely used in assessing genetic relationships among populations. We have analysed the genetic variability among 6 Merino breeds using 18 microsatellites in order to determine their usefulness in tracking differences among populations derived from the same breed in a relatively short timescale. The populations studied (N=253) include Spanish, French Mutton, German Mutton, Portuguese Black, Portuguese White and New Zealand Merino. Genetic relationships were determined using the simple allele sharing statistic and treating each animal as a taxonomic entity. The neighbor-joining method was used to construct an unrooted tree. Genetic variation was highest amongst the Spanish and Portuguese populations although genetic diversity within the other populations was also high. The French Mutton, German Mutton and New Zealand Merino populations could be differentiated from each other and from the Iberian Merinos, indicating that microsatellites are able to track relatively recent changes in the population structure of sheep breeds. Dendrograms constructed on the basis of microsatellite allelic frequencies suggest that populations that have shared selection criteria (meat *versus* wool) tend to cluster together.

**A028**

**A high-resolution comparative gene map of pig chromosome 14**

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Analysis of the human-porcine comparative gene map shows that porcine chromosome 14 (SSC14) has undergone many evolutionary rearrangements, corresponding to at least 6 different human chromosomal segments. Effective utilization of the comparative map for positional candidate cloning of QTLs in swine will require the construction of high-resolution comparative maps, particularly for chromosomes with such complex evolutionary history. A comparative mapping by annotation and sequence similarity (COMPASS) approach was used to select type I loci for mapping. Over 10,000 porcine database sequences were analyzed by COMPASS to identify their corresponding human orthologs with high-resolution RH mapping data. Eighty-four sequence clusters that were predicted to map to SSC14 in addition to resolving evolutionary breakpoints between chromosomal segments were selected. Initially, the INRA somatic cell hybrid panel (SCHP) was used to map genes to their respective chromosomes, followed by WG-RH mapping of SSC14 loci using the INRA-Minnesota porcine radiation hybrid panel (IMpRH). Of 52 loci screened, 71% were successfully amplified and yielded porcine specific products. Currently, 17 new assignments have been made to SSC14 and additional assignments to SSC5 (5), SSC4 (4), SSC10, (4), SSC9 (3), SSC6 (2), SSC3 (2), and SSC13 (1). Thirty-two additional loci are being screened to increase the resolution of the map and further refine evolutionary breakpoints on SSC14.

**A029**

## Parentage control in the Latxa sheep breed using microsatellites

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PCR multiplexes developed for automated fluorescence genotyping were evaluated for parentage testing in the Latxa sheep breed. Multiplexes contain 11 primer pairs recommended by the ISAG Sheep and Goat Comparison Test 1998 (plex2, 5 loci) or by the LABOGENA Lab, France (plex1, 6 loci). The results showed a wide range of variability among the 11 microsatellites. In the Latxa sheep breed the number of alleles per microsatellite locus varied from 6 to 16 (mean = 9.7, n = 145 unrelated ewes) and the polymorphic information content from 0.611 to 0.848 (mean PIC = 0.725). The average exclusion probabilities are >0.9999 and >0.9970 whit and without one parent already known, respectively. In addition, in this paper we compare the results obtained from the analysis of more than 200 families (sire-dam-offspring) using the two PCR multiplexes and the biochemical polymorphism system applied since 1987 at our laboratory. Finally, we see the need for standarization of results from different laboratories, it is necessary to stablish an international panel of microsatellites for sheep paternity control and a common nomenclature.

**A030**

**Linkage mapping ESTs in pigs using single nucleotide polymorphisms (SNPs): SNP discovery**

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Comparative mapping between human and swine genomes requires the localization of conserved genes in both species. Our objective is to improve the resolution of the human/swine comparative map by mapping markers within expressed pig genes. SNPs associated with porcine expressed sequence tags (ESTs) orthologous to genes with known human map positions will be mapped by linkage analysis. Single-pass porcine EST sequence derived from two normalized libraries is subjected to automated PCR primer design aimed at developing primer-pairs that span intron/exon junctions. The results of PCR amplification from porcine, bovine, and ovine DNAs are entered into a relational database (MARCDB). Successful primer-pairs are used to generate amplicons from nine parents of the MARC porcine reference population, and seven animals likely to harbor breed-specific alleles present in mapping animals. Amplicons are purified and subject to fluorescent di-deoxy sequencing. Chromatograms are imported into MARCDB, assembled into contigs, and assessed for SNPs using Polyphred. Potential polymorphisms are interactively evaluated and tagged using Consed. Validated SNPs are exported to MARCDB for automated genotyping assay design. Preliminary assessment of our strategy for SNP-discovery reveals it to be quite efficient. Up to 85% of the amplicons sequenced harbor SNPs. To date polymorphisms have been detected in over 100 porcine genes.

**A031**

## A panel of microsatellites for establishing parentage in domestic sheep

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The Australian sheep industry uses extensive production systems. Hence, even in stud flocks, pedigrees are often unknown or unreliable, resulting in suboptimal rates of genetic gain and unknown genetic relationships among animals selected to parent the next generation. There is a strong demand for reliable and cost effective DNA-based pedigreeing and, in response, we developed several panels of microsatellites that can be multiplexed as part of a DNA parenting system. Our primary pedigreeing set comprises 16 microsatellites, amplified in three PCR reactions, co-loaded and optimised for scoring using ABI's proprietary genotyping software. This panel has been tested extensively in Merino lines. While based on published microsatellites, largely those developed within CSIRO, all primer pairs have been redesigned to ensure high quality peaks and non-overlapping size ranges. Almost all markers are highly polymorphic; 50% have heterozygosities in excess of 80%. In a closed flock of fine-wool Merinos, the average number of alleles per marker was 9.25, and the mean PIC value of the set was 0.73. This set is adequate to assign parentage unambiguously even when the putative parents are highly related. We have, in addition, a supplementary set of 14 markers (in two PCR reactions); together these provide an even coverage of the genome and can be used to estimate genetic relationships where parents are unknown. We believe that these panels should be suitable for use with breeds other than the Merino.

**A032**

**Discovery of a genomic clone with homology to centromeric and telomeric regions of chromosomes among species of *Equus*.**

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A lambda phage clone selected for homology to s*omatostatin* was found to hybridize to the telomeric and centromeric regions of equid chromosomes. The clone hybridized to chromosomes of the following species as detected by fluorescence *in situ* hybridization (FISH): Prezwalski's horse (*Equus przewalskii),* domestic horse *(E. caballus),* donkey *(E. asinus),* Kulan *(E. hemionus kulan),* Grevy's zebra *( E. grevyi),* and Hartmann's zebra *(E. zebra hartmannae)*. For each of these species hybridization was observed at centromeres and telomeres of some chromosomes. To some extent, the patterns of hybridization reflected phylogenetic expectations. In the horses, hybridization was predominantly at acrocentric centromeres. In the zebras, hybridization was at acrocentric and metacentric centromeres and telomeres. In the kulan, hybridization was observed at only one acrocentric centromere. No hybridization was observed to chromosomes of the Burchell's zebra (E. burchelli) or the black rhinoceros (*Diceros bicornis)*. A BLAST search of a portion of the sequence reveals homology to a flanking sequence of a horse microsatellite (*UM007*). This clone appears to contain an equid specific satellite element that appeared after the divergence of the perissodactyls. Currently, sequencing and further characterization of this clone is underway.

**A033**

**A comparative ruminant genetic linkage map based on the deer interspecies hybrid pedigree**

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High levels of synteny observed in sub-chromosomal regions between human and other mammalian genomes allow for information which is already available from human and mouse mapping and sequencing to assist gene discovery in other species. However, the links between the current human and ruminant linkage maps (sheep, cattle, goat) have consisted predominantly of anonymous microsatellite markers which are not conserved in species beyond ruminants. The deer genetic linkage map contains a higher proportion of mapped genes due to the frequency of polymorphism found between Pere David's and Red Deer in the interspecies hybrids used to generate the map (located at Roslin http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=deer). The original deer map shares 147 loci with the human map, 123 with cattle, 95 with sheep and 114 with mouse. We have used a subset of the deer interspecies backcross linkage pedigree to map new genes using a rapid screen with two restriction endonucleases to identify restriction fragment length variants. Over 100 new genes, (cDNA and ESTs) from a variety of species and tissue types have been located on deer linkage groups. The sequence information derived from these cDNAs and ESTs provides further links to the human and mouse maps. An additional 120 mainly ruminant-derived microsatellite markers (cattle, sheep, caribou, gazelle) have been screened in the mapping pedigree to strengthen links with other ruminant maps, and over 270 AFLP markers (amplified fragment length polymorphism) have been mapped to increase the map density.

**A034**

**The nucleotide sequence of *BoLA-DOA* cDNA**

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Studying the *MHC* genes of the class IIb region in cattle, the second exon of *BoLA-DOA*, the bovine homologue of the *DOA* or*DNA* locus of other mammalian species, was identified in cDNA derived from peripheral blood leukocytes. The entire coding sequence and parts of the 5´- and 3´-untranslated regions were amplified using RT-PCR and 5´-RACE with *DOA*-specific primers. A sequence of 1109 nucleotides was determined, which contained an open reading frame encoding a polypeptide of 250 amino acids. The predicted molecule was found to express all of the features expected for functional class II a-chains, as it was composed of a putative signal peptide as well as of putative a1, a2, connecting, transmembrane, and cytoplasmic domains. It also included typical a-chain sequence elements such as the conserved cysteine residues and two potential glycosylation sites. The predicted mature protein showed 93,7 % and 81.1 % identity with the orthologous genes of sheep and pig, respectively. Further studies are required to investigate the expression profile and the function of the *DOA* gene in cattle.

**A035**

## Organization of an ovine keratin associated protein gene cluster on OAR11

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During keratinocyte differentiation in the wool follicle, the acidic type I and basic type II keratin intermediate filaments (KIF) become embedded in a matrix of keratin associated proteins (KAP). Many of the type I and type II KIF genes are tightly clustered in two separate domains in humans and mice, but the organization of the KAP genes has not been described. There is some evidence of clustering of KAP genes in sheep and it is possible that there is a relationship between the organization of these genes and their expression in the wool follicle. A 100kb ovine bacterial artificial chromosome (BAC) containing KAP1.1 was physically mapped to 11q3.1. The presence and arrangement of additional KAP genes within this BAC was investigated by long-range restriction mapping and comparative shot-gun sequence analysis. Sequences were repeat masked and aligned to a human chromosome 17 BAC. This study demonstrated that genes highly similar to KAP1.1, KAP1.3, KAP1.4, KAP2.3, KAP3.4 and one as yet unidentified type I KIF gene were all located within this 100kb BAC. A striking feature of both the ovine and human sequences for this region was the localized, high content of repetitive DNA (45% and 41%, respectively). It is speculated, herein, that since KAP genes lack introns, they require the accumulated repetitive DNA in the extragenic regions for proper regulation or function. Transgenic studies with KAP- or KIF-containing BAC clones would help unravel the complex control of keratin gene expression in the wool follicle.

**A036**

**Radiation hybrid mapping of bovine chromosome 2, assignment of 7 previously unmapped genes**

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At least 38 genes have now been positioned on *Bta*2, either by cytogenetic mapping or by linkage analysis. Most of these genes are mapped at the telomeric end or near the centromeric region, leaving a 'gap' in the middle of the chromosome (*Bta*2q21-31). Recently, we have mapped a trypanotolerance QTL region on *Bta*2. In order to identify candidate trypanotolerant genes and possible homology between cattle and mouse trypanotolerant QTLs we are building up a comparative map between *Bta*2 and the human-mouse genomes. A radiation hybrid map of *Bta*2 has been constructed using a 5000-rad bovine whole-genome radiation hybrid panel. A total of 38 markers were typed: 17 Type I markers (genes) and 21 Type II markers (microsatellites). It includes 7 previously unmapped genes: CACNB4, CTLA4, DES, DPP4, HIS1, IDH, and NCL. The average retention frequency for all markers was 11.7 %. Identical retention patterns were found between two, three and five markers. The remaining 31 markers were linked at LOD score > 3, into four groups including 13 (group I), 3 (group II), 3 (group III) and 12 (group IV) markers. Large discrepancies were found between the large and the small groups with an average retention frequency for I, II, III and IV for 13.8%, 3%, 5.3% and 13.3%, respectively. Comparison with the *Bta*2 cytogenetic map locates radiation hybrid groups I and IV on *Bta*2q12-24 and *Bta*2q35-44, respectively. Comparative mapping confirm the chromosomal homology between *Bta*2 and *Hsa*2, *Mmu*1 and *Mmu*2 as well as the complex gene order rearrangement between cattle, human and mouse for these regions.

**A037**

**A practical program for reconstructing DNA marker haplotypes of half-sib based pedigree structure.**

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Marker haplotype reconstructed from each of individual is important in order to detect population association or linkage between traits and genetic markers. It was better to reconstruct haplotypes of all individuals simultaneously, but it may take much time when the pedigree was big. In case of half-sib analysis based complex pedigree structure that was popular in cattle, we presently could able to divide it into several small pedigrees and reconstruct independently without much loss of information. We made a simple computer program, by which reconstruct haplotypes of half-sib based pedigree structured from haplotypes individual of populations as displayed in the figures.

**A038**

## Phylogenetic analysis and temporal changes in genetic variation of 18 horse breeds

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Temporal changes in genetic variation within and between 18 horse breeds were evaluated using 7 erythrocyte antigene systems (*A, C, D, K, P, Q, U*), 8 polymorphic protein loci (*A1B, ALB, ES, GC, GPI, HBA, PGD, TF*) and thirteen microsatellite loci (*HTG6, HTG4, HMS3, HMS1, HTG10, AHT5, HMS6, ASB2, HMS2, VHL20, HTG7, AHT4, HMS7*). Blood typing data of about 50.000 and microsatellite data of nearly 19.000 samples, sent to our laboratories for routine paternity testing since the year 1988, were analysed. Intrabreed genetic variation was quantified by conventional parameters (e.g. heterozygosity, average number of alleles per locus) and migration by the effective migration rate. The neighbourjoining and UPGMA dendrogram of relationships between breeds was constructed using Nei's $D_A$ genetic distance. In 12 of 19 breeds enough data were available to split samples in two or three generations. All animals within a breed born in a time span of ten years were defined as one generation. Each generation (subpopulation) was represented by at least 50 animals. Heterozygosity remained stable over generations in most breeds. Arabian horses and Throughbreds showed the smallest average heterozygosity values whereas Pintos and Draft horses showed the highest average heterozygosity. Both neighbourjoining and UPGMA dendrogram and non linear map construction showed three well-separated groups of breeds: (1) Icelander, Fjords and Shetland Ponies, (2) Haflinger and South-German Draft horses and (3) all other investigated breeds. Within these three groups further reasonable clustering was observed.

**A039**

## Nucleotide sequence variability at the Horse major histocompatibility complex DQB region

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Existence of at least two major histocompatibility complex (ELA) DQB genes has been suggested (Horín et al., in preparation). PCR-SSCP and nucleotide sequencing of the second exon of the ELA-DQB gene(s) showed that the PCR-SSCP technique specifically detected single nucleotide differences. Based on the nucleotide sequence homology, two different clusters of allelic sequences were obtained, one of them being preferentially, but not exclusively, amplified by the primer pair used. The frequencies of allelic variants differed significantly among breeds. The data provide further support for the existence of two DQB genes in the horse.

**A040**

**Preparation and characterization of bovine/hamster somatic cell hybrid panel**

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Somatic cell hybrid panels have widely been used for physical mapping of genes and DNA markers. In order to locate newly developed bovine microsatellites and ESTs, we prepared a bovine/hamster somatic cell hybrid (SCH) panel and characterized it with more than 300 microsatellites mapped onto the USDA bovine linkage map.

Bovine primary cultured fibroblasts from a Wagyu bull were fused with hamster line $tk^{-}$-ts13neo by 50% polyethylene glycol. Our SCH panel was composed of 30 hybrid clones isolated in the presence of HAT/G418 and grown for DNA preparation (18-137 mg DNA/clone). Retention frequencies of bovine chromosomes were estimated by Genescan analysis of microsatellite loci with an ABI 377 DNA sequencer. Ten clones out of the 30 were analyzed by FISH using total bovine DNA as probe. In the SCH panel the retention frequency in average was 40%. Most of bovine chromosomes in the panel had small deleted regions where one or more successive microsatellite loci were not PCR-amplified. We conclude that mapping of a bovine locus with our SCH panel can be more precisely carried out than expected. Further characterization of the panel is reported.

**A041**

**Comparative mapping of livestock genomes: constructions of cytogenetic banding block counterpart (CBBC) maps**

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The objectives of this study are to exploit the large databases of genes and ESTs that have been mapped and sequenced in human, rodents and other mammalian species to construct Cytogenetic Banding Block Counterpart (CBBC) maps of farm animals in order to facility gene mapping, QTL mapping and comparative mapping in livestock. Human chromosome 4 has been used in a pilot study. Of 294 gene loci that have been recorded in GDBdatabase on the chromosome, 214 designated genes with complete coding sequences were selected to search for homologous gene and EST sequences in livestock species using BLAST. 121 gene loci were found to have the homologous sequences (designated genes or ESTs) in livestock species, including 93 genes of homologous sequences for cattle, 63 for pig, 20 for sheep, 13 for dog, 6 for horse, 6 for cat and 2 for goat, respectively. Primers can be designed to amplify at least 120 fragments of genes on the basis of conserved regions of sequences between human and livestock species (87.5%) or between human and rodents (12.5%). These 120 genes cover 47 cytogenetic banding blocks of human chromosome 4, ranging from one to six genes per block. Once these genes are mapped on the genome of a specific species using RH mapping, their locations of the counterpart cytogenetic banding blocks in the species will be identified. Based on this pilot study and availability of database in GenBank, one can expect that the construction of CBBC maps with 3000 - 3500 gene loci in livestock species is currently possible. The applications of CBBC maps and challenges in their constructions are discussed.

**A042**

**A comprehensive comparative map of pig chromosome 13**

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Pig chromosome 13 (SSC13) habours the locus responsible for *E. coli* F4ab/ac resistance and QTLs for growth and fat deposition. These trait loci make mapping of SSC13 genes particularly interesting. In order to strengthen the possibility of using the positional candidate gene cloning strategy in the hunt for pig genes the resolution of the human-pig comparative map for SSC13 was improved. Eighteen different human chromosome 3 genes with a known human chromosomal localisation were selected and mapped in the pig. Through analysis of somatic cell hybrids, radiation hybrids and dual-colour fluorescence *in situ* hybridisation the genes were physically orientated on SSC13. Our comparison of SSC13 and HSA3 confirms observations from previous studies (Sun et al., 1999 Cytogenet Cell Genet 85, 273; Van Poucke et al., 1999 Cytogenet Cell Genet 85, 279), namely extensive rearrangements in gene order between the two syntenic chromosomes. The new mapping data makes the alignment of SSC13 and HSA3 more precise and enables localisation of the likely chromosomal break-points that have occurred since the divergence between human and pig. The results facilitates the identification of candidate genes from the human chromosome 3.

**A043**

## Genetic diversity and genetic distances in threatened horse breeds from western Pyrenees by microsatellites

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As a part of a larger work on Cantabrian-Pyrenean breeds diversity, the genetic polymorphism of four autochtonous horse breeds has been investigated to characterize their genetic structure and phylogenetics. Two of these breeds, Euskal Herriko Mendiko Zaldia and Jaca Navarra, are genetically analyzed for the first time. In total, samples of more than 300 animals have been collected. A strict sampling strategy was employed: individuals sampled were chosen avoiding relatedness and taking into account their morphological characteristics.

Twelve microsatellite DNA markers included in the set of the ABI StockMark have been chosen for the analysis. The PCR products have been tested automatically by an ABI310 Genetic Analyzer and their fragment size by Genescan software. These microsatellites are quite polymorphic in these breeds with a number of alleles ranging 4 and 12.

For each locus heterozigosity, polymorphic information content and allelic frequencies have been estimated. The levels of genetic diversity observed in the breeds under study are relatively high (mean heterozigosity above 0.65 in all of them). These results suggest that they possess amounts of genetic variation similar to the more common breeds.

The allele frequencies have been used to estimate the genetic distances and to construct phylogenetic trees using different models. This information can provide baseline data for genetic conservation and improvement plans.

**A044**

**The GENETPIG object-oriented environment**

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The GENETPIG project is a european project to accelerate the mapping and to identify GENes controling Economic Traits in PIG, coordinated by J. Gellin (BIO4-CT98-0237). The bioinformatics of GENETPIG is managed by Infobiogen in a custom objet-oriented environment, which is able to cope with the diversity of genomic data and their relationship; it is based on the EyeDB OODBMS (http://www.infobiogen.fr/services/eyedb/). Among others, the database integrates the experimental details (PCR for location, sequencing, different studies), data on the obtention of ESTs, comparative mapping, bibliography, and results of sequence comparison, localization by various methods (SCH, RH, ISH..), polymorphism studies, ZooPCR ... Users have the possibility to fill and update data, make various queries and obtain different synoptic or complete views of the data, consult the experimental details, statistics, results of sequences comparison research against several private and public databases, and visualize cytogenetic and RH maps with a web interface (http, cgi-bin and java). The database also provides a lot of links to relevant other databases and data from other mammalians are being integrated. Finally, an email alert service informs users of new sequence matchs, is used for a redundancy survey and contributes to the exchanges between partners.

**A045**

**A comparative radiation hybrid map of Bovine chromosome 24 with Human chromosome 18**

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Radiation hybrid (RH) mapping technology has been proven to be a powerful approach for gene mapping and genome comparisons between species. A 5000-rad bovine whole-genome radiation hybrid panel was utilized to map 28 markers on bovine chromosome (BTA) 24. The BTA24 RH map integrates 24 Type II loci previously mapped on other bovine linkage maps and 4 Type I loci. Of the Type II loci, six are microsatellites associated with genes. Twenty seven out of 28 loci were ordered with odds of at least 1000:1 in a comprehensive framework map. The observed locus order is generally consistent with results from previously reported BTA24 linkage maps. The observed BTA24 RH map information was used for comparison with human chromosome (HSA) 18 cytogenetic and RH maps. The linear order of genes was not conserved between BTA24 and HSA18. The findings revealed four conserved regions between BTA24 and HSA18.

**A046**

**Bioinformatics resources for genome analysis in farm animals**

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The Bioinformatics group at the Roslin Institute is developing bioinformatics tools and resources for scientists engaged in genome analysis in farmed animals. The resources developed encompass both the databases and the associated analytical and display tools required for genetic and physical mapping of the complex genomes of farm animals. The World Wide Web (WWW) is used to deliver the resources to this user community. The ARKdb genome database: Our ARKdb genome database model has now been fully implemented for pigs, chickens, sheep, cattle, Tilapia, horses, cats, turkey, salmonids and deer genome data. The databases are mounted on the primary node at the Roslin Institute (http://www.ri.bbsrc.ac.uk/bioinformatics/) and subsets are also mounted on nodes Texas A&M University and Iowa State University in the United States. The Comparative Animal genome database (TCAGdb): We have also developed a comparative genome database - The Comparative Animal Genome database (TCAGdb) to capture statement that specific pairs of genes are homologous. We are developing automated methods using Artificial Intelligence to evaluate homology data. Genetic diversity: We have developed a database for genetic diversity data for cattle and are currently implementing this model for data from other domesticated animals. Linkage and QTL databases: We have also developed and operate resource databases to handle raw experimental data for linkage mapping in animals. This resSpecies database also handles trait / performance data and can be used for quantitative trait locus (QTL) mapping experiments.

**A047**

**An ordered comparative map of the cattle and human genomes**

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A cattle-human whole-genome comparative map was constructed using parallel radiation hybrid (RH) mapping in conjunction with EST sequencing, database mining for unmapped cattle genes, and a predictive bioinformatics approach (COMPASS) for targeting specific homologous regions. A total of 767 genes were placed on the RH map in addition to 320 microsatellites used as anchor markers. Of these, 638 had human orthologs with mapping data, thus permitting construction of an ordered comparative map. The large number of ordered loci revealed at least 105 conserved segments between the two genomes. The comparative map suggests that 41 translocation events, a minimum of 54 internal rearrangements, and repositioning of all but one centromere can account for the observed organizations of the cattle and human genomes. In addition, the COMPASS *in silico* mapping tool was shown to be 95% accurate in its ability to predict cattle chromosome location from random sequence data, demonstrating this tool to be valuable for efficient targeting of specific regions for detailed mapping. The comparative map generated will be a cornerstone for elucidating mammalian chromosome phylogeny and the identification of genes of economic importance to the dairy and beef industries.

**A048**

## Replicator network arrays for applications in genomic informatics

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We are developing replicator network arrays to automate the classification and analysis of large complex datasets that have proven resistant to traditional artificial intelligence, neural network, and data mining approaches. We present validated categorized reference datasets to basis vector extractors, which extract custom basis sets for each individual data category. These basis sets are used to construct arrays of associated replicator networks, which we have named Adaptive Focused Replicator Networks or AFRNs. The extracted basis vectors are frozen, so that each AFRN has its own custom-tailored basis vector set for its reference category. In production use, arrays of AFRNs are presented with new data and each AFRN attempts to reconstruct a faithful copy of the new exemplar using its own basis set. A higher-level informatic critic reviews the replication fidelity of the AFRN arrays and decides which AFRN has most faithfully reproduced the novel exemplar. If none of the AFRNs has performed adequately or if multiple AFRNs have inappropriately performed well, the exemplar is tagged for human interpretation and possible inclusion into an auxiliary reference database that can itself be used to build additional AFRNs in future. We demonstrate the method with two applications in genotyping and sequencing. Our genotyping pilot studies indicate that greater than 90% of our STR electropherograms can be scored accurately by AFRN arrays – completely bypassing the manual double-checking step required by other scoring methods in current use. Sequencing pilot studies indicate that we may be able to extend read length and raise the quality scores of distant base calls.

**A049**

## Characterization of the LOSINO HORSE and Phylogenetic Proximity with the Principal American Equine Breeds

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The Losino Horse (*pony sp.*) receives its denomination from the original breeding area, the Valley of Losa, in the north of the province of Burgos (Spain). Is the only autochthonous horse breed of Castilla-León, found to be related geographically and historically to other autochthonous breeds derived from the Cantabrian-Pyrenean branch: Portuguese Garrano, Galician Faco, Asturcón, Pottoka and Meren's Horse. During the Middle Ages it occupied an important place in the Reconquest of Spain and later it participated in the American Conquest. As of now, it is a species in danger of extinction with only 200 animals left.

The study of genetic markers has contributed to the genetic characterization. In order to determine intrapopulation genetic variability between the 2 nuclei of the Losino equine breed (Pancorbo and Quincoces) it was estimated the expected average Heterozigosity Index. It presented an average value for both nuclei of 0.412 for 10 biochemical polymorphism loci, and of 0.733 for 10 DNA microsatellite; being Wright's Fixation Index = 0.25, result that permits to affirm that the Losino Horse has a medium-high genetic variability. It was also established its genetic relation with those equine breeds related geographically as much as historically by using Nei's Genetic Distance. The contribution of the Losino Horse in the Conquest and Colonization of America remains reflected by its genetic proximity to American breeds such as the Paso Fino, the Quarter Horse or the Chilote Horse.

**A050**

**Polymorphic markers within the promotor region of the Horse *NRAMP1 (SLC 11A1)* gene**

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Based on inter-species sequence homology, several pairs of primers identifying parts of equine *NRAMP1 (SLC 11A1)* gene were designed. PCR products obtained were cloned and sequenced. The nucleotide sequence of the 5´UTR was examined for the existence of restriction sites which were subsequently tested for polymorphisms. Three polymorphic markers (Nla III, Taq I, Msp I) were identified either by direct sequencing or by PCR-RFLP. Allele frequencies in two bi-allelic PCR-RFLP polymorphisms (Taq I, Msp I) in two horse breeds were: Old Kladruber: Taq I + 0,66; Taq I – 0.34;Msp I + 0.99; Msp I – 0.01 (N= 81); Akhal-Teke: Taq I + 0,83; Taq I – 0.17; Msp I + 0.37; Msp I – 0.63 (N= 49).

**A051**

**Genetic relationships among genus *Gallus* based on mitochondrial and genome DNA polymorphisms**

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The genealogical origin of the domestic chicken and its relation to four species of jungle fowls, Red Jungle Fowl (*Gallus gallus*), Green Jungle Fowl (*G. various*), Gray Jungle Fowl (*G. sonneratii*), and Ceylon Jungle Fowl (*G. lafayettei*), were explored using DNA polymorphisms of mitochondrial D-loop gene, MHC genes (*B-L*βII and *Y-L*βIII) and AFLP as genetic markers. In addition, Japanese Quail (*Coturnix japonica*) was analyzed as an outgroup of genus *Gallus*. Among four species of jungle fowls, the Red Jungle Fowl showed closest relationship to the domestic chicken. Grey and Ceylon Jungle Fowls were closely related to each other, but most distantly related to the domestic chicken. These results supported the hypothesis that the Red Jungle Fowl is a monophyletic ancestor of all domestic chickens. Among Red Jungle Fowls, that collected in Laos, which is thought to be *G. g. spadiceus*, was relatively close to the domestic chicken in genetic distance. On the other hands, the White Leghorn (inbred CB line) was very far from both native chickens and jungle fowls. This unique genetic position of the White Leghorn might result from intensive inbreeding in this line and/or high selection for egg production ability and immunological characters in this breed.

**A052**

**Genetic characterization of the South American camelids using microsatellite markers**

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The South American camelid (SAC) species are not completely reproductively isolated, as fertile hybrids of any combination of taxa can be produced. This has led to the general belief that in this group a process of speciation is still in progress, being also the origin of domestic species under debate. The main aim of this work was to help to define the genetic diversity and relationships among populations representing these species, alpaca (*Lama pacos*), llama (*L. glama*), guanaco (*L. guanicoe*) and vicuña (*Vicugna vicugna*). This work was done using a set of 23 microsatellite markers developed in our laboratory.

Phenetic analysis based on 38 alpacas, 14 llamas, 16 guanacos and 16 vicuñas showed that each species are clustered separately, with the only exception of some alpacas that were excluded of the alpaca group; these animals correspond morphologically to hybrids ("guarizos"). Interestingly, alpaca and vicuña clusters formed a major group, clearly separated from the llama and guanaco clusters, which were closer among them. This comes to support new evidence based on mithocondial DNA analysis, that suggests the alpacas as descendants from vicuñas (E. Palma, personal communication). Finally, principal component analysis (PCA) based on 193 polymorphic alleles showed that wild species (guanaco and vicuña) had more narrow genetic background than domestic species (alpacas and llamas). The domestic SAC clusters are more heterogeneous, what could be explained by the management of mixed herds, facilitating the generation of guarizos.

**A053**

## Genetic diversity analysis of the 3 Portuguese native horse breeds inferred from microsatellite data

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The study of genetic diversity of autochthonous breeds is very important for programmes related with the conservation of biodiversity in livestock species. Therefore, genetic polymorphism of the 3 Portuguese native horse breeds (Lusitano, Garrano and Sorraia) was investigated in order to characterize their genetic structure. Nine horse microsatellites (ASB2, HMS2, HMS3, HMS7, HTG4, HTG6, HTG10, LEX023 and VHL20), chosen for being highly polymorphic in other Iberian breeds, were used in this analysis. DNA extracted from blood samples was amplified by PCR and the products were separated in 6% polyacrylamide gels using a fluorescence 4200S Li-Cor automated sequencer. For each locus, heterozygosity, allele frequencies and polymorphic information content (PIC) were estimated. The genetic equilibrium according to Hardy-Weinberg, the usual estimators for differentiation between populations (F-statistic) and the genetic distances based on allelic frequencies and multivariate analysis were also calculated. Microsatellites were highly polymorphic for Lusitano and Garrano breeds with the number of alleles ranging from 6 to 11. For Sorraia horse, a rare and very inbred breed, the number of alleles ranged from 2 to 5.

**A054**

**Two major mitochondrial DNA types in the New Zealand Jersey cattle**

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Mitochondrial DNA (mtDNA) was used to verify whether a particular cow was mother to a Jersey bull which had been selected as a donor in artificial insemination. The method used was to PCR amplify and sequence the mitochondrial D-loop region of the two test animals to check whether their sequences were identical. An additional fifteen Jersey animals were included in the test to increase the degree of confidence in the result. In total mtDNA D-loop sequences of seventeen NZ Jersey animals were generated in this exercise. Analysis of the D-loop data revealed the existence of two major mtDNA types in the NZ Jersey animals. When compared to the published mtDNA data of cattle breeds from Europe, India, East and West Africa in our analysis, the two NZ Jersey mtDNA types belong to either the *Bos taurus* group (10 animals) or the *Bos indicus* group (7 animals).

**A055**

## Sequencing and mutation analysis in exon 1 of Horse Tyrosinase gene

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The tyrosinase gene (*Tyr)* is essentially involved in melanogenesis and coat colour as well as in hormone production. Here we first report the sequence of 765 bp of exon 1 in the horse tyrosinase gene (*Tyr)* amplified by PCR using the following primers: AAT GCT CCT GGC TGT TTT GTA (upper primer) and CTG CCA GGA GGA GAA GAA GGA TGC T (lower primer). The horse tyrosinase sequence shows a high sequence identity to the corresponding known sequences in humans, mouse, dog and pig both at the nucleotide and amino acid level. Mutation screening in the horse *Tyr* gene was carried out in 250 individuals of different coat colour and different breeds. One mutation with a simple Mendelian inheritance causing an amino acid substitution (Pro -> Ser) was detected. A possible association of the detected allele with different phenotypes will be elucidated. The results will be shown.

**A056**

**Homology of bovine tyrosinase with human, mouse, and chicken sequences.**

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The cattle tyrosinase gene sequence was obtained by designing PCR primers from human exons (M63235, M63236, M63237, M63238, M63239). Each exon product was amplified separately since the introns in this gene are so large. For the 80% of the cattle tyrosinase sequenced, amino acid homology between cattle and human is 95% with 87% homology in the nucleic acid sequence. Protein homology of 93% was found between mouse and cattle, with a nucleic acid homology of 82%. Lower homologies of 89% for amino acid and 72% for nucleic acid sequences were found between cattle and chicken tyrosinase. Through the course of sequencing the gene a SNP was found, from which a purposeful mismatch PCR-RFLP will be useful in linkage mapping the gene. The tyrosinase gene is implicated in albinism and oculocutaneous albinism in humans and various forms of partial albinism in mice and is therefore of interest in the study of cattle coat color.

**A057**

**High-resolution physical mapping confirms similar gene order with an inversion between human 17 (Hsap17) and porcine chromosome 12 (Sscr12).**

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Comparative mapping between livestock and human provides a powerful tool for exploiting gene map information from the well-studied human genome. Although a large number of conserved chromosome fragments have been identified between human and pig, little is known about the precise gene order between the two species. To better understand the evolutionary relationship of human and pig chromosomes and predict the location of homologous pig genes, we physically mapped 6 genes/ESTs of Hsap17 to Sscr12 and Sscr2, using both somatic cell hybrid panel (SCHP) and radiation hybrid panel (RH) analysis. The primers were designed for the 6 genes/ESTs of human Hsap17 based on pig embryo EST library sequences. The genes/ESTs are *RBP56, H3F3B, PLI, PSA, P311 and EST15*. SCHP analysis located 5 genes/ESTs *(RBP56, H3F3B, PLI, PSA* and *EST15*) on Sscr12. The mapping result indicated an inversion of gene order between Hsap17 and Sscr12 for gene *PLI*. This gene was located on distal Hsap17q, but was assigned to distal Sscr12p. Gene order by RH analysis is *H3F3B-EST15-PSA-RBP56-PLI* on Ssc12, the exact inverse of the order of these genes on Hsap17. By using SCHP and RH mapping, gene *P311* was mapped to Sscr2. Mapping of these six genes confirmed the conserved synteny relationships observed with bidirectional chromosome painting. Furthermore, RH mapping in the present study reduced the number of Sscr12 RH linkage groups from five to four.

**A058**

**The use of bovine microsatellites in the genetic study of African antelopes in European captive populations.**

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The genetic structure of the African antelope European captive population of *Aepyceros melampus* (impala), *Hippotragus niger* (sable antelope), *H. equinus* (roan antelope), *Kobus ellipsiprymnus* (waterbuck) and *Kobus leche* (lechwe) have been studied using bovine microsatellite markers, mapping to 19 chromosomes. The aims of the study are to quantify the genetic variability and to test the usefulness of these markers for parentage testing. The data generated will allow the development of a genetically based management plan for these captive populations. From an initial set of 22 bovine microsatellites tested only 12 were chosen, as the others did not amplify, were difficult to score or were monomorphic. DNA was extracted from blood, tissue, hair and faeces using phenol/chloroform and commercial kits. DNA was amplified by PCR and the products were separated in 6% polyacrylamide gels using Li-Cor 4200 automated sequencer. Preliminary results showed the number of alleles ranging from 2 to 7 and the analysis of a well-characterised impala captive group (n=24) suggest that those microsatellite markers can be used for parentage assignment.

**A059**

**Characterization of a normalized cDNA library constructed from bovine mammary gland tissues.**

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Characterization of mammary-specific gene expression will aid the discovery of biological factors that influence production and udder health in dairy cattle. Expressed sequence tags (ESTs) are a critical component of this investigation, serving as a resource for gene discovery, mapping, and expression profiling. As a resource to generate 10,000 unique bovine mammary ESTs, we constructed a $Cot_{500}$ normalized cDNA library. To encompass a larger repertoire of gene expression, mRNA was isolated from udder biopsies performed on animals from eight different stages of mammary growth, development, and health. Equimolar amounts of mRNA from each stage were combined and used to synthesize cDNA. To make the library amenable for automated high-throughput sequence analysis, 50,000 clones were picked and arrayed into 384 well plates. DNA template for sequencing was generated by PCR amplification of cDNA inserts from bacterial culture. A preliminary analysis of the sequencing data from 4,224 clones was performed using BLAST against GenBank nr and dbEST to assess inter- and intra-library EST redundancy. Clonal redundancy within the library was 35%. Of the uniquely identified ESTs, 61% had no similarity to bovine sequence, and 16% had no identity to any sequence. For the ESTs (39%) with similarity to bovine sequence, 62% of these were redundant with ESTs generated from the four bovine cDNA libraries at ARS-USDA MARC. The EST sequence data indicate ~16,000 clones need to be sequenced to reach our goal of 10,000 ESTs.

**A060**

## Genetic relationships in Swiss sheep breeds based on microsatellite analysis

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The present investigation makes use of a combined set of thirty-one microsatellite markers developed from the bovine, ovine and caprine genomes to characterize the genetic relationships between seven Swiss sheep breeds. Most of these microsatellites are currently in use in an European sheep and goat biodiversity project. The populations studied were the four main Swiss breeds White Alpine, Oxford Down, Black-Brown Mountain, Valais Blacknose and three local Swiss breeds Valais Red, Spiegel Sheep, Engadine Red. The latter three are considered endangered. In addition the wild type Mouflon was included in this study. PCR amplification of 31 bovine, ovine and caprine microsatellites was performed in a total of 307 animals. The average heterozygosity within each population was high in the domestic breeds (0.60 to 0.71) and lower in Mouflon 0.45. The average coefficient of gene differentiation over all loci was 0.17; i.e. only a small part of the variability at the 31 microsatellite loci can be ascribed to the between-breed variability. Cavalli-Sforza's chord measure was used to calculate genetic distances and to build a neighbor-joining tree. The first three components of the principal component analysis could explain 52% of the total variation. Microsatellites developed from the closely related species cattle and goat were useful for estimating genetic relationships among sheep breeds.

**A061**

**Precise mapping of breakpoints in conserved syteny between human chromosome 1 and pig chromosomes 4, 6, and 9.**

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Previous comparative mapping suggested at least five pig chromosomes (Sscr4, 6, 9, 10 and 14) share homology with human chromosome 1 (Hsap 1). Eleven genes, including *Janus kinase 1 (JAK1), prostaglandin E receptor 3 (PTGER3), urate osidoase (UOX), coagulation factor 3 (F3), vascular cell adhension molecule 1 (POU2F1), coagulation factor 5 (F5), prostagladin endoperoxide synthase-2 (PTGS2), myosin binding protein H (MYBPH)* and *antithrombin III (AT3),* were selected to refine the synteic boundaries between Hsap1 and pig chromosomes. Pig STSs were developed from heterologous sequence, then sequenced and physically mapped using a somatic cell hybrid panel. Five genes were also amped by using fluorescent in situ hybridization (FISH) to improve map resolution. This study located *JAK1, PTGER3,* and *UOX* to pig chromosome 6; *F3, RPL5, VCAMI, F5,* and *POU2F1* to *AT3, PTGS2,* and *MYBPH* to Sscr9. Using the GB4 human radiation hybrid information for *PRL5* (258 cR), and for *PRKACB* (237cR) (which maps to Sscr6; Marklund et. al., 1992), we propose the pig Sscr4/9 and Hsap1 we used a heterologous FISH approach using pooled human YACs. The FISH analysis demonstrated that Hsap1q22-24 YAC clones map to Sscr4q15-16 clones map to Sscr9q25, and Hsap1q44 YAC clones map to Sscr14q22. These results suggest the precise breakpoints coreesponding to Sscr4/9 on Hsap1 are most likely on Hsap1q24-25.

**A062**

## New Microsatellite Markers in Chicken

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About 1000 chicken microsatellite markers have been reported in the last decade. However, many more microsatellite markers are still needed for efficient detection of QTL. Here we report 678 new microsatellite markers. A $(CA)_n$-enriched library was constructed according to Takahashi *et al.* (1996) with modifications. CA-positive clones were selected by colony hybridization screening and the DNA sequences were determined. 37.5% of the clones from the library were CA-positive. To date, 1800 CA-positive clones have been sequenced. Of these clones, 1031 clones were subjected to DNA database homology search (DDBJ, GenBank and EMBL). Of the 1031 clones, 678 clones (65.8%) were unique and judged to be suitable for developing PCR primer pairs to detect $(CA)_n$ repeat length polymorphism. Microsatellite markers identified at the National Institute of Agrobiological Resources have reached 800. These markers will be applied to QTL analysis of a Japanese resource family based on Oh-Shamo (Japanese Large Game) and White Leghorn.

**A063**

**Large scale bovine cDNA sequencing toward the construction of the bovine/human comparative map**

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To facilitate the cloning of economic trait loci, we have started to construct a bovine high-density gene map and a precise bovine/human comparative map. We constructed poly A-deleted cDNA libraries from various bovine fetal tissues to overcome the difficulties of sequencing cDNA from the 3' end owing to poly A. We determined cDNA sequences from both 5' and 3' ends, producing 5' and 3' ESTs. Those ESTs were submitted to our database system in which redundancy was checked among our ESTs, followed by blast search against GenBank DB. The ESTs were applied to be located on a human gene map, utilizing the similarity to human EST. Each EST is being assigned to bovine chromosome using a somatic cell hybrid panel. We have determined 35,700 EST sequences in total which are grouped into 7,337 and 6,571 of 5' and 3' ESTs, respectively. 34% of 5' ESTs and 22% of 3' ESTs showed high similarity to the genes or ESTs registered in GenBank DB. About 650 of ESTs were located on a human gene map and fifty of them were assigned to bovine chromosomes by SCH panel.

**A064**

**Rejection of *KIT* as the gene responsible for appaloosa coat color spotting patterns in horses**

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The coat color pattern of the Appaloosa horse appears similar to that caused by the *rump-white* gene in the mouse. Rump-white color pattern in the mouse is the result of an inversion within the *proto-oncogene c-kit* (*KIT*). Therefore, we investigated whether or not *KIT* encodes the appaloosa coat color gene (*Lp*) in horses. *KIT* encodes a transmembrane tyrosine kinase receptor that belongs to the PDGF/CSF-1/c-kit receptor subfamily. *KIT* plays a critical role in hematopoiesis, gametogenesis, and melanogenesis. *KIT* has limited, known genetic variation but is located on horse Chromosome 3 close to *albumin* (*ALB*), *esterase* (*Es*), *Vitamin D binding protein* (*GC*) and microsatellite markers *ASB23, LEX07, LEX57,* and *UCDEQ437*. Family studies were conducted to investigate linkage of *Lp* to these markers using 8 half-sib families in which Appaloosa stallions were mated to solid colored mares. None of the markers demonstrated linkage with the *Lp* gene as indicated by lod scores of 3.0 or greater. Based on cytogenetic studies, ASB23 is likely to be most closely linked to *KIT*. *ASB23* was informative in 7 out of 8 families and lod score analysis rejected linkage at $\Theta = 0.1$ (Lod= -2.16). Therefore, it is concluded that *Appaloosa (Lp*) is not linked to *ASB23*, and thus Appaloosa is unlikely to be the product of *KIT*.

**A065**

**Development of new placental and fetal ESTs for gene discovery in pig reproduction.**

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One major problem that has high economic impact on pig reproduction is the unexplained loss of potential porcine conceptuses during the first month of gestation. To better understand when and how these losses occur, it is imperative to investigate the underlying genetic regulatory mechanisms. We have recently initiated a large-scale cDNA sequencing project to provide molecular information regarding the genes expressed in female reproductive tissues. cDNA libraries are planned for ovary, hypothalamus, pituitary, placenta, uterus, and several stages of embryonic development. Sequence information will also be highly useful in developing sequence-tagged sites for physical mapping and developing comparative links between the human, mouse, and pig genome maps. We have previously reported the creation of 2 cDNA libraries, porcine fetal (day 20), and conceptus (day 17). Sequencing of these libraries produced 220 ESTs, with 180 sequences analyzed by clustering algorithms, and 139 clusters being identified within these sequences. We now report the creation of 2 more libraries from porcine fetal (day 45) and placental tissues. The day 45 fetal library has 971,150 independent clones (average insert: 1.4 kb), while the placental library has 1,320,000 independent clones. Initial sequencing of the fetal library has produced 98 ESTs (81 clusters), while we have obtained 1446 ESTs (1056 clusters) from the placental library. After clustering all sequences thus far obtained, we have identified 1240 unique clusters. Sequences obtained in this project will be deposited into Genbank dbEST, and all comparative homology information will be summarized on a public website.

**A066**

**Refined genetic and comparative physical mapping of the canine copper toxicosis locus**

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Copper is an essential trace element for the survival of all organisms, although it is highly toxic above a certain threshold. A well-regulated copper metabolism is required to ensure a cellular process for copper homeostasis. Only two genetic defects of copper metabolism in man, Menkes and Wilson's diseases have been described. A copper overload disorder, copper toxicosis (CT), is also seen in the Bedlington terrier. CT in Bedlington terriers is an autosomal recessive disorder characterised by an accumulation of copper in the liver leading to chronic hepatitis and, ultimately, cirrhosis. Recently, we assigned the CT locus in Bedlington terriers to the canine chromosome region CFA 10q26, which is homologous to HSA 2p13-21. A radiation hybrid map of the CFA 10q21-26 region was constructed containing 10 DNA markers and 6 genes, to facilitate positional cloning of the CT gene. Using homozygosity mapping, the CT locus was confined to a 42.3 $cR_{3000}$ region estimated to be about 9 Mb. The homologous region of the CT region in man is about 30 $cR_{3000}$ (± 8 Mb). Isolating of a gene for CT will be an important addition to the limited knowledge of the genetic regulation of copper metabolism in mammals.

**A067**

**Microsatellite polymorphism in dog breeds-the AKC Parent Club study**

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The American Kennel Club (AKC) registers 151 breeds of dog, approximately half of all breeds worldwide. Since 1997, 75 breeds have be evaluated with a panel of 17 microsatellite markers to determine 1) the polymorphism at each locus in each breed, 2) the effectiveness of each locus for identity and parentage testing, and 3) the genetic diversity within and between breeds. Herein we report results on the 56 breeds tested in 1998 and 1999 and include data from the previously reported 19 breeds when appropriate. Buccal swab samples (average n=120) were collected at breed specialty shows by AKC personnel and, thus, are not randomly selected but instead tend to include a large number related individuals. All samples were analyzed with a primary and secondary battery of multiplexed, fluorescently-labeled microsatellites containing 10 and 7 loci, respectively. Labeled fragments were electrophoresed on an ABI 377 automated sequencer using an in-lane internal size standard. Overall, the expected heterozygosity for individual loci ranged from 0.01 to 0.92. The mean expected heterozygosity over 17 loci for each breed ranged from 0.45 to 0.74. The number of loci required for a Combined Power of Exclusion to attain 99% in different breeds ranged from 6-14 loci. While the nonrandom structure of the populations tested does not allow absolute assessments of locus informativeness, it is evident that 1) a single battery of 10 microsatellite loci can accurately and efficiently determine identity and parentage in many dog breeds, and 2) a second battery of loci can assist in more inbred populations.

**A068**

**Low genetic differentiation among South American population of the Wood Stork (*Mycteria americana*)**

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The Wood Stork (*Mycteria americana*) is a colonial wading bird of the tropical and lower subtropical zones that breeds in North, Central and South America. We assessed genetic structure within and among six colonies of Wood Stork from the Brazilian Pantanal. Samples of 224 individuals were studied using protein electrophoresis to evaluate their genetic variability and differentiation. Of 23 loci examined, 7 were polymorphic (mean heterozygosity = 0.065), indicating low levels of allelic diversity and low genetic divergence among colonies. The low value of Fst indicates that 0.3% of the total variance can be explained by differences among colonies. Estimated number of migrants per generation based on F-statistics ( Nm = 83.1) suggest high levels of gene flow. Values of Nei's genetic distance among South American colonies ranged from 0.000. Values of genetic distance between South and North America colonies according to Nei ranged from 0.000 – 0.007, while according to Rogers they ranged from 0.010 – 0.049. Our data indicated high levels of gene flow between populations of North and South America, intermediated by a probable interbreeding population in Central America. FAPESP (98/06160-8, 98/16359-6)

**A069**

## RAPD-Analysis of the Genetic Divergence of Nuclear DNA in Ducks (ANAS PLATYRHYNCHOS) in the Course of Selection

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The efficiency of using polymorphic DNA-markers as marker trait which reflect gene pool of duck breeds (Anas Platyrhynchos) and intrabreed lines is shown with a view to determine the degree of intrabreed differentiation conditioned by lineage belonging and the degree of af interlinear intrabreed differentiatin conditioned by selection work and to express this differentiation degree as a quantitative criterion-indices of the genetic distance. The opinion is put forward that the genetic differentiation revealed is an important factor in obtaining heterosis by line hybridization and in gene pool preservation.

DNA-polymorphism of duck intrabreed lineages was demostrated using the method of polymerase chain reaction with 5 different random primers (RAPD-PCR). Genetic distances were calculated by the methods of Nei and Cavalli-Sforza.

Analysis of the segregation of random amplified DNA-markers in F1 progeny was done. It is shown that the parameters of genetic distances calculated on the basis of the RAPD-PCR patterns, objectively reflects even small alterations in the genetical structure of intrabreed lineages in the course of transformation of the initial parental forms.

The results obtained may de used in further genetic improvement of the existing forms and breeding of the new high-yield lineages.

**A070**

## Breeding and biological characteristic of the White Siberian cattle

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The White (black-and-red-eared) Siberian cattle was developed by breeder N.E. Kosov in the state farm of 'Gutovskiy', Novosibirsk Region, in the 1940s - 1960s. The breeding was performed on the basis of the local Siberian cattle with the addition of blood of the Dutch and Friesian breeds. The three leading lines were the following: Lebed 147, Margo 86 and Lobach 122. The herd numbered more than 2000 animals, the average productivity being 4000 kgs per lactation, fat content 4.0 - 4.1%. The milk had wonderful dietary indices, that is the high content of protein, calcium and vitamin D. The animals were distinguished by their excellent adaptability characteristic and the resistance to parching heat and Siberian midges. The analysis of antigen indices of nine blood systems allowed to reveal gene pool of the White Siberian cattle and to estimate the indices of genetic affinity with regard to the animals of other breeds. As for the Black-and-White cattle of the Siberian and European areas, the Dutch and Friesian breeds, the indices were 0.8646, 0.8519, 0.8586 and 0.8405 respectively. The investigation results witness a considerable difference of cattle developed from the animals of initial breeds.

**A071**

**Genetic analysis in two populations of Peruvian Horse: Peruvian Paso and Blood Horse Race**

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Today, our breed of Peruvian Paso Horse is considered a national treasure. The study was realized in order to kwon and compair the genetic variability between two breed of Peruvian Horses.

Two populations, which are males, were analyzed using seven bloods group systems: *A,C,D,K,P,Q,U* and eight serums proteins systems: *Pi, Tf, A1B, Es, GC, Es, PGD, ALB,* and *HBA.* Blood samples were collecte from 329 peruvian horses (116 Peruvian Paso and 213 Pure Blood Race) of diferents peruvian cities and study by polyacrilamide gel electroforesis (PAGE) at pH 7.9, Isoelectric focusing (IEF) pH 4.0 – 6.5 and starch gel electrophoresis (STAGE) al pH 4.6

International recognized alleles were detected in the fifteen systems studied and own variants were founded in every one populations in the systems *Pi, Tf, Es and A1B.*

Allelic frequencies in that groups were compared and our results indicate that of thirteen allelic variants identified in the *Pi* systems, five of them: *H, J, L3, R, S** were detected only in PP Horses (the new unrecognized variant [S*] was similar to S) and the allelic variant *I* and *L2* was identified only in Blood Horse Race. Also in *A1B* and *Es* systems the allele *S* (f=0.349) and *G* (f=0.3157) respectively were observed only in Peruvian Paso Horse.

Finally, in the *Tf* system was detected only one individual for allele *F1* in Peruvian Paso Horse in comparison whit nineteen individuals in Blood Horse Race.

**B001**

## Survey of allele occurrences of DNA marker-loci used in the selection of the Hungarian Prolific Merino sheep

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The Hungarian Prolific Merino sheep as a new breed was registered in 1992 in Hungary. Booroola rams imported from Australia were used to establish this breed. The selection criterion of breeding rams and ewes is to carry the $Fec^B$ allele. The selection is based on the ovulation rate (OR) and the reproduction performance of the ewes, and in the case of the rams, it is based on the OR of their daughters. We also selected animals for bigger body weight and without horns. To improve the selection efficiency molecular genetic information besides the traditional selection methods will be used to determine the genotype of animals regarding fecundity gene. As a first step of this program, the OarAE101 and BM1329 microsatellite markers linked to FecB gene were analyzed in the whole population. DNA was prepared from blood samples. Visualization of the microsatellite alleles was achieved by using an ABI 310 Genetic Analyzer. With microsatellite OarAE101, five alleles were observed, and with microsatellite BM1329, four alleles were observed. The next step in the study is to perform test mating and segregation examinations. Using the results of microsatellite analysis and the OR and reproduction data within families, marker-assisted selection can be achieved in this population.

**B002**

**Mapping QTL affecting milk production traits by means of selective milk DNA pooling in a daughter design, using a false discovery rate criterion**

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In 1996, a project was initiated aimed at mapping QTL affecting economic traits in the Israel-Holstein dairy herd, using selective milk DNA pooling and dinucleotide microsatellite markers. In the first stage, 2300 milk samples were collected from the high and low daughters of seven sires, with respect to milk protein percent. A complete genome scan involving 150 markers was carried out. In the second stage, reported here, 6500 milk samples were collected from high and low daughters and 10 sires, with respect to protein percent, milk yield and protein yield. Markers found to be significant in the first scan, and additional markers adjacent to these, were tested in the pools for the three traits. To date, 160 markers have been tested for protein percent; and 72 of those were tested for the other two traits as well. At an experiment-wise false discovery rate (FDR) of 0.05, 120 marker-by-trait combinations were significant, indicating the presence of more than 30, 10, and 20 QTL affecting milk protein percent, kg protein, and kg milk, respectively. Thirty-six sire-by-marker-by-trait combinations were significant for more than one trait. In accordance with the known genetic correlations, the effect for protein percent was in the same direction as kg protein in 6 of 9 instances, but in the opposite direction as kg milk in 17 of 20 instances; effects for kg milk and kg protein had the same direction in 6 of 7 instances.

**B003**

**Epistatic interactions with myostatin for carcass composition in beef cattle**

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A search to detect quantitative trait loci (QTL) in families segregating an inactive copy of myostatin was conducted. An objective of this search was to identify epistatic interactions between myostatin, on chromosome 2, and several chromosomal regions. Two half-sib families were developed from a Belgian Blue x MARC III (n=246) or a Piedmontese x Angus (n=209) sire. Traits analyzed were fat depth (cm) and meat tenderness, measured as Warner-Bratzler shear force (kg) at 3 and 14 d postmortem. In the family from the Belgian Blue x MARC III sire, the presence of an interaction for Warner-Bratzler shear force 3 d postmortem at 19 cM from the beginning of the linkage group on chromosome 4 was detected (expected number of false positives=1.48). In the family from the Piedmontese x Angus sire, three interactions were detected. For Warner-Bratzler shear force 14 d postmortem, an interaction was detected at 69 cM from the beginning of the linkage group on chromosome 5 (expected number of false positives=1.26). Two interactions were identified for fat depth; one at 30 cM from the beginning of the linkage group on chromosome 8 (expected fraction of false positives=.1) and the other at 14 cM from the beginning of the linkage group on chromosome 14 (expected number of false positives=1). Myostatin is considered a major gene with large phenotypic effects and this data demonstrates that interacts with other loci throughout the genome in the expression of carcass composition traits.

**B004**

## Mapping chicken QTL affecting obesity by selective DNA pooling in an advanced generation full-sib intercross line

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A full-sib intercross line (FSIL) was initiated in 1989 by mating a single White Rock broiler line male to five semi-inbred Leghorn females (ADOL Line-0). The F1 progeny were intermated to produce an F2 generation, and the line was continued by intermating each generation. Biometrical analysis of the F2 through F9 generations showed high phenotypic variation and high heritability for obesity as measured by abdominal fat pad weight as a proportion of total body weight (pAF). In the F10 generation, three hatches, each comprising 200 birds (100 males and 100 females) were reared and phenotyped for pAF, and blood samples were taken from each bird. The 20% high and 20% low males, and 20% high and 20% low females were chosen in each hatch. DNA was extracted from the chosen birds and pooled separately by hatch, sex and phenotypic group (high and low) to give 12 pools. Each pool was independently prepared in duplicate, and densitometrically genotyped using the ABI automatic sequencer. A total of 47 markers were examined. Of these, 6 gave highly significant effects ($P<.01$), and 5 gave significant effects ($P<.05$); these probably represent about 7 or 8 true effects. By the F10 generation, the FSIL would have reached a state of three- or fourfold genome expansion; 47 markers thus provide about 15 to 20% genome coverage. Therefore, there may be 30 to 40 mappable QTL affecting pAFW segregating in this population.

**B005**

## Mapping of QTL for egg quality and egg production traits in chicken

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A genome scan is the first step in localizing economically important gene loci. The aim of this study was to find QTL for important traits in egg layer chicken. Eggshell strength and egg white thinning are two major traits affecting egg stability and economics of production. A genome scan was carried out in an $F_2$-mapping population derived from a cross between two genetically and phenotypically extreme egg layer lines. Three hundred-twenty $F_2$ hens were scored for egg quality and production at given periods. A total of 115 microsatellite markers were genotyped in the pedigree and mapped to 14 linkage groups covering over 80% of the chicken genome. QTL analyses were performed using interval mapping by multi-marker regression. Empirical significance thresholds were calculated using a permutation test, and confidence intervals for QTL positions were obtained by bootstrapping. In the initial genome scan, 23 QTL areas were found at 5% chromosome-wise significance level. These were fitted as cofactors in subsequent analysis. In all, 8 different QTL affecting egg quality, egg production, body weight and sexual maturity were significant at the 5% genome-wise significance level. For the next step, fine mapping, $F_2$ individuals were back-crossed with the grandparent lines. The fine mapping of the chromosomal areas containing significant QTL is in progress.

**B006**

## A genome scan for milk production QTL in Finnish Ayrshire cattle

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A genome-wide scan for milk production QTL was carried out in Finnish Ayrshire cattle. Twelve half-sib families with a total of 494 sons were genotyped and used in a granddaughter design. More than 150 microsatellite markers were genotyped to construct a 2700 cM (Haldane) male linkage map. Interval mapping was performed with a multiple-marker regression approach with both one-QTL and two-QTL models. Empirical values for chromosome-wise significance thresholds were determined using a permutation test. Putative QTL (chromosome-wise $p < 0.05$) were detected for milk yield on chromosomes 1, 5, 6, 12, and 20; for protein yield on chromosomes 5, 6, and 25; and for fat yield on chromosomes 6 and 14. Protein percentage QTL were found on chromosomes 6, 14 and 23. A QTL for fat percentage at marker ILSTS39 on chromosome 14 is significant at the genome-wise level after correcting for the number of autosomes and the number of traits ($F=5.03$; genome-wise $p < 0.01$). Analysis with the two-QTL model supports two distinct QTL affecting milk production traits on chromosome 6. The location and effects of QTL found on chromosomes 6, 14, and 20 are similar to previous results in Holstein-Friesian cattle. This indicates that identical major QTL may still be segregating in different cattle breeds.

**B007**

## Detection of CA repeats insertion in IDVGA-48 interrupted microsatellite

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Microsatellite IDVGA-48, located on bovine chromosome 19, was tested for association to beef performance traits in a population obtained by crossbreeding Piemontese and Chianina breeds. In 7 out of the 109 crossbred F1, the microsatellite region could not be amplified under the published experimental conditions and with the published primers (EMBL Accession Number X85065). Because the presence of 'null' allele was suspected, two more primer pairs were designed in order to amplify different portions of the flanking regions and different experimental PCR conditions were performed. Primers designed in the 3' flanking region of the microsatellite repeats successfully amplified the right fragment in all animals. A primer designed in 5' flanking region of the CA repeats, utilised with a primer of the 3' flanking region under different PCR conditions, amplified the DNAs of all animals. In the progeny suspected to contain the 'null' allele, a 550 bp amplification fragment was observed instead of the 210 bp fragment observed in normal animals. The 550 bp fragment was sequenced and represents the IDVGA-48 microsatellite sequence containing an insertion of about 165 CA repeats. In the parental animals, the same amplification fragment (550 bp) was observed together with the normal fragment (210 bp). These results indicate the heterozygotic condition of the microsatellite in the parents of the 'null' allele animals. The new IDVGA-48 allele, previously unknown, may represent an interesting marker for future association to QTL.

**B008**

## Molecular cloning and characterisation of the porcine methylmalonyl-CoA mutase locus

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Inherited defects in the gene coding for methylmalonyl-CoA mutase (*mut*) leading to reduction or complete loss of mutase activity are well known in humans. Yet no mutations within the *mut* locus are shown to correlate with genetic disorders in animals. Here we report cloning and characterisation of the porcine methylmalonyl-CoA mutase locus. A porcine genomic PAC library was screened using 5´- and 3´-DNA-probes. Two overlapping recombinant clones harbouring 13 exons and spanning >130 kb were analysed by sequencing. Coding regions revealed high conservation among different species. Several dinucleotide repeats were found within the introns at least one of them is an informative polymorphic microsatellite. In the 5´-upstream region we identified several GC-boxes, which are binding sites for the Sp1 transcription factor. The porcine *mut* locus was assigned to chromosome 1q13 - q14 by in-situ hybridisation demonstrating linkage with human chromosome 6p and mouse chromosome 17E-F. This study provides a foundation for mutation analysis to clearify the pathophysiologic effects arising from impairment of the methylmalonyl CoA mutase.

**B009**

**Mapping quantitative trait loci controlling genetic resistance to gastro-intestinal nematode parasites in Red Maasai sheep**

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Gastro-intestinal nematode parasites are the most important disease constraint limiting small ruminant productivity in sub-Saharan Africa. Use of anthelmintics as a method for control is too costly for smallholders in many developing countries. A potential alternative means of control is the breeding of genetically resistant livestock. The Red Maasai sheep of East Africa have developed genetic resistance as a result of intense worm challenge over a long period of time. The aim of this research is to identify molecular markers linked to specific regions of the ovine genome that are responsible for resistance to gastro-intestinal parasites in this breed. Six double backcross resource families are being generated in this experiment by mating six Red Maasai-Dorper $F_1$ rams to both Red Maasai (resistant) and Dorper (susceptible) ewes. The aim is to phenotype about 200 backcross lambs per $F_1$ sire family. To date, 29 Dorper, 57 Red Maasai and 541 backcross lambs have completed phenotyping. Phenotyping involves the exposure of the backcross lambs to endoparasite (predominantly *Haemonchus contortus*) challenge at pasture (2-6 month old lambs), followed by a 5-7 week experimental trickle challenge with *H. contortus*. Their resistance/susceptibility status is then assessed by faecal egg counts, blood packed cell volume and worm counts after necropsy. After phenotyping, the most resistant and most susceptible lambs will be genotyped using about 200 microsatellite markers evenly spread over the entire ovine genome. Quantitative trait loci (QTL) controlling parasite resistance will then be located by integrating the phenotypic and genotypic data through linkage analysis.

**B010**

**A partial genome scan reveals three QTL affecting fat deposition in sheep**

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A genome scan is underway to detect chromosomal segments carrying major genes affecting fat deposition traits in divergent selection lines of Coopworth sheep backcrossed using a conventional reciprocal design. These lines were selected for 6 generations (1981-1992) for and against weight-adjusted ultrasonic fat depth. They differ in fat content by 3.5 standard deviations, and also differ in live weight, litter size, lamb survival and fleece weight traits (Morris *et al.*, 1997 Anim. Sci. 65, 93). Large half-sib families of about 150 progeny per sire generated from four F1 sires have been extensively phenotyped for body composition and meat traits including colour, tenderness and ultimate pH. Genotyping with DNA microsatellites at about 20 cM intervals has been completed for about one-third of the sheep genome to date. Using regression-based QTL analysis procedures for half-sib families (Knott *et al.*, 1996 Theor. Appl. Genet. 93, 71) and after adjusting for carcass weight, single QTLs affecting subcutaneous fat depth have been detected (P=0.00102 and P=0.0117) on two different chromosomes. A QTL has also been detected on a third chromosome affecting total internal fat (P=0.000033). The values reported are of nominal significance (significant thresholds are P<0.0016 and P<0.000052, respectively) and equivalent genome-wide suggestive. No significant QTL were detected on sheep chromosome (OAR) 18, to which the *Rib-eye muscling* (*REM*) QTL has been mapped, or on OAR4, to which *Leptin* (*OBS*) has been mapped.

**B011**

**Rib-eye muscling (REM) locus in sheep: phenotypic effects and comparative genome localization**

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A locus that increases the area and weight of the valuable rib-eye muscle (*Longissimus dorsi,* LD) by 11% and 7%, respectively, has been detected using genetic linkage analysis in the progeny of two Poll Dorset rams originally from the Carwell stud, Armidale, NSW, Australia. This *REM* locus was mapped to the sub-telomeric region of sheep chromosome 18. This location overlaps with the *Callipyge* (*CLPG*) locus that increases the overall muscularity of sheep and decreases their level of fatness. However, *REM* differs from *CLPG* in that it, (1) affects only the LD muscle and has no other effects on body composition; (2) appears to act as a dominant gene (i.e., a single copy inherited from either the sire or the dam has virtually the same effect as two copies inherited from both parents) whereas *CLPG* shows global overdominance, such that the inheritance of a maternal copy of the gene completely inhibits the expression of the paternal copy; and (3) has minor effects on meat tenderness, unlike *CLPG* which significantly increases the LD toughness. Mapping of known genes in the distal region of sheep chromosome 18 has established that this region is equivalent to the distal regions of human chromosome 14q and mouse chromosome 12, and to a segment of pig chromosome 7. No obvious functional candidate gene for *REM* presents itself from these genomes.

**B012**

**Using mammary gland ESTs as candidate genes for QTL affecting milk production traits in cattle**

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As part of a genome scan with microsatellite markers in a granddaughter design with 1099 progeny tested bulls, ESTs were derived from the mammary gland of a lactating cow and used as candidate genes for quantitative trait loci affecting milk production traits. Resource families were genotyped with 247 microsatellite markers and 3 polymorphic ESTs. It was shown by linkage analysis that one of these ESTs, KIEL_E8, mapped to the centromeric region of bovine chromosome 14, where a quantitative trait locus for milk production traits has already been reported. Regression analysis confirmed the location of the QTL in the centromeric region of chromosome 14 and its significant effect on milk production traits. Analysis of variance revealed a strong linkage disequilibrium between the marker KIEL_E8 and the QTL. Effects of the marker KIEL_E8 were estimated to be 140 kg milk, - 5.02 kg fat yield and 2.58 kg protein yield for the first hundred days of lactation. Although a strong linkage disequilibrium between the EST marker and the QTL has been observed, it is up to now not known whether they are identical or not. Nevertheless our results show that KIEL_E8 will be an efficient marker to perform marker assisted selection in the German Holstein-Friesian population.

**B013**

**Development of physical and genetic maps for the bovine Y-chromosome and the pseudoautosomal boundary region (PBR)**

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We have constructed and screened a bovine Y-chromosome (BTY) specific library for microsatellites (MS). Approximately 66,000 plaques were screened with end-labeled $(AC)_{12}$ oligos. A total of 318 MS positive plaques were isolated. Dot blot analyses with bovine genomic DNA, bovine TSPY and BTY0920 probes showed that 69 out of 318 clones contained repetitive sequences. Seventy-nine and 52 clones were homologous to TSPY and BTY0920, respectively. A total of 118 clones have been sequenced. These sequences can be classified into two major sets: homology to previously reported sequences (set A), and newly identified sequences (set B). Set A contains 67 clones, and set B 51 clones. For set A, 60% of the sequences have been reported by other laboratories as localized in the Y-chromosome, confirming the quality of our BTY-specific DNA library. We have developed 45 new MS for BTY. Of these new MS, 12 have been mapped to the pseudoautosomal region (PAR) and 22 to the Y-specific region. Systematic analysis of our newly developed MS and their related sequences allowed us to identify the bovine Y chromosome PBR. A SINE sequence (309bp) has been found at the boundary that defines the Y-specific region from the PAR. A bovine cosmid library is being screened to develop a PBR contig map.

**B014**

## Towards a whole genome radiation hybrid panel in chicken

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The chicken is of great interest since its genome is only one third that of mammals and comprises 9 pairs of macrochromosomes and 30 pairs of microchromosomes. International efforts towards detailed physical and linkage mapping are being made, and a consensus linkage map was published recently that contains 1889 loci localized on 50 linkage groups. A radiation hybrid (RH) panel would help a lot in integrating physical and genetic maps and in localising non-polymorphic markers such as ESTs or genes. We used 44 unlinked markers (20 on the macrochromosomes and 24 on the micro-chromosomes, or small linkage groups) to determine the extent to which chicken genomic fragments are retained in our 102 first hybrid clones. Our results show that the macrochromosome retention rate is only 9.4% while the microchromosome one is 14.5%, leading to an overall retention rate of 12.2%. Half of the hybrids can be selected to get a microchromosome RH panel with a retention rate close to 25%, suitable for high-resolution microchromosome mapping as shown by the construction of a radiation map of the biggest microchromosome (chromosome 10). Only 20% of the hybrids produced would be suitable to build a whole genome RH panel with a retention rate close to 24%, implying the development of more than 500 hybrids. Pooling of hybrids to increase the retention rate will be discussed.

**B015**

**Mapping of HSA17 genes in the common shrew (*Sorex araneus*)**

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Comparative gene mapping has shown a complete conservation of human chromosome 17 gene group for many mammalian species studied including, cattle, pig, cat, mink, and sheep. In other mammalian species the chromosomal material corresponding to human chromosome 17 is presented as a single gene group with insertions of genes from other syntenic groups (mice) or numerable amount of gene associations (dog). For our investigation we used a set of 35 pig, cattle, mice, human, and consensus PCR primer pairs specific for HSA17 genes for a mapping effort of the common shrew (*Sorex araneus*) using a shrew-rodent somatic cell hybrid panel. As a result, we have assigned a set of genes (*ACADVL, GLUT4, NF1, MPO, MYL4, THRA*) to shrew chromosome *hn* confirming the homeology of SAR*hn* and HSA17 which is in complete correlation with zoo-FISH data (Dixkens C et al., 1998 Cytogenet. Cell Genet. 80(1-4)). The assignment of HSA17 gene *MYH2* to shrew chromosome *ik* suggests a breakage of a small chromosome region corresponding to human 17p13.1-pter in shrews. PCR product of shrew *THRA1* and *MPO* were partially sequenced and demonstrated about 95% homology with sheep *THRA1* and 91% with human *MPO* sequences, respectively using the NCBI BLAST program. Our data suggest that the using of heterologous PCR primers for shrew genome mapping is possible but high levels of positive results (about 20%) were not obtained. Our current goal is to assign at least 10 HSA17 gene markers in shrews suitable for comparative analysis.

**B016**

## Candidate gene markers connected to litter size in German Pigs

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The application of marker assisted selection (MAS) seems to be especially promising for the pig breeding industry. The important litter size trait is an ideal candidate due to the low heritability and the existence of appropriate genetic markers. The decision about the incorporation of genetic markers into MAS schemes requires reliable information about additive and dominance effects of each marker in the population of interest and depends on the genetic background.

Four different candidate genes for litter size (estrogen receptor (ESR) gene, prolactin receptor (PRLR) gene, osteopontin (OPN) gene and retinol-binding protein 4 (RBP4) gene) were evaluated for their association with the number of piglet born alive in German pig breeds. A total of 8302 litter records from 2144 sows were used in the analyses. The females were all housed in a single farm and belonged to three different genetic groups (German Landrace, Duroc and a synthetic line). The ESR locus showed polymorphism in the synthetic line only, but no significant effect of the extremely rare B allele could be observed. The dialleclic PRLR marker was polymorphic in all three lines and a difference of more than one piglet born alive between the homozygotes could be identified for the Duroc line. One out of seven alleles at the OPN locus seems to have an influence on litter size in all three lines. Before including this information in MAS possible pleiotropic effects will be examined.

**B017**

**The analysis of new *G*-system of serum alpha-2-globulin allotypes in different pig populations**

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The phenotype distribution of 4 allotypes of serum alpha-2-globulins among 824 pigs in different breeds and populations have been analysed. The allotypes were named G1, G4, G5, G7, and were revealed by agar gel double immune diffusion precipitates with antibodies. The specific antisera were prepared by special isoimmunizations. Only 9 pigs (from 824) were observed to be complete negatives for all 4 G-allotypes, and no pigs were observed to have all 4 simultaneously. Only 10 animals were G4-negatives, but 814 pigs were G4-positives (including 560 with G4 as a single G-allotype in their sera). The results obtained from the study point to a possible hypothesis of genetic control of G-allotypes by *G*-system.. It consists of two subloci that controlled, respectively: "common" allotype G4, and "specific" allotypes G1, G5, G7, and maybe G0. The *G*-system has probably formed a "basic" allele $G^{4,0}$ and evolved from it new alleles $G^{4,1}$; $G^{4,5}$; $G^{4,7}$. Very rare (9 from 824), absolutely G-negative pigs point to a possible "zero"-allele $G^{0,0}$, or maybe a supression of G-locus expression.

**B018**

## Least squares interval mapping of QTL based on selective DNA pooling

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Selective DNA pooling is an efficient method to identify markers that are linked to QTL based on marker frequency differences between phenotypic extremes. Current single marker analysis methods cannot separate QTL position and effect. Objectives of this study were to develop and evaluate a least squares interval mapping method to detect and map QTL based on selective DNA pooling data from multiple markers and multiple half-sib families. Analysis of simulated data from 6 evenly distributed markers on a 1 M chromosome gave nearly unbiased estimates of QTL position and effect. At 5% significance, the method had 90% power to detect a biallelic QTL with an additive effect of 0.25 phenotypic standard deviations based on upper and lower pool frequencies of 6 markers in 7 families, for a total of 84 genotypings. Family size was 2000 progeny, 10% was selected per tail, and the standard deviation of measurement errors on observed marker frequencies was 0.053. Power and precision of mapping increased with QTL effect, number of families and progeny per family, and decreased with magnitude of errors. Optimal percent selected decreased with increasing size of measurement errors. We conclude that least squares interval mapping is a powerful method to detect and map QTL through multi-sire selective DNA pooling.

**B019**

**Adaptation of a linkage disequilibrium method to QTL mapping with granddaughter design data**

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The granddaughter design has successively been used in linkage studies to map chromosome regions affecting milk and health traits in dairy cattle. New tools are now needed to fine map these regions. Here we present a likelihood-based linkage disequilibrium approach (Terwilliger, 1995 Am. J. Hum. Genet. 56, 777). The method allows for multiple markers and alleles. Prior to fine mapping, it is assumed that the linkage analysis is performed and sires that are heterozygous for a bi-allelic QTL are identified. The marker haplotypes of the sires are also assumed to be known. We looked for associations between the marker alleles received from the dam and the trait of interest. The data consisted of daughter yield deviations of the sons, QTL alleles received from the sires, and maternal marker alleles. Marker allele frequencies were assumed to be known. A likelihood function was constructed. Following the idea of Terwilliger, the association parameter is λ. This parameter is a function of the proportion of the chromosomes that carry the favourable QTL allele and descend from the founder chromosome of interest, the recombination frequency between the QTL and the marker, and the number of generations since the founder. Further, residual variance, the three genotypic values, polygenic effects of the sires, and allele frequency of the QTL were estimated. The likelihood ratio test statistics was computed. The method was tested with simulated data.

**B020**

## Cytogenetic and physical mapping of the rabbit MHC using a BAC library

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The major histocompatibility complex (MHC) region is a high gene density DNA segment of two-four megabases (depending on species) which is divided into three regions referred to as classes I, II and III. Each region contains groups of genes involved in the host immune response, as well as many other non-related genes. In mammals, the MHC region is associated with immunological functions in addition to various economic traits. The rabbit MHC (RLA complex) has not yet been mapped, and in contrast to other mammalian species, the class I and II regions were assumed to be adjacent while a physical link with the class III region remained to be established. We screened a rabbit BAC library produced in the laboratory and recovered clones for: *DRA*, *DRB*, *DQA*, *DQB*, *TAP1*, *TAP2*, *DMA*, *DMB*, *DPA* and *DPB* class II genes; *TNFA*, *HSP70*, *TNX*, *G13*, *PBX2* and *Notch4* class III genes; and *R27* and *R19* class I genes. We show that the RLA complex maps to a single locus at position 12q1.1, as revealed by fluorescent in situ hybridization. Overlapping BACs containing the class II genes indicate a well conserved gene order as compared to other species. We have found one BAC clone containing both *R27* and *R19* class I genes, suggesting that the likely non-classical class I *R27* gene and some classical class I genes are in close vicinity. Work is in progress to assess the specific organization of the MHC in Lagomorphs.

**B021**

## Generation of a 12,000 rad radiation hybrid (RH) panel for the porcine genome

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Radiation hybrid mapping has proven to be an efficient method for mapping the human genome and, more recently, the genome of various mammals including swine. Whole genome radiation hybrid panels offer the unique ability to map polymorphic and non-polymorphic markers, integrating the linkage and cytogenetic maps essential for fine mapping of economically important trait loci (ETLs). We have produced a first generation (7000 rad) Whole-Genome Radiation Hybrid Panel IMpRH (INRA-Minnesota porcine Radiation Hybrid panel) (Yerle et al., 1998 Cytogenet Cell Genet 82, 182), and provided an initial RH map of the porcine genome containing 757 markers (Hawken et al., 1999 Mamm Genome 10, 824) with an estimated ratio of 70 kb/cR. This initial map is already a valuable resource for medium-resolution mapping in pigs, with 1500 markers ordered on the panel to date. It is available to the research community through IMpRH Server (http://imprh.toulouse.inra.fr/). The next level of development of a porcine comprehensive map requires a higher resolution template to facilitate construction of a genome-wide BAC contig map and generation of high resolution STS maps. Therefore, we started the construction of a 12,000 rad hybrid panel. We currently (March 2000) have 70 clones. Retention frequency is being established by PCR with 43 markers (mainly ESTs) well dispersed over the porcine genome. We are estimating the resolution at this radiation dose by PCR analysis of markers in a 1Mb region (RN BAC contig). A ratio of around 10 to 20 kb/cR is expected.

**B022**

## Mapping quantitative trait loci for twinning rate in Norwegian cattle

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Results from a granddaughter design in Norwegian cattle suggest QTL for twinning rate on chromosomes 5, 7, 12 and 23. Among these, the QTL positions on BTA5 and BTA23 are strongly supported by the literature. Our results also confirm previous mapping of a QTL for twinning to BTA7, but definitely suggest a different location on the chromosome. The most convincing QTL peak was observed for a region in the middle part of BTA5, close to the insulin-like growth factor 1 (IGF1) gene. Since IGF1 plays an important role in the regulation of bovine folliculogenesis, a mutation search was performed by sequencing more than 3.5 kb of the gene in actual families. However, no functional mutations were detected in IGF1. The mapping resolution achieved by our granddaughter design is rather poor, and linkage disequilibrium (LD) mapping-based methods were developed in order to obtain more precise QTL positions. Analyses by these methods on BTA5 narrowed the QTL position to an area of approximately 3 cM. Encouraged by these results, we are now testing candidate genes that have been localised to that region through comparative analysis.

**B023**

## Microsatellite variation across chromosome 20 in sheep: implications for detecting selection at MHC linked microsatellites

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We analyzed genetic variation at several microsatellite loci mapped on chromosome 20 in sheep. Microsatellites inside the MHC block on chromosome 20, and others spanning the rest of the chromosome were typed. Four domestic breeds of sheep (*Ovis aries*) were included in this study, chosen as representatives of breeds under different selection pressures (e.g. wool or milk) and with different geographic origin: Sarda (from the south of Europe) and Friesain (from the North) have been selected for milk, while Merino (from the South) and Leicester Longwool (from the North) have been selected for wool. Samples of the wild mouflon were also included for comparison. A clear pattern of variation can be seen across chromosome 20, with higher heterozygosity and number of alleles in the microsatellites within the MHC region and immediately adjacent to it, and decreasing values towards the two ends of the chromosome. The high variability at microsatellites within and around the MHC block can be attributed to the effects of overdominant selection at the closely linked MHC genes. Interestingly, the pattern of variation differs between breeds (although the general pattern is consistent) and shows less variation for the northern breeds along the whole chromosome 20.

**B024**

**Characterisation of porcine 3-beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase genes**

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The enzyme 3-beta-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3β-HSD) is essential for the biosynthesis of all classes of hormonal steroids in classical steroidogenic, as well as in peripheral tissues. Different isoenzymes have been reported in the 3β-HSD enzyme family that function either as dehydrogenase/isomerase or as reductase. 3β-HSD is one of the enzymes involved in the formation of the pheromone androstenone (5α-androst-16-ene-3-one) which contrib-utes to the unpleasant odour present in the meat of uncastrated boars, also known as 'boar taint'. A porcine adipose tissue cDNA library was screened with an RT-PCR probe containing part of exon 3 and a large part of exon 4 of the enzyme 3β-HSD. Both strands of the positive clones were sequenced and the putative coding sequence of 1122 nucleotides encodes 374 amino acids. Comparison of the putative open reading frame with the bovine and the human type I homologues was performed using the GCG program package. Porcine 3β-HSD showed 85.6% identity with the bovine and 79.3% identity with the human cDNA. Fluorescence in situ hybridisation was performed with labelled PAC clone containing the gene of interest. The 3β-HSD gene was mapped to the porcine chromosome 4q16-4q21. The different types of the 3β-HSD gene family appear to be expressed in different organs. Various tissues will be analysed to identify and characterise further members of the 3β-HSD gene family.

**B025**

**Full sequence of the SLA region containing all class I genes**

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The major histocompatibility complex (MHC) represents an outstanding genetic system for genome evolution studies in mammals. The recent publication of the complete sequence of the human HLA complex allowed fine comparisons. As a first step of the establishment of the complete sequence of the pig SLA complex, we report here the sequence of 465 kb DNA containing all the class I loci from the SLA H01 haplotype. The 465 kb sequence corresponded to two segments of the class I region, one of 155 kb spanning from the LTB gene to the HCR gene and a second segment of 310 kb centromeric to the RFB30 gene. In contrast to the anchor genes, which are strictly conserved in both species, orthologous relationships do not exist between MHC class I genes. From centromere to telomere, the 155 kb segment contains three potentially active class I-b genes, one truncated MIC-like sequence and a full length MIC-like gene, whereas in HLA the corresponding segment displays the two MIC-B, MIC-A loci and the HLA-B and C class I-a genes interspersed with 17 pseudogenes. The 310 kb segment harbors 7 SLA class I-a loci, including the 3 loci that encoded the serologically defined SLA molecules. The orthologous position in the human contains only the class I-b HLA-E locus plus 4 pseudogenes. Finally, the telomeric HLA-A, F, G cluster has no counterpart in the pig. The organization of LINE, MER and TIGGER-like elements suggests a possible scenario for the duplication of the class I locus in pig. Furthermore, a gene conversion-like event between two class I-b genes is suggested.

**B026**

## Radiation hybrid (RH) mapping of expressed sequence tags from a 28-day-old pig embryo cDNA library

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In the analysis of our swine resource family at the National Institute of Animal Industry (NIAI), a genetic region on swine chromosome (SSC) 6q was identified as responsible for the termination of conceptus development. In order to obtain candidate genes responsible for the termination of embryo development in the region, we identified genes expressed in the early stage of embryo development, and assigned these genes to swine physical and comparative maps. The comparative map would help identity candidate genes by referring to the corresponding chromosomal region of other species. A 28-day-old normal pig embryo was used to produce the cDNA library. In order to assign genes efficiently to the RH map, from a collection of expressed sequence tags (ESTs) selected after comparison with the human ESTs available in Genbank, we PCR amplified the 3' untranslated region (3' UTR). Pig-specific oligonucleotide primers that amplified the expected fragment size were chosen for the assignment in INRA-University of Minnesota porcine Radiation Hybrid panel (IMpRH). Comparative maps between pig chromosome SSC6 and human chromosomes (HSA) 1, 16 and 18 were constructed using physically assigned pig genes and ESTs. We identified extensively conserved chromosomal region synteny with human HSA1 and SSCs 2, 4, 9 and 13. At present, we have mapped 13 genes in the region. RH mapping also revealed new synteny between SSC2 and HSA16, SSC3 and HSA22, and SSC15 and HSA19. These assignments provide additional benchmarks for the comparative map and help define the precise correspondence of genes between pig and human.

**B027**

## A genome scan for umbilical hernia gene in cattle

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The frequency of umbilical hernia at birth (UH) in the Israeli-Holstein cattle is 1%. Progeny of the Canadian Holstein sire, Enhancer, have a much higher than normal incidence of UH. Ten sons of Enhancer had 1% to 21% frequency of UH, in their Israeli offspring. The most likely explanation is that Enhancer is heterozygous for a major gene with partial penetrance for UH. Genomic DNA of gene carrier and non-carrier animals was compared by Representational Difference Analysis (RDA). Two sons of Enhancer, with 18% UH in their offspring were assumed to be gene carriers, while two other sons with <2% UH in their offspring were assumed to be non-carriers. Two reciprocal RDA experiments with the non-carrier group as "driver", and the carrier group as "tester", and vice versa, yielded 12 DNA bands. These fragments were cloned, sequenced and analyzed by PCR. None of the bands showed specificity to a single group. We also initiated a genome scan to detect and map the UH gene by linkage to genetic markers. Blood and hair were sampled from 139 progeny of the sire Elvis, a son of Enhancer assumed to be an UH carrier. Thirty-nine of these progeny had UH, while the remaining 100 were normal. These animals have been genotyped for 33 microsatellites spanning 18 chromosomes. No significant differences were found for paternal allele frequency between groups of UH carriers and non-carriers for any of these markers. Additional markers will be analyzed to complete the genome scan with maximum marker spacing of 50 cM. Power of detection with this experimental design will be about 85%, assuming that 75% of the progeny genotypes are informative with respect to allele origin.

**B028**

## AFLP mapping of a resource family for muscular dystrophy in chicken

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A chicken line of muscular dystrophy (MDS) has been maintained by Nippon Institute of Biological Science as a closed colony. To reveal the causative gene for the genetic defect, we employed a method of positional candidate gene approach making a resource family consists of parents, a hybrid hen and 55 backcross chicks produced by crossing of White Leghorn cock and Fayoumi hen. About 340 AFLP from 71 primer sets, 56 microsatellite and 6 expressed genes were used as DNA markers to make up linkage groups using the MapMaker software. For AFLP analysis genome DNA were digested with ***Eco*** RI and ***Mse*** I and fluorescence primers against ***Eco*** RI adapter were used for analysis on Licor DNA sequencer. Thirty-nine linkage groups were built up using these 400 markers, in which 39 did not converge into these linkage groups. The chromosome 1 (Ch1) consists of 2 linkage groups and Ch2 consists of 4 and Ch3 of 3 and so on. The causes that these linkage group did not converge into 39 groups are due to i) mistyped AFLP markers still remained, and ii) numbers of DNA markers are still not good enough for complete linkage map. As significant linkage and synteny around chicken MDS gene with an human linkage group were found, a candidate gene is now on cloning. We express our sincere thanks to Dr. H. A. Cheng for providing MS markers.

**B029**

## Isolation of potential candidate genes for congenital splay leg in piglets by differential display/reverse transcriptase PCR (DD/RT PCR)

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Congenital splay leg is a hereditary disorder in newborn piglets characterized by muscular weakness of the hind limbs. Affected piglets are unable to stand and walk properly. These piglets often die of starvation or are crushed by the sow. The pathogenesis of the disease is still unknown. We isolated cDNAs differentially displayed in skeletal muscle of healthy and affected piglets in order to identify potential candidate genes for the disease. Total RNA from M. biceps femoris was prepared and subjected to reverse transcription with poly-dT-primers. The second strand synthesis was facilitated by using a kit of optimized arbitrary primers (Biometra, Germany). Resulting cDNAs were separated by PAGE and differentially displayed fragments were recovered for further analysis. A total of 30 ESTs were reamplified, sequenced and partly submitted to GenBank (Acc. No. AJ 133887 - 133891; 271011 - 271019 and 279581 - 279591). Homology searches revealed no similarities to published ESTs isolated from porcine tissues or already identified porcine genes. This indicates specific gene expression in neonatal skeletal muscle. Fourteen of the isolated fragments were consistently stronger displayed in preparations of splay leg muscle than in those of healthy piglets. Two of them, a transcription factor and a protein kinase inhibitor are involved in the regulation of the cell cycle in different human cells. The genes were physically mapped onto SSC 3q21-q27 and SSC 1q23-q27, respectively using a Chinese hamster/mouse x pig somatic cell hybrid panel. Our data support the supposed pathogenetic mechanism of an immaturity of the skeletal muscle at birth.

**B030**

## PCR-RFLPs and linkage analysis of the porcine acid beta-glucosidase (*GBA*) gene

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Acid beta-glucosidase (GBA; glucocerebrosidase; EC 3.2.1.45) is a lysosomal membrane protein that cleaves the O-beta-D-glucosidic linkage of glucosylceramide and aryl-beta-glucosides. Polymorphism of the porcine *GBA* gene was earlier studied by *Taq*I RFLP with the use of a human *GBA* cDNA probe. The gene was localized to a chromosome 4 *ATP1B1-GBA-EAL* linkage group (Marklund *et al.* 1993 Anim. Genet. 24, 333). Because the RFLP technique is labour intensive and costly we have aimed at developing a PCR-RFLP test. Three PCR primers were designed from the human *GBA* sequence (EMBL, access. no. AF023268): Primer F1: 5'-TCA GCC GCT ATG AGA GTA CAC-3' (from exon 3); Primer F2: 5'-CCA GAC CTG GGC CAG ATA CTT-3' (from exon 6); Primer R: 5'-GGC GGA CAT TGT GGT GAG TAC-3' (from exon 7). With the use of primers F1 and R a 2 kb fragment was amplified by PCR; primers F2 and R gave a fragment of 450 bp. Both fragments were cloned (pUC18; *E. coli* DH5) and sequenced to verify that the products corresponded to the *GBA* gene. Polymorphisms in the F1-R fragment were revealed with *Bam*HI and *Sdu*I, and in the F2-R fragment by double digestion with *Hae*III/*Kpn*I. Codominant inheritance of the polymorphisms was confirmed in the Hohenheim Meishan x Pietrain and Wild Boar x Meishan families. Linkage analysis was performed that encompassed genes *GBA*, *TSHB*, *NGFB*, *AMPD1*, *EAL*, *PKLR*, *ATP1B1* and *V-ATPase*.

**B031**

## Mapping quantitative trait loci in Danish Holstein Cattle

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An experiment is currently being performed using a granddaughter design to detect QTLs in Danish Holstein dairy cattle. At present, 19 grandsires with a total of 1397 sons are being genotyped for 186 microsatellites covering the genome, using an ABI 377 DNA sequencer. The phenotypic traits analysed so far are daughter yield deviations or predicted breeding values for mastitis, calving ease, fertility and milk production traits. Preliminary analyses of marker data on chromosome 6, 14 and 23 have been conducted using all markers simultaneously for each chromosome position in a marker-interval based least squares regression analysis. The results indicate the presence of QTLs for mastitis, fertility and production traits.

**B032**

## Use of a genetic map from the SALMAP project to localise the *NRAMP*-ß gene on the rainbow trout (*Oncorhynchus mykiss*) genetic linkage map

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Apart from serving a function as carriers of divalent cations, the *NRAMP* genes appears to be involved in the general resistance to infectious pathogens. Genetic variation in the *NRAMP* genes has been found to be associated with resistance to intracellular parasites in different species. Aquacultural species are exposed to a large variety of pathogens in their environment and the *NRAMP* genes are therefore interesting as potential candidate genes for QTLs for genetic resistance towards infections. The genetic localisation of these genes in the present genetic maps of the species makes it possible to study the effect of the genes in resource families. A PCR primer set spanning an intron of the *NRAMP*-ß gene was designed based on the available cDNA sequence of the gene in rainbow trout and identification of intron-exon boundaries by comparison to homologous human sequences. A single nucleotide polymorphism (SNP) was detected by direct sequencing of the PCR product from 6 individuals. An allele specific PCR was designed to genotype the SNP. The SALMAP reference family lot 25 was genotyped for the SNP and linkage analysis was performed against the approx. 300 primarily anonymous markers in the current map (Sakamoto et al. in press. Genetics) generated within the EU-funded SALMAP project. This made it possible to locate the *NRAMP*-ß gene within the genetic map of rainbow trout. The inclusion of a potential candidate gene for a QTL of considerable interest for the aquaculture industry along with future incorporation of additional genes will greatly improve the value of the genetic map of the rainbow trout, when utilising the information for conducting genome scans for QTL studies and genetic mapping of single gene effects.

**B033**

**Physical mapping of the chicken genome**

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The chicken is intensively being studied for genes affecting polygenetic traits (quantitative trait loci or QTL) which drive the international efforts towards detailed physical and linkage mapping in chicken. To obtain a more detailed chicken-human comparative map, chicken BAC clones were isolated with markers located at 20 cM intervals on the chicken linkage map. Sequence scanning of these BAC clones is used to identify chicken genes with homologs in human. In addition, for five microchromosomes and part of chromosome 8, a physical map is under construction by making a BAC contig by chromosome walking. Additional sequence scanning of BACs from these areas resulted in more than 100 genes with a known human location. In total more than 1000 BAC clones have been isolated from these chromosomes. This work is part of a bigger effort to develop a physical map of the complete chicken genome.

**B034**

**Linkage mapping of ESTs in pigs using single nucleotide polymorphisms (SNPs): II. High-throughput genotyping and mapping**

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Existing swine genetic linkage maps, developed primarily with microsatellite loci, have been used to scan the porcine genome for chromosomal regions that affect economically important traits. However, direct investigation of the nature of the underlying genetic variation requires knowledge of the position of genes within the existing map. To improve the efficiency of the labor intensive comparative mapping process and take full advantage of the vast information generated by the human genome sequencing effort, a program has been implemented to directly integrate variability detected in swine expressed sequence tags (ESTs) into the existing genetic map. Our program objectives are to map porcine ESTs orthologous to genes with known human map positions using SNPs. The SNP discovery phase has identified polymorphic positions within amplicons for automated design of genotyping assays. Observed SNPs in the MARC swine reference population were genotyped via microsequencing and MALDI-TOF mass spectrometry. Map positions of ESTs were determined by linkage analysis. Genotypic data from SNP assays within amplicons developed from EST sequences are being used to develop an integrated, high density, type-I marker map of the porcine genome.

**B035**

## A radiation hybrid map for a defined region of bovine chromosome 6

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In order to resolve the candidate chromosome region for QTL affecting milk production traits on bovine chromosome 6 (BTA6), exact localisation of available markers and candidate genes as well as additional informative markers are required. Therefore, a radiation hybrid (RH) framework/comprehensive map of bovine chromosome 6 (BTA6) was produced using a 12,000 rad whole genome RH panel. Available markers covering a region of 45 cM were selected from genetic markers maps and new targeted markers were isolated from a microdissection library specific for BTA6q21-31. Statistical analysis using the RHMAP package showed that retention frequencies of 31 markers typed range from 9.8% to 28.5% and averaged 15.8%. Three marker pairs showed no obligate chromosome breaks. All loci typed were successfully ordered in one linkage group using the LOD score criterion of 3.0. The order of markers obtained by multipoint analysis principally agreed with the order on the genetic maps. The length of the RH comprehensive map integrating all 31 markers spans 1,490.1 $cR_{12,000}$. Thus, 1 cM corresponds to 33.1 $cR_{12,000}$. The RH map from the defined region of BTA6 presented here provides valuable information regarding the physical distances of markers flanking the QTL and a prerequisite to identify possible candidate genes that map within the QTL region.

**B036**

## Marker assisted selection of composite beef cattle for meat quality traits

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A commercial trial of marker assisted selection for carcass and meat quality attributes in beef cattle is being conducted in Australia. Partners in the project are The North Australian Pastoral Company (NAPCo), a vertically integrated company with breeding, grazing, feedlot and processing resources; Genetic Solutions (GS), a private genetic information technology company responsible for commercial delivery of MAS and the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which developed the initial QTL associations. A total of 1093 progeny of 16 composite sires (Brahman, Shorthorn, Charolis and Belmont Red) from the NAPCo nucleus herds were bred for the project and backgrounded, feedlot-finished and processed through to commercial specifications. A large range of carcass and meat quality traits, including taste panel assessment has been recorded. Multiple markers from QTL regions associated with carcass and meat quality traits have been analysed on DNA samples from each of the progeny and sires. Analysis is currently underway to determine gene marker profiles of sires that will be candidates for selection in the nucleus herds. Through the project GS is developing systems for enabling commercial delivery of MAS services including marker systems and data analysis modules. Results of the marker analyses and selection decisions will be presented.

**B037**

**A radiation hybrid map of bovine X chromosome (BTAX).**

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We present a comprehensive radiation hybrid map of the bovine X chromosome containing 40 markers, including microsatellites, CATS primers and other type I loci. This study was conducted with a 5000rad whole-genome RH cell panel established from gamma-irradiated bovine fibroblast donor cells fused with hamster fibroblast recipient cells. Ninety-four hybrid cell lines were typed for the 40 markers using agarose gels. Retention frequencies of individual markers range from 7,7% for XBM24 to 31,1% for TGLA325. Statistical analysis with the use of RHMAP package showed that all loci formed three linkage groups under lod score criterion 3.0. All the type I markers included in this RH map were tested previously for linkage, but couldn't be added to the map due the lack of polymorphism. RH mapping represents a significant improvement, and seems to be an ideal mapping tool for placing conserved genes on an ordered map, while at the same time integrating them with existing microsatellite linkage maps.

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**B038**

## Sequences flanking microsatellite loci in pigs are a rich source of SNPs

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The aim of this project is to determine the degree of sequence variation in the sequences flanking microsatellite repeat loci by sequencing multiple (length) alleles from each locus. As pigs are outbred, for highly polymorphic loci as exemplified by microsatellites a high proportion of individuals will be heterozygous. The PCR amplification products will be a complex mixture of two alleles plus the associated stutter band artefacts. For each microsatellite locus to be characterised the locus is amplified separately for each of twenty pigs. The pigs represent a range of different breeds. The PCR products are cloned and sequenced. We have characterised sequences flanking the repeat motifs in over fifty porcine microsatellite loci. The sequence analyses have revealed a rich source of length variants, single nucleotide polymorphisms (SNPs), and insertion/deletion events. Although the cloning and sequencing approach will not reveal all the alleles present in the sample of twenty pigs it should identify most of the common alleles. In practice, rare alleles would be of limited value for a DNA chip-based genotyping system. In order to exploit the high throughput potential of DNA chips it is essential that the PCR steps are carried out efficiently. To this end we have optimised the PCR conditions for six multiplexes each containing eight microsatellite markers.

**B039**

**Targeted development of microsatellite markers from bovine BACs.**

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Development of microsatellite markers in targeted genomic regions was motivated by a paucity of markers in regions containing specific QTL and the need for additional markers for fine resolution mapping. Comparative map information was used to identify genes in the regions of interest. Oligonucleotide probes (n=17) were developed from bovine DNA sequence information if available, or from human sequence information. Half of a bovine genomic BAC library was screened with pooled, labeled probes in order to identify BAC clones containing loci in the regions of interest. Positive BACs were subsequently re-screened by PCR using primer pairs which identify the same loci but were independent of the original oligonucleotide probes. Confirmed positive BACs were used as a source of microsatellites for genetic marker development. An average of 7.7 BACs were identified per probe. Microsatellites were subcloned using several different procedures including BAC restriction digest followed by biotinylated-microsatellite oligonucleotide/steptavidin bead capture, or PCR amplification with RAPD and microsatellite oligonucleotides in combination. Southern blot and probe hybridization ($TG_{12}$) of digested BAC DNA indicated the occurrence of from one to four microsatellites per BAC. Cloned microsatellites that were subsequently evaluated for polymorphism varied from five to 24 uninterrupted dinucleotide repeats, and number of alleles per polymorphic locus ranged from two to eleven. This strategy was effective in developing additional markers for QTL and comparative mapping efforts.

**B040**

**Studies in the inheritance of scurs in *Bos taurus* breeds.**

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Polled is a trait of interest to many cattle breeders. However, unless cattle are smooth polled (lacking scurs) their value is diminished. Traditionally the scurred trait has been reported as sex-influenced. Long and Gregory (1978 J. Hered. 69, 395) suggested that homozygous polled masks scurs unless homozygous for scurs. With the development of a linkage test for polled, this theory could be tested. Using the Canadian Beef Cattle Reference Herd, four full-sib families had scurred male offspring although none of the parents had scurs. All scurred males were heterozygous polled. No female offspring developed scurs. The size of scurs within families was relatively consistent although size varied greatly among families. In a total genome scan, the scur gene was mapped to chromosome 19. This confirms that the gene for scurs is different from the polled gene which has been mapped to the centromeric region of chromosome 1.

**B041**

## Genetic distance between individual sires and dam-line's DNA pools assessed by microsatellites, predicts heterosis for egg production

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Several principles and laboratory procedures, which theoretically and experimentally are well evidenced and documented, were the basis for the present experiment: 1) Heterosis is linearly proportional to the dominance deviation of the analyzed trait and quadratically to the genetic distance between the parents. 2) Genetic distance can well be assessed by molecular markers, including microsatellites. 3) Egg production is characterized by large dominance effects. 4) DNA pools made of equal amount of DNA from the individuals comprising the sample pool, represent faithfully the gene frequencies of the analyzed sample. Each of forty sires from a egg-type sire-line A was crossed to about 10 hens of a dam-line B, and 3 hen-housed daughters from each dam were recorded for egg number (total, 1200 daughters). DNA pool of 30 randomly sampled females from line B were used to estimate the gene frequencies of line B, for 24 microsatellite markers that were chosen from the largest eight autosomes and from Z chromosome. These frequencies and the genotype data of each of the 40 sires for these 24 microsatellites were used to estimate the genetic distance between each of the 40 sires of line A and the pool of the females from line B (software *microsat2* – Reynolds estimates). Highly and positive significant ($p < 0.005$) association was found between the average egg number of sire's daughters and the genetic ditances between sires and the female line.

**B042**

## Estimation of quantitative effects associated with marked loci in populations under linkage disequilibrium

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Stochastic simulation was used to study the estimation of quantitative effects at candidate loci by two frequently employed methods of QTL detection using data from populations with or without selection. Methods used were a candidate gene approach, where the quantitative effect was assumed to be due to a mutation that is in complete linkage disequilibrium (LD) with the marker used for genotyping, and a family segregation approach, where the marker is assumed to be linked to the QTL but not necessarily in complete LD. Both methods were evaluated when applied to data from large halfsib families. The families were simulated to be from a population that was not selected and therefore in Hardy-Weinberg and linkage equilibrium, or from a population in which directional selection had been applied, causing LD between the QTL and polygenes. LD between QTL and polygenes was found to bias the estimates of QTL effects downward for both methods of analysis when the marker was in LD with the QTL. This downwards bias is present, even when an unselected sample is genotyped, due to a residual negative correlation between the QTL effect and the polygenic effects. When no LD between marker and QTL was present in the population, estimates from a candidate gene analysis were regressed towards zero but unbiased estimates were obtained from a family segregation analysis, even when the population was selected. Marker-QTL LD was found to cause an upward bias in estimates from the family segregation analysis. This upward bias became smaller with increased marker informativeness and was no longer observed when markers were completely informative.

**B043**

**Linkage mapping of *TYRP2* on cattle chromosome 12.**

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Tyrosinase related protein 2 is known as the slaty locus in mice. Two SNPs were identified (Genbank AF152005), but no restriction enzyme was found to detect either mutation so a purposeful mismatch forward primer was designed. *TYRP2* was mapped 5 cM from *INRA005* (LOD=3.092), which is at 83 cM from the centromere on the USDA map of cattle chromosome 12 (Kappes et al., 1997 Genome Res. 7, 235) and about 11 cm up from *BMS2724* (LOD=2.101), the most telomeric marker at 105 cM. *TYRP2* was previously mapped by in situ hybridization to 12q23 (Hawkins et al., 1996 Mamm. Genome 7, 474) and therefore these linkage data make *TRYP2* another locus between the physical and linkage maps.

**B044**

**BAC clones used for the mapping of five genes to G-banded chromosomes of the dog (CANIS FAMILIARIS)**

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In order to obtain cytogenetic markers for individual dog chromosomes, clones from a canine genomic BAC library (Li et al., 1999 Genomics 58, 9) were hybridized to metaphase spreads from normal dogs. The clones used contained the following genes: *PGR* (*progesterone receptor*), *COMP* (*cartilage oligomeric matrix protein*), *HTR1B* (*5-hydroxytryptamine (sero-tonin) receptor 1B*), *TGFB1* (*transforming growth factor, beta 1*) and *LHX3* (*LIM homeobox protein 3*). FISH conditions had to be optimized for each clone. Localizations were made to five different autosomes, namely CFA 1, 9, 12, 20 and 21, which were identified on the basis of their GTG-banding patterns. These chromo-somes all belong to the group of larger, standardized chromosomes within the G-banded canine karyotype (Switonski et al., 1996 Chrom. Res. 4, 306). Since hybridization with the BAC clones resulted in very clear fluorescent signals, they can serve as markers for the respective chromosomes, both in metaphase spreads and in interphase nuclei. The mapping data presented here add information to the cytogenetic map of the dog and enable refinement of the canine-human comparative map.

**B045**

## Generation of a dense genetic map in a region of a QTL affecting corpora lutea in a Meishan × Yorkshire cross

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Previously a genomic scan revealed a quantitative trait locus (QTL) on porcine chromosome 8 (SSC8) as significantly affecting the number of corpora lutea/ovulation rate in swine. Statistical evidence for the putative QTL was found in the chromosomal region flanked by the microsatellites (MS) SW205, SW444, SW206 and SW29. A YAC library was screened for these MS by PCR using the corresponding primers. From five positive YAC clones 10 MS were isolated and mapped to SSC8 using the INRA-University of Minnesota porcine Radiation Hybrid (IMpRH) panel. The map position of the QTL has been refined by addition of these 10 markers. The QTL evaluation included pedigrees of $F_2$-intercross Meishan × Yorkshire design, with phenotypic data of 108 $F_2$ female offspring and genotypic data for 30 MS markers on SSC8. The analysis was performed using least square regression method. The calculated QTL effect for corpora lutea showed a maximum at position 84 cM between three MS derived from a YAC containing SW205 and SW1843 spanning an interval of 7.2 cM. The pointwise (nominal) *P*-value was $1.2 \times 10^{-5}$. The estimated QTL effect explained about 50 % of the root mean square error.

**B046**

## A depository for standardized digital DNA signatures in animals

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Single nucleotide polymorphisms (SNPs) are based on mutational events leading to nucleotide substitutions or the deletion / insertion of individual nucleotides. These markers are of a digital nature, since each polymorphic site can be queried for the presence or absence of a specific base. The digital nature of SNPs and the unique sequence context of each SNP are the basis for a straightforward standardization of genotyping. The principle is as follows: An SNP position is defined for a species by the sequence of about 100 nucleotides on either side of the polymorphism. The actual SNP site can be queried with any suitable method for the presence or absence of two alternative (or of all four possible) nucleotides. Forty SNPs (frequency of minor allele $\geq$ 0.3) will yield individual specific signatures with a probability of identity = $10^{-15}$. The task is now to compile a set of SNPs that can be used for individual identification in all populations of a given species. This can only be accomplished if a large collection of SNPs is available from which an ideal set of SNPs can be chosen. We have taken the initiative to set up a database as depository for SNP information, initially in cattle and swine. This database includes information on the actual SNPs, flanking sequence and population-specific allele frequencies. Registered submitters provide the information via email. The data base can be queried under www.snpzoo.com by the general public and is open to hold SNP-information for other animal species as well. We envisage that - after a certain period of collection - the selection of the actual standard sets will be accomplished by an internet poll.

**B047**

**Body weight and fat content in the Mouse are determined by a complex web of interacting genes**

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Genes influencing body composition as well as serum concentrations of leptin, insulin, and IGF-I in non-fasting animals were mapped in an intercross of the extreme high growth mouse line DU6i and the inbred line DBA/2. The animals of line DU6i show twice as high body weight and three times as high fat content compared to unselected control mice. The extreme phenotype is accompanied by hyperleptinemia, hyperinsulinemia, and significantly elevated IGF-I serum concentration. For genome wide linkage analysis the data were analyzed by multiple regression. Quantitative trait loci (QTL) with major gene effects (F>7.07) for body weight, obesity and muscle weight were found on eight chromosomes, the net effect of all detected QTLs explained 35 %, 34 %, and 28 % of the phenotypic variance in the $F_2$ population, respectively. Loci influencing leptin, insulin, and IGF-I serum concentrations were identified on three chromosomes, together, these loci accounted for 25 %, 9 %, and 21 % of the phenotypic $F_2$ variance, respectively. For the examination of interaction between QTLs we used the general linear model of variance analysis. The model included the effects of sex, subfamily, parity, pupsize, the single effects of the QTL identified for the specific trait at two selected loci, and the interaction between these loci. The interaction analyses provided evidence for epistasis and pleiotropy. The net effects of identified QTLs, epistasis, and pleiotropy accounted for about two third of the phenotypic variance of body weight, fat accumulation, and serum proteins. These results emphasize that the estimation and consideration of QTL interaction effects may significantly contribute to more efficient utilization of QTL information in marker assisted selection programs.

**B048**

**Isolation of Porcine acetyl- coenzyme A carboxylase α (ACACA) exon5, and assignment to Pig chromosome 12 by in situ hybridization and confirmation by genetic mapping.**

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Acetyl-coenzyme A carboxylase α catalyzes tae carboxylation of acetyl-CoA to form malonyl-Coa, which is the rate limitant and committed step in the novo fatty acid biosynthesis. Previously, *ACACA* gene has been cloned and sequenced in several species such as rat, chicken, sheep and human. Whereas, the pig ACACA remains unassigned the human *ACACA* has been located to human (HSA) chromosome 17 (17q12).

We have isolated porcine exon 5 *ACACA*, using degenerated primers. Comparing the nucleotides and the deduced amino acid sequences of the pig, human, rat, ovine and chicken exon 5 *ACACA* revealed considerable similarity between species. Using the exon 5 information, we have screened a porcine BAC library. Using the positive BAC for exon 5 *ACACA*, we also carried out fluorescent in situ hybridization (FISH), assigning the gene to chromosome 12. This BAC clone contained a CA microsatellite. The polymorphism of the microsatellite was used to genotype the PigMap reference pedigrees. A kinship between the results of FISH and linkage analyses in this study corresponds with the comparative map information (human *ACACA* maps to HSA17 which has extensive homology with SSC12).

**B049**

## Effect of *H-FABP* gene variants on intramuscular fat in commercial pigs in Australia

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A recent study (Gerbens *et al*. 1999 J. Anim. Sci. 77, 846) has shown variants of the *heart fatty acid binding protein* (*H-FABP*) gene had a significant affect on intramuscular fat content (IMF) and backfat thickness (BFT) in Duroc pigs. Contrasts between homozygous *H-FABP* RFLP genotype classes for IMF and BFT were .4% and .6mm, respectively. A trial of the *H-FABP* RFLP test was undertaken on commercial pigs in Australia. As part of a recent QTL mapping experiment, progeny from Large White and Landrace sires had been scored for IMF and BFT at P2 under test station conditions at Bunge Meat Industries. A subset of these progeny were genotyped for the *HinfI*, *HaeIII* and *MspI* RFLP within the *H-FABP* locus. Primers and PCR conditions were as described in Gerbens *et al.* (1997 Mamm. Genome. 8, 328). The *MspI* RFLP was shown to be monomorphic in the resource population. A high degree of linkage disequilibrium was found between the two restriction sites *HaeIII* and *HinfI*. For the 169 progeny genotyped for both sites, 74% were concordant. A statistical analysis was performed using the ASREML program. A mixed linear model was used where membership of the different *H-FABP* RFLP classes was included as an independent variable. An animal's genetic effect and pedigree information from three preceding generations was also included. The heritabilities of IMF and BFT were taken from a recent variance component estimation experiment performed on Bunge populations. The results showed no significant effect of the *H-FABP* genotype from either restriction site on either IMF or on BFT at P2.

**B050**

## QTL mapping in an Iberian x Landrace $F_2$ pig intercross: 2. Composition and metabolic ratios of fatty acids.

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Fatty acid composition of subcutaneous backfat and its metabolic ratios (average chain length of fatty acids -*ACL*- , double bond index -*DBI* - and unsaturated index -*UI*-) were measured in an experimental $F_2$ cross between Iberian and Landrace breeds. The available pedigree consists of 3 pure Iberian boars, 31 pure Landrace sows and 77 F1 individuals (6 boars and 71 sows). Here we report results corresponding to 250 F2 animals genotyped for 100 microsatellite markers covering the 18 porcine autosomes. Data adjusted by carcass weight were analysed for QTL detection using a regression approach. QTLs were found (position in cM, F value; additive and dominance effects ± s.e.) for percentage of linoleic acid (C18:2) in chr. 4 (79, 17.4; 0.77 ± 0.13; -0.12 ± 0.19); *DBI* in chr. 4 (80, 8.65; -1.21 ± 0.29; -0.44 ± 0.44) and *ACL* in chrs. 6 (45, 8.7; -0.95 ± 0.35; -1.37 ± 0.45) and 8 (93, 11.6; -1.64 ± 0.35; 0.46 ± 0.50). Results for *DBI* in chr. 4 are due primarily to the strong effect on linoleic content of the QTL detected in the same map position. No effects for fatty acid composition were detected in chrs. 6 and 8 where QTLs found for *ACL* could be related to differences in chain elongation reactions of fatty acids.

**B051**

**Genetic analysis of seven Italian horse breeds using microsatellites.**

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The genetic variability and relationship among seven Italian horse breeds (114 Bardigiano horse, 64 Giara Horse, 223 Haflinger, 114 Italian Trotter, 147 Maremmano Horse, 50 Murgese Horse and 76 Thoroughbred) were studied using eleven microsatellites (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS2, HMS3, HMS6, HMS7, ASB2). In addition, gene frequencies of Spanish Pure Breed horse have been included.

Allelic frequencies, genetic equilibrium according to Hardy Weinberg, inbreeding coefficient (Fis) and average observed heterozygosity for each locus were estimated by GENEPOP statistic package.

The allele frequencies were used to estimate the genetic distances according to Nei, Cavalli-Sforza and Reynolds by PHYLIP statistic package. The phylogenetic trees were constructed using Neighbour-Joining algorithm.

Microsatellites were polymorphic in all breeds. All populations, but Haflinger and Thoroughbred were in genetic equilibrium according to Hardy Weinberg. The inbreeding coefficient ($F_{is}$) was very close to zero in all breeds.

Two main clusters have been identified. The first including Bardigiano Horse and Haflinger seems to confirm the common origin from the barbaric horses. The second including Thoroughbred and Italian Trotter, was in accordance with the historical origin of the breeds.

**B052**

**Isolation and mapping of expressed sequence tags from porcine skeletal muscle: a contribution to the genomic transcript map of this tissue.**

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Skeletal muscle is one of the target tissues for the isolation of genes with important effects on meat quality and production traits in pig. For this reason, we developed a cDNA library from *biceps femoris* muscle of an adult pig and generated 675 expressed sequence tags (ESTs) derived from single pass sequencing of both ends of 484 cDNA clones. Database search showed similarities to 192 different genes already identified in other species and to uncharacterised ESTs and mitochondrial genes. Considering data available in other species, 26.6% out of the 192 genes were classified as muscle prevalent or muscle specific transcripts, while 73.4% as housekeeping genes. The genes with prevalent or specific muscle expression have been attributed, in relation to their biological role, to the following categories: cell structure and motility (59.6%), metabolism (21.6%), cell signalling and communication (13.7%) and gene expression (7.8%). So far, 81 out of 192 identified genes have been physically mapped and 52% of the localised genes were assigned to 6 chromosomes (i.e. chromosomes 1, 2, 5, 7, 9 and X). These mapping results suggest that the distribution of skeletal muscle genes on porcine chromosomes could be unequal as already reported for some human chromosomes. The ESTs isolated from our library represent a first contribution to establish a gene expression profile of the porcine skeletal muscle tissue. The mapping of 81 of these genes enriches the transcript map of the porcine genome that is an important tool for the identification of candidate genes in QTL studies.

**B053**

## An SNP based digital DNA signature for individual identification in cattle

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SNPs (single nucleotide polymorphisms) are the most frequent type of genetic polymorphisms. Potential uses of SNPs as genetic markers include DNA fingerprinting, genetic mapping, phylogenetic analysis and biodiversity studies. In farm animals they can be used to trace the origin of animal products. The digital nature of these diallelic markers makes them ideally suited for DNA chip applications and other high-throughput genotyping methods. We have developed a system where the genotype of each animal at all analyzed SNP loci is represented by a unique individual specific Digital DNA Signature (DDS). Each polymorphic site can be queried for the presence or absence of a specific base, resulting in “10” (homozygous for one allele), “01” (homozygous for the other allele) or “11” (heterozygous) genotypes. Since an animal's DDS does not depend on any specific genotyping method it can take full advantage of rapid technological advances in this field.

In this study, comparative direct sequencing of genomic DNA from a test panel of major cattle breeds yielded a total of 113 SNPs. Their chromosomal locations were determined by FISH. For SNP genotyping we established a multiplex oligo ligation assay with fluorescent labels in combination with an ABI 377 genetic analyzer. So far, 18 of the most informative SNP loci (those with allele frequencies of the rarer allele of 30% or more in the economically important breeds) have been selected to be part of the DDS. Our goal is to define a set of about 40 SNP loci (probability of identity = $1.3 \times 10^{-15}$) to become an international standard for animal identification.

**B054**

## Isolation and chromosomal localization of the fatty acid synthase (FAS) gene in cattle

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The synthesis of long chain fatty acids from acetyl-CoA, malonil-CoA and NADPH is a complex process catalized by Fatty Acid Synthase (FAS). This process requires seven enzymes activities that, in animals, are integrated into a single polypeptide chain. Previous studies have shown some evidences of the association between FAS gene polymorphisms and fatness variability in turkeys. We used rat fatty acid synthase gene specific primers in order to partially amplify the bovine gene. Putative exons 31, 32, 33 and introns 31 and 32 were established by comparison with the FAS rat sequence. We assigned FAS to BTA19 using the INRA hamster-bovine somatic cell hybrid panel. Primers amplifying a 180 bp product were used to screen a bovine BAC library and two BAC clones were identified. One of these was mapped to BTA19q22 by fluorescence in situ hybridization. Two microsatellites were isolated from one BAC clone after subcloning and hybridization with a poly (AC) probe. One of these is polymorphic and was used in order to genetically map the FAS gene in the international bovine reference panel.

**B055**

**Physical assignment of *adipocyte determination and differentiation factor*-1 (*ADD1*) and *pyruvate dehydrogenase E1-alpha* (*PDHA1*) in the pig.**

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Improvement of meat quality has become one of the top priorities of the pork industry in recent years. Many studies have found correlations between traits associated with fat metabolism and differences in meat quality traits. *Adipocyte determination and differentiation factor-1* (*ADD1*) and *pyruvate dehydrogenase E1-alpha* (*PDHA1*) were mapped in the pig for study as potential candidate genes for pork quality. *ADD1* is a transcription factor believed to play a role in encoding enzymes of lipid biosynthesis and may also be involved in the control of plasma cholesterol levels. *PDHA1* has been found to catalyze the conversion of pyruvate into acetyl-CoA. A deficiency of the enzyme pyruvate dehydrogenase is one of the most commonly defined genetic defects of mitochondrial energy metabolism resulting in lactic acidosis. Primers were designed using porcine cDNA sequence. Results of a pig-rodent somatic cell hybrid panel indicated that *ADD1* was located on pig chromosome 12 (SSC 12) with 100% probability and the regional assignment was SSC12q11-q15. *PDHA1* was determined to be on SSCXp22-p23 with 100% probability. These results are similar to the mapping locations predicted from human-pig comparative mapping studies. Currently, linkage analyses to confirm these results are being conducted for both genes, as well as association studies to characterize their effects on meat quality in the pig.

**B056**

**Identification and mapping of polymorphic loci between two pig populations using representational difference analysis**

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Representational difference analysis (RDA) was performed using genomic DNA from pigs differing in genotype at the *skeletal muscle ryanodine receptor 1* (*RYR1*) locus. DNA samples were obtained from a Landrace pig population selected for increased loin eye area. RDA difference products obtained using the restriction endonuclease *BamHI* were cloned into the plasmid vector pGEM-3Z and a total of 70 clones were isolated. Southern blot analysis of 23 clones indicated inserts contained repetitive DNA elements. Seven inserts were sequenced and five exhibited high homology to intronic or repeat regions of well-characterized pig sequences in GenBank. The remaining two inserts were homologous to pig-specific centromeric satellite DNAs. PCR primers were designed and used to cytogenetically map insert MSURDA7 to the q arm of SSC7 using a pig-rodent somatic cell hybrid panel (Yerle et al., 1996. Cytogenet. Cell Genet. 73:194-202). A single-stranded conformational polymorphism (SSCP) was detected in MSURDA7 and used to genotype the PiGMaP reference families. MSURDA7 showed significant linkage to 28 markers on SSC7q with $\theta = 0.00$ for markers S0334, S0420, SW1614 and MYH7 (LOD scores 28.60, 27.09, 23.18 and 10.54, respectively). The development of RDA derived markers will contribute to the further development of high-resolution genome maps. These markers could also potentially lead to the identification of genes contributing to the variation in traits observed between pigs of differing *RYR1* genotype.

**B057**

## Mapping feed intake genes in mice

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The cost of feed is a major proportion of the total cost in any animal production system. Selecting for net feed intake has the potential to improve the gross efficiency of the production system because selection is based on feed intake independent of growth and body weight. However the current cost of measuring feed intake in cattle is around $500 per animal which is prohibitively expensive for phenotypic selection. A cheap alternative with the potential to significantly reduce generation interval would be to use a DNA test for markers of genes affecting intake. The aims of this study were to divergently select mice from a base population on high or low net feed intake and to map the gene (s) associated with feed intake. Seven generations of selection for high or low post-weaning net feed intake were carried out on a randomly mated base population of mice. The high line ate 21 % more per day post-weaning. Net feed intake was genetically correlated with feed intake but genetically independent of growth traits. For mapping the intake genes, 15 mice from the low intake line were crossed with 15 from the high intake line to produce an $F_1$ generation. Four $F_1$ males were randomly selected and mated to 32 $F_1$ females to produce 400 $F_2$ progeny. Families from the two $F_1$ males with greatest standard deviation in net feed intake were selected to be genotyped. Approximately 80 microsatellite markers will be run over 120 $F_2$ progeny and the 10 $F_1$ parents at a distance of 20 cM. This information will be utilised in a cross-species comparative genome mapping, physiological studies and development of tests for genes or quantitative trait loci affecting feed efficiency in commercial livestock.

**B058**

## QTL analysis for milk production and health traits on candidate chromosomes 4, 6, 14, 20 and 23 using a dual purpose cattle German Simmental and German Brown

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Quantitative trait loci (QTL) mapping was performed in two dual-purpose cattle breeds using the granddaughter design (GDD). Thirteen half-sib families of German Simmental and one of German Brown cattle were used (42 sons ± 17 (mean ±SD) per family). The presented study focuses on five chromosomes (4, 6, 14, 20 and 23) on which QTL were previously described in Holstein-Friesian and/or German Brown cattle. The families were genotyped for 33 microsatellite markers. For five milk production traits (milk, protein and fat yield, protein and fat percentage) and one health trait (somatic cell score), an across-family multimarker regression analysis was made. Significance thresholds were obtained by permuting phenotypes within families. A QTL affecting protein percentage was observed in the region of marker RM188 on chromosome 4 ($P<0.05$). On chromosome 6 evidence for a QTL affecting protein ($P<0.01$) and fat percentage ($P<0.05$) were observed. These two QTL segregate in different Simmental families and share different haplotypes. On chromosome 20 we observe indications for three QTL, one affecting fat ($P<0.05$) and protein yield ($P<0.06$), one for protein percentage ($P<0.05$) and one affecting somatic cell score ($P<0.05$). Regression analysis and identification of shared haplotypes in heterozygous families clearly support this observations. Interestingly, in thirteen German Simmental and one German Brown family, we didn't find any evidence for a QTL affecting milk production traits on chromosome 14.

**B059**

**Physical mapping of a putative recombination hotspot on BTA23**

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The *D23S22-D23S23* interval on BTA23 exhibits significant variation in meiotic recombination rate (θ) between bulls. In the present study, we have constructed a partial YAC and BAC contig to further characterize this region and to identify potential DNA sequences or chromosomal segments responsible for variation in θ. Primary screening of a bovine YAC library yielded 17 YAC clones ranging in size from 180-2200 Kb. Inverse or vectorette PCR was performed for YAC-end rescue resulting in 23 new sequence tagged sites (STSs) from YAC ends. Chimerism of YAC ends was tested using a somatic cell hybrid panel and was estimated at 65% for this region. BAC library screening resulted in 20 additional clones ranging in size from 30-200 Kb. Direct sequencing of BAC ends produced 27 new STSs. Contig assembly was performed by STS content mapping. Two contig islands were produced within the *D23S22-D23S23* interval. Size estimates of the *D23S23-D23S36* and *D23S7-D23S22* subintervals are 250 Kb and 5 Mb, respectively. A large distortion in ratio of physical distance to genetic distance in the *D23S23-D23S36* interval was noted (100 Kb/cM) indicating the presence of a recombination hotspot in this region. A completed contig will provide a framework for determining the underlying molecular basis for hotspot activity and θ variation on BTA23.

**B060**

## Genetic Mapping of *agouti* in a Mangalitza x Piétrain cross

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The agouti protein is an antagonist of the α-melanocyte-stimulating hormone with respect to melanocortin-receptor 1-binding. It causes a switch from eumelanin to phaeomelanin production in the hair bulb. The *agouti* locus is central in the expression of coat colour traits in animals. In mice, ectopic expression of the agouti protein due to specific alleles at *agouti* is responsible for obesity and diabetes. *Agouti* can be considered as a candidate locus for feed intake and fat deposition traits in farm animals. In swine, *agouti* was physically mapped to chromosome 17q21-23 by fluorescence in situ hybridization (FISH) of a porcine BAC clone containing the *agouti* gene. For genetic mapping, a microsatellite marker *AgCA1*, was derived from the same BAC clone. Four different alleles were detected in a Mangalitza x Piétrain cross consisting of two Mangalitza boars and 22 Piétrain sows in the parental generation. The Mangalitza animals were postulated to be homozygous at the *agouti* locus for a recessive allele responsible for the light belly in the otherwise black-coloured animals. Sixty Mangalitza coloured F2 animals were genotyped along with 43 F1 animals and the Mangalitza and Piétrain grandparents at *AgCA1*. In 31 animals, both marker alleles could be traced back to the Mangalitza grandparents. In 29 animals, at least one of the two alleles was unequivocally of Mangalitza origin. However, there was no evidence for one of the alleles originating from Piétrain. These findings confirm the hypothesis that the colour pattern observed in the “swallow-bellied” Mangalitza is caused by a recessive allele at the agouti locus, most likely corresponding to the $a^t$ allele.

**B061**

**The expected Information Content of Markers in QTL Mapping in Outbred Crosses**

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The information content (e.g. Marklund *et al*., 1996 Anim. Genet. 27, 255; Knott *et al*., 1998 Genetics 149, 1069) gives an indication as to how informative the multiple markers are at any location. This information content helps in choosing the density of the marker map. We consider an outbred cross where two lines will be crossed yielding an $F_1$ generation and an $F_2$ generation. In order to get a high probability of detecting QTL a small number of very great families will be used (Alfonso, Haley, 1998 Animal Science 66, 1).

After having genotyped the $P_0$ and $F_1$ generation with respect to a primary marker set and before beginning genotyping the large number of $F_2$ animals, it should be decided, wether more markers are necessary. At this moment, the marker genotypes of the $F_2$ generations are unknown. Therefore only the expected content of marker information can be calculated. The expectation value has to be calculated over all possible marker genotypes of the $F_2$ generation, given a mating design. Since in this mating structure there are large groups of half sibs the animals are highly dependent. Therefore the calculation of the expected information content is very time consuming. To minimize this time it would be sensible to use only the one ore two most probable haplotypes of the Fathers.

The information content consists of components of the information about the additive genetic effect and the dominance effect of a QTL assumed at a special location. In some situations also the information content with respect to the imprinting effect can be given.

**B062**

**An enriched version of the Dog RH-map.**

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The creation of a dog radiation hybrid panel (Vignaux et al. 1999), the characterization and typing of microsatellite and gene markers using this RH panel led us to the first canine RH map ever constructed (Priat et al. 1998), comprising a total of 400 markers. Then through a collaborative effort, an integrated map has been constructed by placing a total of 724 markers, some of them being localized by genetic linkage and RH mapping (Mellersh et al. 2000). The assignment of most of the RH groups to the 38 canine autosomes and heterochromosomes has then been achieved using FISH techniques (in prep.). Recently, our main effort has been focused on increasing the map density by the identification and positioning of an ever-increasing number of markers. This led us to produce an enriched version of the RH map (Jouquand et al. in prep.) comprising as much as 1500 markers including 1200 polymorphic microsatellites, 600 of them being typed through a 5' nuclease assay based on fluorescence transfer energy (Jouquand et al. 2000) and 300 canine gene markers. Pairwise-analysis of the data using Lod scores greater than 8 resulted in 64 radiation hybrid groups. A framework map with statistical support >1000 :1 and comprising nearby 750 markers has been drawn, the remaining markers being positioned relative to those in a comprehensive way.

Positioning of a greater number of genes on this map allows us to explore the syntenic relationship between dog and other species such as human, mouse and rat. This results on pinpoint regions where gene sequences are conserved in all these species or conversely enables us to refine the limits of the synteny disruptions highlighted in the first generation RH map. Finally, the selection of a set of highly polymorphic and well-spaced markers is now possible and would make genome-scanning studies required for gene hunting feasible.

**B063**

**High Resolution Mapping of 148 Type I Markers in Pigs.**

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In the frame of the European program GENETPIG, 4 groups (Denmark, France, Germany, Italy) are localizing about 700 markers on the pig genome. We present here the localization of 148 new type I markers from different origins (58 anchorage markers, 30 human ESTs and 60 pig ovarian ESTs), using either Somatic Cell Hybrid Panel (SCHP) or the whole genome INRA-University of Minnesota porcine Radiation Hybrid panel (IMpRH). A total of 133 markers were localized on SCHP and 141 markers were significantly linked to a marker of the first-generation radiation hybrid map. Seventy-four of these markers correspond to an identified human gene, which gives information on comparative mapping between pigs and humans. These data show that the localizations on SCHP give valuable information on cytogenetic map, in agreement with IMpRH mapping, except for chromosomes 2 and 5q. The results of comparative mapping are in agreement with previous data and sometimes give new correspondences. For example, our results precise the limit of the correspondence between Sscr5, Sscr14 and Hsap22. They confirm the correspondence of the non painted Sscr 3p16-p17 zone with Hsap 7. IMpRH tool (available at http://imprh.toulouse.inra.fr) allows us to map the new markers relatively to reference markers, and to draw the most likely resulting map. Thus we order markers on Sscr8 and construct an improved framework map for Sscr14. These results significantly improve pig transcriptional map, since they increase by 30% the number of type I markers on this map. They also contribute to the development of the irradiated map, which now counts 186 type I markers.

**B064**

**Intrabreed genetic differentiation of cattle in answer to different ecological stress factors**

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The analysis of intrabreed differentiation on transferrin, ceruloplasmin, purine nucleoside phosphorylase, haemoglobin, amylase-1, receptor to vitamin D (GC), post-transferrin 2, intron of a gene leptin and 4-th exon of a kappa-casein gene in connection with influence of biotic and abiotic factors of ecological stress was carried out. The following groups of animals were included in the investigation: Red steppe, infected ("sensitive" group) and not infected ("resistant" group) by bovine leukose virus in the same farm; Holstain, born in an experimental economy (New -Shepelichi) in conditions of 10 km of Chernobyl's zone and had three generations of selection to ionizing pollution ("resistant" group), and also in a rather "clean" zone ("sensitive" group); the Grey Ukrainian breed in native zone of Ukraine ("sensitive" group) and in region of its introduction in 1982 y in Siberia ("resistant" group); three Pinzgauer groups, reproducing in mountain (optimum for the given breed - stress "sensitive"group), high-mountainous and land regions ("resistant" groups). "Sensitive" and "resistant" intrabreed groups differed on distribution of allele frequencies and interloci associations on some structural genes. The similarity of such differences between different breeds and effects of the different factors of ecological stress was revealed. It was assumed, that increase of the frequencies, in particular, allele A on GC, the changes of the interloci associations between syntenic and not syntenic loci were the universal population-genetic answer in generations of cattle to action of the different factors of ecological stress.

**B065**

## Chromosome assignment of differentially expressed bovine sequences by RH mapping

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One of the major efforts in the field of positional candidate cloning is to identify coding sequences or transcript units in chromosome regions associated with performance traits. Identification of expressed sequence tags (ESTs) for QTL regions can provide access to potential positional candidate genes and will allow definition of complex phenotypic traits by much more complex epigenetic networks of interacting genes, proteins, and environmental signals.

The subject of the present study was physical mapping of DNA sequences that are identified as differentially expressed in liver and intestinal tissues of lactating cows differing in metabolic type (milk type, meat/milk type, meat type) using the mRNA differential display method. Primers derived from these sequences were used for the physical mapping of the EST using a Bovine Somatic Hybrid Panel and a Bovine 5000 rad Whole Genome Radiation Hybrid Panel. With this mapping approach 70 differentially expressed sequences were ordered into the known bovine syntenic groups and 60 of these sequences were integrated into the recently published first generation radiation hybrid map of the cattle genome.

The presented mapping data, based on a combined approach using methods of mRNA differential display and physical mapping, allowed us to identify potential positional candidate loci in regions of mapped QTL according to chromosome assignment of different expressed sequences and to contribute to completion of the physical map in bovine.

**B066**

**Development and mapping of Type-I (gene-related) markers in the bovine genome by large-scale Expressed Sequence Tag (EST) sequencing and single nucleotide polymorphism (SNP) detection**

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Genetic linkage maps of the bovine genome, consisting mainly of Type-II (microsatellite) markers, have been applied to identify chromosomal regions carrying loci that affect production traits (quantitative trait loci; QTL). In order to populate the linkage map with gene-related markers, providing a source of positional candidate genes and tying the bovine map to the more highly developed human map, we have initiated a program to develop single nucleotide poly-morphisms (SNPs) from expressed gene loci. The EST sequencing phase of this program utilizes four normalized libraries constructed with RNA pooled from multiple tissues having importance to production traits. As of the abstract deadline, over 32,000 bovine EST sequences had been deposited in GenBank, with approximately 2,500 new sequences collected each week. Sequences are compared with GenBank using BLASTN, to identify clones with orthologs on the human map. In the SNP discovery phase, BLAST output is used to design primers predicted to flank introns in target loci. Amplicons of primers that successfully amplify bovine genomic DNA (approximately 60% of primers designed) are sequenced to identify polymorphisms in the MARC mapping population. The first 34 amplicons sequenced have identified 21 with SNPs. Mapping ESTs is the final phase and uses MALDI-TOF mass spectrometry-based assays to genotype the reference population. Our goal is to generate sequence from 40-50,000 independent bovine genes, and map 500-1,000 ESTs on the bovine genome. This program will significantly improve the comparative map of cattle with other mammals and provide a resource for SNP-based high throughput genotyping.

**B067**

**Cloning, mapping and mutation analysis of bovine candidate genes for growth and carcass traits.**

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Genes which are part of biomolecular mechanisms that influences growth or carcass traits were selected as candidate genes as were those who were identified as the causative genes of abnormal carcass or growth fenotypes. Neurotransmitters, hormones and their receptors are key players in controlling feeding behaviour. For this reason melanocortin 4 receptor, dopamine 1A receptor, carboxypeptidase E and emerine can be regarded as candidate genes for growth and carcass traits. Primers were designed based upon bovine sequences when available or upon conserved regions between different species. The expected PCR fragment was cloned and sequenced for confirmation. SSCP and sequence analysis were used to scan for possible polymorphisms/mutations in 3 different types of cattle (dairy, dual purpose and beef cattle). Once identified they were further characterized by PCR-RFLP or OLA (oligo ligation assay). The amplified PCR fragment was also used for screening a bovine brain cDNA library. The nature of positive cDNA clones was confirmed by sequencing and by comparison to known sequences from other species. The chromosomal locations of the candidate genes was determined using an irradiation hybrid panel

**B068**

## An initial EST Radiation Hybrid Map of Porcine Chromosome 13

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Radiation hybrid (RH) mapping provides a powerful and efficient method for fine-structure mapping and the generation of long-range genomic maps of chromosomes of different species, using both polymorphic and non-polymorphic markers. Our goal is to order several thousand porcine expressed sequence tags (ESTs) on the IMpRH 7000 rad panel (Hawken et al., 1999: Mamm Genome. 10:824-30) to build parallel (comparative) RH maps, of the human, rodent(s) and domestic livestock genomes.

ESTs were constructed from ten oligo(dT)-primed individually tagged, directionally cloned and normalized cDNA libraries. Individual tissues included peripheral blood cells, spleen, thymus, lymph node, and bone marrow from immunologically naive and challenged pigs as part of an implant-associated orthopedic infection model. All markers were mapped on the 7000 rad IMpRH panel using RHMAP Version 3.0 (Boehnke et al., 1996) and a cut off value of LOD 4.86. Currently, we have ordered 32 ESTs on porcine chromosome 13 (SSC13). The ESTs were randomly distributed over the chromosome. Synteny with regions of Human Chromosomes 3 (HSA3) and 21 (HSA21) were confirmed. One EST mapped to HSA11q13 (SSC2, lod 19.8) suggesting a translocation of this gene in the pig from its position on HSA3 near the CCK gene.

**B069**

**Genetic mapping in salmonids.**

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A collaboration with the title: Generation of highly informative DNA markers and genetic marker maps of salmonid fishes (SALMAP), funded by the European Commissions FAIR program (Agriculture and Fisheries), was established aiming at constructing genetic maps of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). The project was running from 1997-1999 and involved five European countries and Canada.

Microsatellite PCR-assays were developed in Atlantic salmon and rainbow trout. Approximately 2,300 clones containing microsatellites have been identified from Atlantic salmon and approximately 600 clones from rainbow trout. At the end of the project approx. 950 clones from Atlantic salmon and 400 clones from rainbow trout has been sequenced and used to construct genetic markers. The markers were tested in all three species to identify those that show cross-species amplifications and subsequently used for creating comparative maps.

Linkage maps have been constructed for all three species using standard reference families. For Atlantic salmon and rainbow trout maps containing approximately 300 markers each has been constructed. The maps consist mainly of microsatellite markers but other markers such as minisatellites and genes are included. For brown trout 200 markers already mapped in Atlantic salmon or rainbow trout was used for constructing a framework map. In order to map the loci relatively to the centromeres meiotic gynogens have been analysed in all three species.

**B070**

## Genetic polymorphism and mapping of genes involved in adipocyte development in the pig

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Beta- 3-adrenergic receptor (b3AR), cyclic-adenosine monophosphate protein kinase regulatory subunit II beta (CAMPPKRIIB) and CAAT- enhancer binding protein alpha (C/EBPa) have been shown to be expressed and influence the development and signaling in adipocyte tissues. For this reason they can be regarded as candidate genes for meat and carcass traits in pig. Primers were designed based on known porcine sequences or conserved sequences between human and mice.The expected PCR fragments were amplified, cloned and sequenced to confirm their nature. Subsequently the primers were used to screen a porcine BAC library. The positive BAC clones were used to determine chromosomal localizations by FISH mapping (fluorescent *in situ* hybridization) and to identify polymorphic microsatellites. Irradiation hybrid mapping was used as an alternative and/or as confirmation of the FISH mapping. SSCP analysis and sequence analysis were used to identify possible polymorphisms/mutations in the species Meishan, Piètrain, Large White and Belgian Landrace. Once identified they were further characterized by PCR-RFLP (restriction fragment length polymorphism) analysis and OLA (oligo ligation assay).

**B071**

**Identifying *Trypanosoma congolense* resistance genes by positional cloning.**

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*Trypanosoma congolense*, the agent of sleeping sickness in African cattle, causes anaemia, loss of production and death. The wide distribution of these parasites in Africa limits cattle raising to resistant breeds. Different mouse strains also show a range of susceptibilities to *T. congolense* infection. We have used the mouse model of *T. congolense* infection to identify five QTL in mice that are associated with the extreme resistant and susceptible phenotypes. In the F6 intercross the *Tir1* QTL is a 1.2 cM region immediately proximal to the mouse MHC on chromosome 17. A contig with a minimum tiling path of 16 PAC clones, containing approximately 40 STS, across the *Tir1* QTL is presented. The contig spans a number of synteny switches between mouse and human. A putative *γ*-actin pseudo-gene and a second Glp1R locus, syntenic with human chromosome six have been detected. Additional genes within the contig are being detected by cDNA library screening and cDNA selection. These will be used to compile a short list of *Tir1* candidate genes with the aid of high-resolution mapping using interval specific congenic lines.

**B072**

## Mapping QTL for meat quality, carcase traits and growth in commercial pigs in Australia

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A QTL mapping project has been carried out on mostly purebred, commercal pigs in Australia. A half-sib design was used in which two Landrace and two Large White boars were each mated to a random selection of dams to produce on average 100 progeny. The families were genotyped for a total of 100 markers. Thirteen chromosomes had between 3 and 12 markers per chromosome at an average spacing of 20cM. The remaining chromosomes in the genome had fewer than 3 markers per chromosome. Approximately 75% of the genome was covered. Phenotypic data for the study were recorded in test station facilities at Bunge Meat Industries. Initially, traits were analysed separately, within each sire family, using both maximum likelihood and regression, semi-composite interval mapping software. Multi-trait interval mapping was also used to increase power and to test for pleiotropic effects of QTL. Semi-composite interval mapping meant that significant, unlinked regions were included as cofactors in the analyses. Using an experiment-wise error rate (EWER) of 5% only one QTL affecting fat deposition at the p2 site was significant. Under an EWER of 50% a further QTL, again affecting fat deposition was detected. By considering false discovery rate (FDR) as an alternative to EWER, 34 null hypotheses of no QTL can be rejected. It is expected that 75% of these hypotheses were correctly rejected and do represent “true” QTL. The 34 QTL affected 14 traits of the 18 traits tested. One QTL was shown to be pleiotropic for several fat deposition traits while another was shown to be pleiotropic for fat deposition, meat depth and pH of meat post slaughter. This study gives hope that MAS will make a significant contribution in practical breeding programs in Australia.

**B073**

## Identification of genomic regions for muscle pH in pigs

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Genomic regions associated with muscle pH were identified in pigs. A Yorkshire half-sib family showing large muscle pH variation (5.68 ± 0.37) compared with five other families was selected for this study. This half-sib family was produced from mating a sire with 15 unrelated dams producing 115 offspring. Marker genotypes were used to evaluate genomic effects. Selective genotyping was performed to identify genomic regions potentially associated with muscle pH using 67 informative microsatellite markers. Markers identified as nominally significant ($P<0.05$) were subsequently typed in all individuals. Chromosomal regions linked to putative QTL were identified using interval mapping. Chromosome substitution effect was evaluated by regressing muscle pH value on the probability of inheriting the paternal haplotype arbitrarily designated as haplotype 1. Two genomic regions nominally associated with muscle pH were identified on chromosomes 3 ($p<0.03$) and 10 ($p<0.02$). However, neither region surpassed a suggestive linkage threshold which accounts for multiple comparisons in a genome-wide search (pointwise p-value = 0.0034). The allele substitution effects on chromosomes 3 (0.29 ± 0.07) and 10 (0.33 ± 0.07) accounted for 9% of the total phenotypic variation of average muscle pH. Replication of this study is required before any conclusion can be drawn concerning the existence of muscle pH QTL on SSC3 and SSC10.

**B074**

## The association of an *agouti-related protein* (*AGRP*) gene polymorphism with growth and meat quality traits in commercial lines of pigs

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Agouti-related protein (AGRP) is a neuropeptide that mediates the orexigenic and metabolic effects of leptin signaling. The underlying physiological roles of the AGRP protein and the relationship to other neuropeptides suggest that *AGRP* is a candidate gene for feeding behavior and fatness in the pig. The objective of this study was to investigate associations between a DNA polymorphism of the porcine *AGRP* and economic traits in commercial lines of pigs. Over 1,800 animals from several different pig lines of PIC were tested with the *Drd*I PCR-RFLP of the porcine *AGRP* gene. No overall significant associations were detected, but results from some of the individual lines suggested that the rare allele 2 is preferred for better growth and overall meat quality traits in some of the lines investigated. In one population, the heterozygote animals (since only a few animals were the allele 2 homozygote) tended to exhibit lower ham Minolta score (46.65) and drip loss (2.27), higher ham pH (5.71) and loin depth (62.04) than the allele 1 homozygote animals (47.7, 2.65, 5.68 and 59.37, respectively). These heterozygote animals also showed higher daily gain (918 g/day) than allele 1 homozygote animals (897 g/day). In a second population the allele 1 homozygote animals showed better Japanese loin color score (3.74) than the heterozygote animals (3.32), but the presence of allele 2 showed better growth as in the first population (allele 2 homozygote animals were not present in the sample). These results indicate that this *AGRP* polymorphism is possibly associated with several economic traits based on linkage-disequilibrium in two commercial populations of pigs.

**B075**

**Mapping BAC clones by FISH: integrating the bovine genetic map with the physical map.**

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The genetic map for cattle (*Bos taurus* or *Bos indicus*) is now of sufficient resolution for the identification of regions of the genome contributing to genetic trait variation. This map is predominately composed of anonymous microsatellite markers and includes few gene polymorphisms. The bovine physical (cytogenetic) map, however, includes many assignments of orthologous human genes, and can be used for extrapolation of candidate genes from the human transcript map. Therefore, progression from genetic localization to identification of candidate genes requires sufficient integration between the bovine genetic and physical maps.

A panel of nine ovine bacterial artificial chromosome (BAC) clones harboring microsatellite markers from three of the bovine linkage groups (BTA8, BTA13 and BTA16) was obtained to anchor the consensus genetic map with the physical map. These DNA clones were cross hybridized to bovine metaphase chromosomes by fluorescence in situ hybridization (FISH). There was sufficient unique microsatellite flanking sequences, and conservation of these sequences between sheep and cattle, for the BAC clones to be successfully physically assigned.

These assignments increased the number of physically mapped genetic markers 2 to 3-fold for these 3 chromosomes. The results demonstrate that the consensus genetic maps span most of the physical distance for the chromosomes. The increased integration between the genetic and physical maps should facilitate the identification of orthologous human candidate genes for quantitative trait loci (QTL) localized on the bovine genetic map.

**B076**

**Development of genetic markers using end sequences of swine BAC clones**

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We are attempting to determine genetic regions responsible for quantitative traits by linkage analysis using a swine population constructed in our institute. Currently, more than a thousand markers are available for linkage analysis. However, since marker density varies from one region to another, the number of makers in a specific region is not sufficient for precise linkage analysis. As most of the swine BAC clones contained at least one microsatellite as well as marker sequences in our laboratory, we decided to assign as many swine BAC clones as possible to the INRA-University of Minnesota porcine Radiation Hybrid (IMpRH) map. This effort provides “land marks” for construction of BAC clone contig. DNAs of swine genomic BAC clones were subjected to PCR using a primer “6-MW” and a primer (Vect1) that was designed based on the vector sequence close to vector cloning site. The PCR products (BAC-end) thus obtained were then subjected to sequence analysis using a primer developed from the vector sequence located between Vect1 and the cloning site. Based on the BAC-end sequences, primer sets were designed to perform locus-specific DNA amplification (100-300bp fragment) in genomic DNA from swine, but not from hamster DNA used for the construction of IMpRH. Each primer set was then subjected to RH mapping (kindly supplied by Dr. Yerle, INRA, Toulouse). Currently we have located 96 of 112 swine genomic BAC clones (85.7%), for which primers were designed. We will continue this effort to increase the number of BACs on RH map to close current gaps as well as contribute to the construction of BAC contig.

**B077**

**Physical mapping of markers and candidate genes in a QTL region on bovine chromosome 20**

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A considerable number of quantitative trait loci (QTL) has been indirectly mapped on bovine chromosomes by linkage with microsatellite markers. In order to refine the maps of QTL regions, we employ bacterial artificial chromosome (BAC) – cloning of markers and candidate genes and subsequent physical mapping by fluorescence in situ hybridization (FISH) of BACs containing these specific markers and genes. Here, we present data for a lactation QTL region on chromosome 20. First, BACs were isolated for the closest flanking microsatellites by screening of a BAC – library (RPCI-42; http://bacpac.med.buffalo.edu) and mapped by FISH to 20q16-21 and 20q22-24, respectively, to delineate the physical boundaries of the QTL mapping interval. Two obvious candidate genes (*GHR* and *PRLR*) and two other genes (*ANPRC* and *IL7R*) were identified in regions of human chromosome 5 and mouse chromosome 15 with approximate evolutionary correspondence to the QTL region on bovine chromosome 20. PCR – probes, obtained with primers either from available bovine sequence or from highly conserved regions as determined by inter – species alignment of the sequences were used for screening of the gridded BAC libraries RZPD – No 750 and No 754, respectively (http://www.rzpd.de). Resulting BAC clones were confirmed with regard to their gene content by PCR. FISH mapping placed all four genes within the physical support interval, thus moving their candidate gene status to the positional level.

**B078**

**The hereditary disease 'congenital progressive ataxia and spastic paresis in pigs' maps to chromosome 3**

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The congenital progressive ataxia (CPA) and spastic paresis in pigs is a disease with unknown aetiology. It manifests itself within shortly after birth as a severe neuropathy. The disease seems to be controlled by a recessive allele designated as *cpa*. The studies were conducted to confirm the autosomal recessive inheritance of CPA and map the *CPA* phenotype to the porcine genome. Up to 139 inbred animals revealed linkage of *CPA* with microsatellites *Sw1066* and *Sw902* located on pig chromosome 3. The LOD scores of the two-point linkage analyses of *Sw902-CPA*, *Sw1066-CPA* and *Sw902-Sw1066* were 16.9, 11.6 and 47, respectively. The recombination frequency between *Sw1066* and *CPA* was estimated to be 0.05 while no recombination occurred between *Sw902* and *CPA*. *Sw902* (allele 189) co-segregated 100% with the recessive allele, thus revealing a single best fitting order coinciding with *Sw902* and *CPA*. The $\chi^2$-test, calculated from the segregation data showed that the observed ratios of the *cpa* vs *CPA* alleles did not deviate significantly from the expected 1:3 ratio ($\chi^2$=0.01; 0.9<P<0.95; 1df). In order to find the genetic factor causing this disease in the pig a comparative gene mapping approach will be used to find candidate genes.

**B079**

## Cloning and physical mapping of Horse (*Equus caballus*) EST Markers

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The horse gene map needs markers with homologues on the human gene map so that we can use information from the Human Genome Project to benefit horse research. Therefore, two major goals of this project were: 1) to identify the points of homology between the human and horse gene map and 2) to create an accurate physical/linear horse gene map by physically mapping genes. A cDNA library was constructed using RNA from a day 60 horse embryo. The cDNA library was directionally cloned into the Uni-Zap vector and produced an average insert size of 2.5 kb. Random clones were sequenced from the 3' end and human homologues identified by BLAST searches. Primers were designed to exclude amplification in the mouse and to produce PCR products of about 150-300 bp. Primers were sent to INRA and thirty positive BAC clones were isolated. The clones represented genes from human chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17 and X. The human map position was unknown for some genes. DNA from the BAC clones was prepared and labeled for mapping by fluorescence in situ hybridization (FISH). Also, partial inserts were sequenced to confirm gene identity. The BAC clones were mapped to horse metaphase chromosomes.

**B080**

**Nucleotide sequence, genomic organization and allellic variation of the ovine interleukin 2 gene**

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The cytokine interleukin 2 (*IL-2*) represents a central link in cellular and humoral immune defense mechanisms and therefore is a candidate gene for genetic resistance or susceptibility to infectious diseases. To determine the genomic organization of the gene and to demonstrate genetic variation between and within different breeds, the gene has been amplified from 5‘UTR to 3‘UTR for cloning and cycle-sequencing by primer walking. The cloned gene has a lenght of approximately 4.9 kb. Sequence information has been obtained from 11 domestic sheep of six different breeds (Merinolandschaf, Texelschaf, Heidschnucke, Romanov, Ostfriesisches Milchschaf, Kamerunschaf) and one Argali (*Ovis ammon ssp.*). The gene containes four exons and three introns. Although breeds of very different origins were investigated, few mutations could be demonstrated. Beside polymorphisms in intronic regions, a A/G transition in exon 1 has been identified and typed by SSCP. Frequency of the A allele was 0.6 in Merinolandschaf (n = 20) and 0.375 in Rhönschaf (n = 24). Half-sib family material was used for linkage mapping of ovine IL-2. Preliminary results yielded a recombination frequency of 0.15 between *IL-2* and the microsatellite OarCP 16. Since it is unlikely that the polymorphisms detected so far have an impact on biological function of IL-2, analysis of the 5‘ and 3‘ flanking regions of the gene is in progress to find further genetic variations in regulatory elements.

**B081**

**A molecular genome scan analysis to identify chromosomal regions influencing meat quality in the pig.**

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Genome scans can be employed to identify chromosomal regions and eventually genes (quantitative trait loci or QTL) that control quantitative traits of economic importance. A three-generation resource family was developed using two Berkshire grand sires and nine Yorkshire grand dams to detect QTL for meat quality traits in pigs. A total of 525 F2 progeny from 65 matings from 9 F1 litters were produced. All F2 animals were phenotyped for birth weight, 16 day weight, growth rate, backfat, loin eye area, drip loss, water holding capacity, firmness, color, marbling, percent cholesterol, ultimate pH, fiber type and several sensory panel and cooking traits. Animals were genotyped for 125 microsatellite markers covering the genome. Linkage analysis was performed using CRIMAP version 2.4 (Green et al. 1990). Regression interval mapping (Haley et al. 1994) was used for QTL detection. Significance thresholds were determined by permutation tests. Significant QTL at the chromosome wide 5% level were detected for growth (chromosomes 3, 4, 7, 8, 9, 12, 13 ,14, 15, 16, X), backfat (chromosomes 1, 5, 6, 7, 13, 14, 18) and meat quality traits (chromosomes 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18). Additional marker analysis and examination for positional candidate genes is underway. This research was supported by an industry consortium consisting of National Pork Producers Council, Iowa Pork Producers Association, Iowa Purebred Swine Council, Babcock Swine, Danbred USA, DEKALB Swine Breeders, PIC, Seghersgenetics USA, and Shamrock Breeders.

**B082**

## A New Whole-Genome Radiation Hybrid (WG-RH) Panel for Cattle

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Radiation Hybrid (RH) panels have already been recognised as a powerful tool in genome mapping as they allow the quick mapping of both polymorphic and non-polymorphic markers and the integration of linkage and physical maps into one comprehensive genome map. Within this context, we are constructing a new 7,000 rad bovine RH mapping panel. Approximately $18 \times 10^6$ fibroblasts from a bovine primary culture were irradiated with 7,000 rads and fused to $HPRT^-$ Wg3hCl2 hamster cells. Fusions were plated onto RPMI-1640 plus 10% fetal bovine serum and 1X HAT and incubated at 37°C. Non fused irradiated bovine and hamster fibroblasts were plated onto 1X HAT medium and incubated at 37°C as well as controls. Single colonies were picked and grown in four 300-$cm^2$ flasks for DNA extraction. Hybrid clones were randomly chosen to determine donor DNA content by FISH. In addition, sixty-two microsatellites (ms) spanning all bovine chromosomes were used to estimate the chromosome retention frequency. To date, 92 hybrid clones have been isolated and characterised with the sixty-two ms. Currently, the retention frequency ranges between 15% and 50% and closely reflects the results from FISH. Once characterised, the panel will be used to construct a medium density map using available and new ms as well as EST markers. We expect the bovine RH panel to be a platform to facilitate construction of regional YAC/BAC/cosmid contig maps underlying individual ETLs.

**B083**

## Genomic organization of *ELA* class II region

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To investigate the structure and organization of *ELA* class II region, we isolated and characterized microsatellite (mSAT) markers from *DQ* , *TAP-LMP* and *DM* regions.

*TKY107* and *TKY108* were isolated from *TAP-LMP* region, *TKY151* was obtained from *DM* region, and *TKY155* was found at 2 kb 3 from putative poly (A) adenylation signal of *DQB1* gene. Three BAC clones harboring each region were screened by PCR in INRA laboratory. And retention of the genes and mSAT in the clones were confirmed by direct sequencing and Southern blotting.

Using these markers in addition to *DRA*- and *DQB1*- polymorphisms, pedigree analysis was performed. These data demonstrated that *DR*-, *DQ-, TAP2, LMP7, TAP1, LMP2* and *DM* sub-region cluster into the respective order about 250 KB region of *ELA* class II complex. In FISH experiments, three BAC clones were positioned on to ECA20 21.1 region.

The genomic organization of *ELA* class II region was similar to *HLA* class II region, but strikingly different from *BoLA* class II region.

**B084**

## Mapping quantitative trait loci (QTL) affecting type, fertility and beef traits in a dual purpose cattle breed German Simmental and German Brown

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Thirteen German Simmental and one German Brown family, with a total of 583 sires, were analysed in a granddaughter design for marker-QTL association. Breeding values of nineteen type traits, four fertility traits and three beef traits were subjected to an across-family multimarker regression analysis. Here we report the results from 33 microsatellite markers located on 5 chromosomes (4, 6, 14, 20 and 23) chosen primarily as candidate chromosomes for milk production traits (see Förster et al., *this issue*). Significance threshold levels were set empirically by permuting the phenotypes within families. The critical value of 0.05 (chromosome-wise) was chosen to avoid high Type II error rates (i.e. to prevent missing true QTL due to conservative tests). With this methodology we detect four putative QTL affecting udder traits (teat length on chromosome 4 ($P$<0.05), udder depth on 4 ($P$<0.02), 6 ($P$<0.01) and 23 ($P$<0.01)), four QTL affecting body traits (hip width and body depth on 4 ($P$<0.05), stature on 6 ($P$<0.05), and body depth on 14 ($P$<0.05)), one QTL affecting rear leg set on 14 ($P$<0.05), one QTL affecting calving ease on 14 ($P$<0.05) and one QTL affecting daily net gain on 23 ($P$<0.01). Certainly not all of these observations are real QTL but we present them to encourage confirmation by other authors. For example, our evidence for a QTL affecting teat length confirms reported findings in US Holstein. Furthermore, our QTL affecting daily net gain could be the same as published for living weight in Finnish Ayrshire dairy. Analysis of additional markers and families to confirm these results are in progress.

**B085**

## Identification of a recessive-lethal genetic region in the National Institute of Animal Industry (NIAI) swine family by genome scan

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A genome scan of 198 animals in a F2 swine population constructed from single G tingen miniature bore and two Meishan sows revealed that no F2 pigs homozygous for a genetic region near centromere of chromosome 6q derived from the boar were generated in the population, indicating the region contains a recessive lethal gene(s). Genotypes from an additional 134 F2 population demonstrated that the region was confined to a 3.6 cM interval between microsatellite markers RYR1 and Sj086. In order to determine the stage at which the recessive lethal gene is expressed during conceptus development, F2 embryos at different stages were collected and genotyped. At day post conception (dpc) 11, 35 F2 embryos were collected from uterus of two F1 females. Sixteen embryos were homozygous for the interval, 5 embryos were homozygous unaffected, and 14 embryos were heterozygous. Embryos homozygous for the interval were spherical but smaller in size (<4 mm vs. 7-10 mm). At dpc 14, 11 additional F2 embryos were collected, three embryos were homozygous for the interval and remained spherical similar to conceptuses at dpc 11. Two embryos were homozygous unaffected, and filamentous. Six embryos were heterozygous and 5 of them were filamentous. These results suggest that a gene(s) located with the region RYR1-Sj086 expressed prior to dpc 11-12 is homologous lethal for embryo development.

**B086**

**Construction of a radiation hybrid map of bovine chromosome 28 using microsatellites and AFLPs.**

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Radiation hybrid map offers a very efficient approach to gene mapping and ordering of markers and genes without the need of a high level of polymorphism and the typing of large families. AFLPs are now widely used to investigate biodiversity in farm animals, however little information is presently available on their map position.

A 5000 rad mapping panel was typed with 10 microsatellites known to map on BTA28 and with 110 AFLPs produced from 8 EcoRI/TaqI primer pairs. The overlapping between AFLP bands amplified from hamster and bovine genomes was reduced increasing to 4 the number of selective nucleotides used at the 3' end of the EcoRI primers. Microsatellite and AFLP data were analysed using the program RHMAP 3.0, either separately or jointly. Retention frequency of individual microsatellite markers ranged from 0.213, for BMS2658 to 0.292, for BMS2200. Retention of AFLP bands averaged 0.155, spanning from 0.034, for E35+A/T33m01, to 0.701, for E35+C/T33m14. Microsatellite analysis revealed a single linkage group at LOD 6.0. The retention frequency of these three AFLP markers (0.203, 0.217 and 0.264) was similar to that of BTA28 microsatellites, suggesting that they are likely to identify single loci homozygous in the bovine parental of the RH panel. RH mapping seems an efficient method to rapidly locate on the genome biallelic AFLP bands.

**B087**

**Genetic mapping of the BoLA complex using BAC contigs.**

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Abstract: The MHCs of mice (*H-2*) and humans (*HLA*) are among the most completely characterized regions of mammalian genomes. Significant changes have occurred over evolutionary time between *H-2* and *HLA* to alter gene numbers and arrangements, but the genetic content of *H-2* and *HLA* remain essentially intact and accommodated within about 4mb of DNA. In contrast, genes of the bovine MHC (*BoLA*) on BTA 23 occur in two clusters separated by a genetic distance of about 20cM. Evidence suggests that a large inversion with the distal breakpoint within the class II region is responsible for the disruption of *BoLA*; hence the two clusters are designated *BoLA* IIa and IIb. Ordering of loci on chromosome 23 by FISH analysis and radiation hybrid mapping revealed that the telomeric breakpoint of the inversion occurred within the class II region near the contemporary *DQ* locus, and the location of the centromeric breakpoint was near the bovine homologue of human EST *AA298919*. Here we describe a physical map of *BoLA* IIa and IIb based on restriction mapping of 37 bovine BAC clones that contain genes homologous to *HLA* class I, II, and III loci. The BAC clones have been ordered into two contigs that respectively span the *BoLA* IIa, III, and I region and the IIb region. Together the contigs contain more than 3.5 mb of DNA and the order of genes within each contig suggest that no additional chromosomal rearrangements distinguish *BoLA* from *HLA*. Supported by a grant to LCS from the USDA-National Research Initiative Competitive Grant Program (NRICGP).

**B088**

## Genetic mapping of Spinal Dysmyelination in cross-bred American Brown Swiss cattle and evaluation of a comparative positional candidate gene

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The autosomal recessive inherited neurological disease Bovine Spinal Dysmyelination has been diagnosed in several national cattle breeds upgraded with American Brown Swiss (ABS) cattle. This is also the case in the Red Danish Dairy breed where all diagnosed cases of Spinal Dysmyelination have been genetically related to one single ABS bull. The disease is characterised by congenital recumbency, opisthotonus and extension of the limbs.

In a family material we conducted a genome scan covering all 29 autosomes with approximately 200 microsatellite markers. Based on this scan we were able to locate the Spinal Dysmyelination locus to a 17 cM interval on the proximal end of BTA#11. In the family we found a disease-specific haplotype consisting of three markers. Nearly all of the affected calves were homozygous with the same alleles. From the general population of Red Danish Dairy cattle, further nineteen cases were sampled. In 16 of these the calves were homozygous with respect to the same disease-specific haplotype. Based on the combined genotyping results the most likely candidate region can be restricted to an interval of approximately 4 cM, spanning three markers. These results have enabled us to initiate a screening programme of breeding bulls in the Danish population of cross-bred ABS cattle.

Available human-bovine comparative maps predict that a large part of BTA#11 is conserved on human chromosome 2 (HSA#2). A gene encoding a transcription factor, *EGR4* maps to a relevant position on HSA#2. This gene is highly homologous to *EGR2*, which in humans have been shown to be involved in hypomyelinating diseases, when defect.

We cloned the entire coding region of the bovine *EGR4* gene from genomic DNA, constituting an open reading frame of 486 amino acids. Furthermore, we isolated a number of cDNA clones from a bovine brain cDNA library. Sequencing of the *EGR4* gene from both a heterozygous carrier and an affected individual did not reveal any mutations supporting the theory that *EGR4* is the Bovine Spinal Dysmyelination gene.

**B089**

**QTL mapping in an Iberian x Landrace $F_2$ pig intercross: 1. Growth and carcass traits.**

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A cross between a highly inbred strain of Iberian pig and a maternal Landrace line was performed to map quantitative trait loci for several productive traits. The pedigree consists of 3 pure Iberian boars, 31 pure Landrace sows and 77 $F_1$ individuals (6 sires and 71 sows). Here we report results for growth and carcass traits corresponding to 250 $F_2$ animals genotyped for about 100 micosatellites spanning the 18 autosomes. The traits measured were carcass weight and length, backfat thickness, and weight of different pieces after a commercial cutting procedure. The two breeds are highly divergent for these traits.A linear regression procedure for *QTL* detection was employed. Carcass weight was adjusted for age at slaughter, and the other traits were adjusted for carcass weight. The main *QTL* results are (position in cM, F value, additive and dominance effects ± s.e.): carcass weight in chrs. 2 (71, 7.84, 3.05±0.82, 1.06 ±1.21), and 5 (130, 6.7, -2.02±0.69, -1.88±0.98); carcass length in chr. 4 (79, 21.8, -1.54 ±0.23, -0.17 ±0.34); backfat thickness in chrs. 2 (81, 7.7, 2.91±0.74, 0.23 ±1.07); 4 (83, 15.8, 3.65±0.67, -0.45 ±1.02); 6 (98, 24.8, 4.54 ±0.68, -2.00 ±1.07); and 7 (166, 7.0, -1.99 ±0.63,-2.02 ±0.94); and ham weight in chr. 13 (74, 9.74, -0.29±0.07, 0.25±1.12).

**B090**

**Restriction fragment length polymorphism at the bovine insulin-like growth factor binding protein-2 (IGFBP-2) locus in Angus cattle divergently selected for serum IGF-I concentration**

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*Insulin-like growth factor binding protein-2* (*IGFBP-2*) was selected as a candidate gene for growth in cattle. An Angus population that has been divergently selected for serum insulin-like growth factor-I (IGF-I) concentration since 1989 was used for this study. Primer pairs for the polymerase chain reaction (PCR) were designed from bovine *IGFBP-2* cDNA sequence (GenBank accession no. 4154260) and DNA sequence variation (polymorphism) at this locus was evaluated. A restriction fragment length polymorphism (RFLP) was identified in a 1,200 bp fragment of the bovine *IGFBP-2* gene by using the restriction endonuclease *Hind III.* Genotyping of 19 high and 20 low line individuals (born in fall 1997 or fall 1998) indicated no differences in allelic frequencies between the high and low IGF-I lines (allelic frequencies: 0.26 A/0.74 B high line; 0.35 A/0.65 B low line) or between males and females (allelic frequencies: 0.26 A/0.74 B bull calves; 0.35 A/0.65 B heifer calves). Previous analysis of this *IGFBP-2* fragment in other cattle breeds showed the presence of an additional RFLP by using the restriction endonuclease *Nla III* but this polymorphism was not segregating in the IGF-I selection lines (allelic frequencies 1.0 C/0.0 D). RFLP identified in this study could potentially serve as markers for variation in expression of important beef traits such as muscle yield, and also could provide new insights with respect to how divergent selection for serum IGF-I concentration affects other IGF-I system genes in this unique population.

**B091**

**Polymorphism of microsatellite loci and parentage identification in some Italian dog breeds**

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Canine microsatellites are powerful tools for parentage verification to increase the value of pedigrees and to give selective criteria in dog breeding. The ENCI (Ente Nazionale della Cinofilia Italiana) only recently has started to test dogs in our laboratory to resolve doubtful breeding cases; the interest in a correct litter's registration in stud books is growing up. In the present study we investigated the variability of ten canine microsatellites loci (AHT121, CXX20-123-263-403-2159-2137-2138-2001-2132) in ten Italian pure breeds (Bergamasco, Bolognese, Bracco Italiano, Cirneco dell'Etna, Corso, Fonnese, Lagotto, Maremma sheepdog, Segugio Italiano, Spinone Italiano, Volpino Italiano). All the samples have been collected from animals unrelated in two generations. DNA extracted from blood or buccal swabs was PCR amplified and labeled fragments were electrophoresed on ABI 377 automated sequencer. The differences in the observed allele frequencies between the breeds and the Polymorphism Information Content were examined and evaluated for each population and for each locus. We investigated also the most polymorphic microsatellite markers for parentage control and we calculated the cumulative exclusion probabilities for all the breeds and for all the analysed loci. In addition the obtained allele frequencies were used to estimate the genetic distances for all the considered breeds. Analysis of exclusion power (PE), ranging from 98,50% and 99,93%, using the most informative markers, demonstrates, the microsatellite efficiency and their potential use in stud book registrations of Italian pure breeds.

**B092**

## Mapping quantitative trait loci in commercial pig populations

The PigQTech consortium*

The segregation of Quantitative Trait Loci (QTL) affecting traits of economical importance in pigs, such as growth and backfat, is well-documented from experimental crosses. The aim of this project is to demonstrate how the farm animal breeding industry can use gene mapping technology to identify QTL for utilization by marker-assisted selection. Optimal sampling designs for detecting QTLs segregating in commercial pig populations have been evaluated by statistical modeling and the results indicate that additional power is obtained by sampling the large half-sib groups and those showing the largest within-family variance. A total of about 5,000 pigs representing ten commercial populations, including Pietrain, Landrace, Large White, Hampshire, and Meishan composite breeds, have been sampled. Phenotypic data on growth and carcass traits have been collected, and genomic DNA isolated from all animals. Ten chromosomal regions have been selected, including those containing major QTL in experimental crosses, and a set of microsatellites covering these regions are being genotyped. The association between markers and phenotypic traits is being explored using a variety of statistical tools ranging from simple sib pair analysis to more sophisticated approaches in a Bayesian framework. MtDNA typing is included to test for possible phenotypic differences associated with the two major European and Asian clades detected in a recent study (Giuffra et al. 2000, Genetics in press). Preliminary results of the QTL scan will be reported.

**The PigQTech consortium is composed of researchers at Swedish University of Agricultural Sciences, Sweden; Roslin Institute, UK; Pig Improvement Company, UK; Quality Genetics AB, Sweden; COPAGA, Spain; Institut de Recerca i Tecnologia Agroalimentàries, Spain; Universitat Autònoma de Barcelona, Spain.* *PigQtech is a demonstration project funded by the EC Biotechnology program.* *Presenting author: L. Andersson, Sweden.*

**B093**

## Detection of porcine ESTs that are preferentially expressed in liver

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The detection of expressed sequence tags, ESTs, can largely contribute to the identification of QTL. Detection of ESTs deals just with cDNA, devoid of intronic and intergenic sequences, and facilitates comparative mapping. Many catabolic and anabolic processes take place in hepatocytes and thus many genes of key-enzymes of these metabolic cycles are expressed in the liver. ESTs derived from genes that are preferentially expressed in liver may not only serve as anchors in the porcine genomic map but may also represent candidate genes for QTL. In the framework of the EC-project GENETPIG we used two approaches to detect porcine ESTs that are preferentially expressed in liver: Firstly, differential displays from liver, small intestine, ovary, leucocytes, muscle, uterus, pituitary gland, mammary gland, thyroid gland and adrenal gland were compared, liver-specific bands were recovered, sequenced and specific primers were designed for mapping. Secondly, in order to detect ESTs corresponding to genes known to be involved in hepatic metabolic pathways public databases were screened for sequence information of such genes and heterologous PCRs were performed. Regional assignment was done using a somatic hybrid panel (Yerle *et al*., 1996 Cytogenet Cell Genet 73, 194-202). By differential display 180 distinct ESTs were detected that appeared to be present only in liver or no more than three other organs. 52 clones were homologous with known genes, 128 clones had no match in databases. Ten porcine ESTs representing genes active in liver were detected by heterologous PCR. Until now 70 ESTs were physically mapped.

**B094**

**Comparative mapping of a region on pig chromosome 2 containing a QTL for backfat thickness**

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A maternally imprinted QTL for backfat thickness is located on the p-arm of porcine chromosome (SSC) 2 (Rattink et al, 2000). The comparative map shows homology between SSC2p and HSA11p-11q13 and between SSC2cen-2q21 and HSA19p. To improve the comparative map between pig, man and mouse, an RH map consisting of microsatellite markers and genes was constructed for SSC2p and the proximal part of SSC2q. A total of 25 genes and 25 microsatellite markers are mapped on this RH panel (Research Genetics). Eight new genes were mapped to SSC2 and 17 genes were mapped more precisely, that previously had been assigned to SSC2.

In addition, a BAC library was screened with markers and genes known or expected to map to SSC2. Shotgun sequencing and a BLAST database search of these BACs revealed high similarity with 30 expressed sequences. In total, approximately 50 expressed sequences were placed on SSC2p-2q23 by RH mapping and shotgun sequencing. The comparative map shows that SSC2p15 is homologous to HSA11p15.5. Comparing gene order on HSA11p15.4-11q13 with SSC2, one intrachromosomal rearrangement is observed on SSC2. This high-resolution comparative map of the SSC2 region will facilitate selection of positional candidates for the observed maternally imprinted QTL for BFT. Although imprinting has been reported for the IGF2 region on HSA11p15, this is not the case for the obesity related QTLs located in the region HSA11p15.4-11q13.

**B095**

**Development of genetic markers from a BTA29-specific library**

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Chromosome-specific libraries are an important resource for directed marker development. A BTA29-specific library, constructed by chromosomal microdissection of a 1:29 Rb-fusion cell line, was screened for presence of the dinucleotide repeats $(CA)_{15}$ and/or $(GA)_{15}$. Approximately 1200 primary clones were recovered and over half of these secondarily screened. DNA sequences were determined for 458 positive clones. From these, a total of 90 (19.7%) primer pairs were designed and 82 (17.9%) of these successfully amplified bovine genomic DNA by PCR. In addition to these 82 loci, primer pairs have been developed for 9 putative genes. Two somatic cell panels were used to test for synteny of the new loci with two BTA29 markers on the MARC bovine linkage map (BMC2228 and BMC3224). Results of these tests show that 85% of the loci are syntenic ($\varphi > 0.74$) with the previously mapped BTA29 loci. Non-significant synteny ($0.74<\varphi<0.59$) was calculated for approximately half of the remaining markers. Two loci mapped to chromosome 1 (BMS4017, $\varphi > 0.74$) and four loci did not map to either chromosome 29 or chromosome 1 (3 of these constitute one UN linkage group). The results of this effort will significantly increase the marker density on BTA29.

**B096**

**Dog genome intra and inter-breed polymorphism study using SNP markers.**

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Owing to the growing interest of the dog species as a genetic model to study inherited diseases, genetic, radiation hybrid and cytogenetic maps have been developed. The search for genes involved in genetic diseases requires the use of either existing pedigrees, generally highly inbred, or to construct one's pedigree. In both cases, the knowledge of the general level of polymorphism in the studied breed is crucial. The general idea is that a set of markers might be polymorphic in a breed but not necessarily in another one. To appreciate the degree of both, intra-breed homogeneity and inter-breed heterogeneity, we focused our study on the analysis of SNP (Single Nucleotide Polymorphism) markers.

The use of SNPs as landmarks along the genome, will be of great value in gene hunting since they’re likely to reveal regions which have been submitted to selective pressure during the course of breed creation, leading then to the characterization of genes governing these breed-specific traits.

A small insert genomic library has been constructed and randomly sequenced. 250 STS, have been designed from these sequences and amplified by PCR on 12 different breed DNA samples. Purified PCR products have then been submitted to single-pass sequencing. Alignments have been performed, and final comparisons by visual inspections of the patterns seen among the several individuals have led to the identification of roughly 90 polymorphic sites, representing an average SNP occurrence rate of 1/1200 bp. Observed heterozygous frequency of these SNP markers ranges from 0.13 to 0.54 and the transition to transversion ratio is 3:2. A detailed analysis of the observed inter and intra breed polymorphisms will be also reported.

**B097**

## An initial EST Radiation Hybrid Map of Porcine Chromosome 7

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The large scale development of ESTs from porcine immune tissues and their subsequent mapping using an RH panel will allow dissection of local and systemic immune responses, while improving the overall map resolution of the swine genome. Expressed sequence tags (ESTs) were constructed from ten oligo(dT)-primed individually tagged, directionally cloned and normalized cDNA libraries from peripheral blood cells (PBC), spleen (Sp), thymus (Th), lymph node (LN) and bone marrow (BM) from immunologically naive and challenged pigs as part of an implant-associated orthopedic infection model. The ESTs mapped using the 7000 rad IMpRH panel (Hawken et al., 1999 Mamm Genome. 10:824-30) represent sequences that show significant homology to classical MHC genes as well as novel sequences with low homology to MHC genes. Novel sequences were chosen for primer design only if an open reading frame, a polyadenylation signal and/or a poly(A) tail could be detected. Markers were mapped using RHMAP Version 3.0 (Boehnke et al., 1996) and a cut off value of LOD 4.86. Currently, we have assigned 47 markers clustered around TNFB (SLA class III), TCRA (HSA14) or SSC2B02 telomeric on SSC7q. Additional ESTs were randomly distributed throughout regions syntenic with Human Chromosomes 6, 14 and 15.

**B098**

**Canine malignant hyperthermia is linked to the gene encoding the skeletal muscle calcium release channel (*RYR1*)**

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Malignant Hyperthermia (MH) is an inherited muscle disorder, characterized by rhabdomyolysis, generalized skeletal muscle contracture, cardiac dysrhythymia, renal failure, and typically an elevated body temperature, that develops when susceptible patients are exposed to succinylcholine or volatile anesthetics. All swine and 50% of human MH is caused by a mutation in the skeletal muscle calcium release channel of the sarcoplasmic reticulum, also designated as the ryanodine receptor (*RYR1*). To determine the molecular basis for canine MH, a breeding colony was established with a male Labrador Retriever who survived a reaction to halothane. He was mated to three unaffected females to produce four litters, and backcrossed to an affected daughter to produce one litter. An affected son of his was mated to an unaffected female to produce one litter. All dogs were phenotyped with an in vitro contracture test (IVCT), and they were diagnosed as MH susceptible (MHS) or MH normal (MHN) based on the North American protocol. There were 21 MHS and 18 MHN pups in the five outcross litters. In the backcross litter there were two MHS and one MHN, and five pups that did not survive past two months. Pedigree analysis revealed MHS in this colony to be transmitted as an autosomal dominant trait. *RYR1* has been mapped to canine chromosome 1 (CFA01) (Priat *et al*., 1999 Mamm. Genome 10, 803), and eight CFA01 microsatellite markers were tested for linkage to MHS. The marker closest to *RYR1*, FH2294, is linked to MHS at a distance of 5 cM with a LOD score of 8.9, strongly suggesting that canine MHS co-segregates with a mutation in *RYR1*.

**B099**

**Physical and Linkage mapping of the bovine Acetyl-CoA carboxylase –α encoding gen (*ACACA*) on BTA 19.**

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Acetyl –CoA carboxylase (*ACC*) is the rate limiting enzyme in the long-chain fatty acids synthesis. It catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, wich is only generated by *ACC* and is the substrate for fatty acids synthesis. We've been working in the isolation and characteritation of the cDNA and genomic *ACACA* Here we report the phisical and genetic maps of bovine *ACACA* as well as the cDNA sequence .We used an EMBL3 clone containing an insert of 17 kb bovine genomic ADN encoding for several exons of *ACACA* to perform FISH. That clon maps to BTA 19 q1.3-1.4 We've also found several microsatellites into some genomic clones and used one to perform the linkage analysis in the IRBP animals. Multipoint analysis showed the localitation of that locus on the proximal part of BTA 19.

**B100**

**Characterization of a Single Nucleotide Polymorphism in the coding sequence of bovine transferrin gene**

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A Single Nucleotide Polymorphism was identified in the coding sequence of the bovine transferrin (*TF*) gene. Two alleles ( SSCP 1 and SSCP 2) were detected by SSCP analysis. The point of mutation was also confirmed by direct sequencing of the PCR products. The relationship between protein and genetic polymorphism was established. Protein variants A, D1 and E correspond to SSCP allele 1 and the variant D2 corresponds to SSCP allele 2. DNA sequences of the genotypes AA, AE, AD2, D1E, D2E and D2D2 reveal an A/G substitution at position 1455 of the cDNA which causes a Gly/Glu substitution, which could be responsible of the mobility differentiation of the D1 and D2 variants. This suggests that other SNPs exist in the bovine transferrin gene. A linkage analysis between SSCPs and two microsatellites (UWCA46 and CSSM019) mapped the transferrin gene to BTA1. Two-point analysis revealed a hight linkage within the transferrin protein variants and the SSCPs.

**B101**

## Radiation hybrid mapping of STS markers derived from a bovine chromosome fragment-specific library of *Bta* 5q21-q24

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In a preliminary search for quantitative trait loci (QTLs) in a cattle F2 resource population segregating trypanotolerance, two large candidate regions were identified on cattle chromosome *Bta* 5. In order to narrow down the positions of these QTLs, there is a need to increase the resolution of the bovine map within the QTL peak regions. Using chromosome fragment-specific libraries covering the peak region at *Bta* 5q21-24, we have sequenced approximately 150 clones, which were pre-selected for an insert size of at least 200 bp. From the obtained sequences, 15 sequence tagged site (STS) markers have been developed so far. To determine the positions of the STSs relative to known markers they were incorporated into a 5000rad radiation hybrid (RH) framework map of *Bta* 5, which comprises twelve type I markers (genes) and 18 type II markers (microsatellites). Six of the STS markers were found to map to the peak region and therefore also were integrated into the more accurate 12,000rad RH map of *Bta* 5. These markers are currently being used to screen a bovine BAC library (provided by the Resource Center of the German Human Genome Project, Berlin; library No. 750). From corresponding BAC clones, microsatellites will be isolated, tested for informativness in the F2 population and used to refine linkage mapping of the trypanotolerance QTL regions on *Bta* 5.

**B102**

## *H-FABP* gene association study for body composition in pigs

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One QTL affecting backfat thickness (BFT), intramuscular fat content (IMF) and eye muscle area (MA) was previously localized on porcine chromosome 6 on an $F_2$ cross between Iberian and Landrace pigs. This work was done to confirm the location and effects of this QTL and to study the effect of the *H-FABP* gene on these traits. The QTL mapping analysis was performed with a regression method using records and genotypes for seven microsatellite markers of 369 $F_2$ animals. The presence of a QTL with high effect and significance on BFT (104cM, F=35.6), IMF (101cM, F=27.1) and MA (117cM, F=17), was confirmed. Further, 508 $F_2$ animals were genotyped for a PCR-RFLP located in the second intron of the *H-FABP* gene. The effect of the polymorphism was analyzed using an animal model where the *H-FABP* genotype was included as fixed effect. Linkage mapping of the *H-FABP* gene allowed its location in the position 84.7 cM. The *H-FABP* polymorphism showed significant effects on IMF (DD-dd=0.33, p=0.002; Dd-0.5(DD+dd)=0.06, p=0.35) and MA (DD-dd=-1.81, p=0.029; Dd-0.5(DD+dd)=1.25, p= 0.012), but not on BFT. The effect of the different *H-FABP* alleles is opposite to that of previous reports and the *H-FABP* association results explain only partially the QTL effects observed.

**B103**

**QTL for marbling maps to cattle chromosome 2.**

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Studies to map many traits, including those associated with meat quality, are being conducted using the Canadian Beef Cattle Reference Herd. This herd is composed of purebred cattle representing the five most common Canadian beef breeds and a double-muscled breed. The herd was designed to yield full-sib information by employing embryo transfer technology, and resulting calves were both purebred and crossbred. (See http://skyway.usask.ca/~schmutz.) Fat traits measured included grade fat, backfat, and marbling. A QTL for marbling was found in the middle of cattle chromosome 2. *Nebulin (NEB)* is a gene which maps to this region and is involved in the structural integrity of muscle fibers. *Glucagon (GCG)*, a hormone that promotes glycogen and lipid hydrolysis, also maps to this region. Both *Nebulin* and *Glucagon* are potential candidate genes for marbling, based on their muscle specificity and lipid involvement respectively. A QTL was also found near the centromeric region of chromosome 2 common to all three fat measurements. Myostatin maps to this region, however none of the families contributing to this QTL involved a double muscled breed.

**B104**

**Assignment of previously unassigned genes to Bovine chromosome 13 (BTA13)**

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Bovine spongiform encephalopathy (BSE) is probably caused by feeding on animal protein preparations containing the scrapie/BSE-agent. Evidence has accumulated, that the infectious agent causing BSE is a protease-resistant, insoluble isoform of the physiologically expressed prion protein ($PrP^C$). In species other than cattle, expression of spongiform encephalopathies is clearly dependent on polymorphisms in the prion protein gene (*PRNP*). Within the framework of a matched case-control-study aimed to detect genetic associations between BSE and markers of the bovine *PRNP* region, we first focus on further characterizing the vicinity of the gene. *PRNP* has been assigned to (Womack & Moll, 1986, J. Heredity 77, 2-7) and mapped on BTA13 (Schläpfer et al. 1997, Chromos. Res. 5, 511-519, Schläpfer et al. 2000, J. Anim. Breed. Genet. 117, in press). BTA13 is homologous to parts of human chromosome 10 and 20 (HSA10 and HSA20) (e.g. Solinas-Toldo et al. 1995, Genomics, 27, 489-496), in that it is composed of a HSA10 segment sandwiched by centromeric and telomeric HSA20 regions. Based on sequence information of published human homologous genes, PCR primers were designed to amplify cattle DNA. Sequence homology of amplified fragments in cattle was confirmed by nucleotide sequence analysis. Homologous gene fragments were then typed in a somatic cell hybrid panel in order to assign the conserved loci to BTA13. In this ongoing process, we have as of yet assigned six previously unassigned genes to BTA13. Our results will provide further insights into the chromosomal evolution of BTA13 as compared with man and thus elucidate the vicinity of the prion protein gene.

**B105**

**A QTL for behavior maps to cattle chromosome 9.**

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The Canadian Beef Cattle Reference Herd is being used for a QTL study. A total of 136 ET calves born during the summer of 1996 made up the 17 full-sib families, which ranged in size from 2 to 17 calves per family and were the offspring of 5 sires and 13 dams. Angus, Belgian Blue, Charolais, Hereford, Limousin and Simmental purebred cattle were the parental generation but the calves were both purebred and crossbred. A modified identical-by-descent analysis was used, where the phenotypic differences between like and unlike genotype sib-pairs were determined using an unpaired, one-tailed t-test. Evidence of linkage was suggested when the sum of $t^2$ values exceeded the threshold probability value of $P< 0.00156$ using a $\chi^2$ distribution. The two behavioral traits measured in this study were isolation response at weaning which was measured by the amount of movement in an enclosure during 1 minute and habituation which was the difference in this measurement between weaning and several months later. The first which may be a measure of temperament was calculated to have a heritability of 0.36 and habituation 0.46 in this herd. Six QTL were found for both of these behaviors. One was found at ILST013, at 44 cM on chromosome 9. Cannabinoid receptor (CNR1) is a gene which was previously mapped to this region by in situ hybridizaiton (Pfister-Genskow et al. 1997 Mamm Genome 8, 301). This neurochemical receptor has been associated with behaviors such as motor activity and may therefore be a candidate gene.

**B106**

**Development of a physical contig containing the *callipyge* gene on ovine chromosome 18**

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The *callipyge* mutation causes pronounced muscular hypertrophy in sheep. Animals expressing the callipyge phenotype produce leaner, higher yielding carcasses, but there is some concern with decreased tenderness of the loin. The gene is characterized by nonmendelian inheritance called polar overdominance, in which only heterozygous offspring inheriting the mutation from their sire exhibiting heavy muscling. Linkage analysis using large paternal half sib families has localized *callipyge* (*CLPG*) to an interval flanked by microsatellite IDVGA30 and OY3 on ovine chromosome 18. As a preliminary approach for positionally cloning the gene, a physical contig of the callipyge region has been constructed. The contig consists of 6 ovine and 39 bovine BAC clones that were isolated by PCR screening or filter hybridization and the contig spans 1274 kb. Average depth of the contig is 7.7 clones. New sequence tagged sites (STSs) were generated by direct BAC-end sequencing, exon-trapping and random subcloning. The 50 STSs that were characterized include 3 Type I genes, 7 microsatellites, and 40 newly generated sequences. This contig will be an essential tool in the isolation and characterization of the *callipyge* gene.

**B107**

**Bovine *CAPN1* maps to a region of BTA29 containing a QTL for meat tenderness**

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Micromolar calcium activated neural protease (*CAPN1*) was investigated as a potential candidate gene for a quantitative trait locus (QTL) on BTA29 affecting meat tenderness. A 2,948 base pair (bp) bovine cDNA containing the entire coding region of the gene was obtained, showing 91% identity to human *CAPN1.* The 716 amino acid (aa) protein predicted from this sequence shows 97% similarity (95% identity) to the 714 aa human protein. Analysis of the gene structure revealed that *CAPN1* mRNA is encoded by at least 20 exons, and 9,800 bp of the gene were sequenced including 17 of the introns. Two single nucleotide polymorphisms (SNP) were detected in intron 12 and used to map bovine *CAPN1* to the telomeric end of the BTA29 linkage group. This approximately coincides with the position of the QTL, demonstrating that *CAPN1* protease is a positional candidate gene potentially affecting variation in meat tenderness in a bovine resource mapping population.

**B108**

## Genetic polymorphism in the *mhc* B-L locus of Camperos, a mixed-breed of broilers

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Camperos is a mixed-breed of broiler chickens produced by cross-breeding different breeds of chickens in INTA. This mixed-breed is more adapted to the conditions of the argentine farm. They are bred like free-range broilers. Camperos (F1) are obtained by cross-breeding of 10% of a breed of males with 90% of a breed of females. The male breed was produced by random cross-breeding of Anak females with White Rock males. The female breed was produced by random cross-breeding of Cornish Red males with Rhode Island Red females. Both breeds were unselected and produced during the course of seven generations of random breeding. The aim of the work presented here was to determine the degree of polymorphism in Camperos at the B-L locus. As a first approach we used a 773 bp probe obtained by PCR for exon 2 (β1 domain), exon 3 (β2 domain) and exon 4 (trans-membrane domain) and the introns between them of a Y-LB III gene from a Camperos chicken. This probe shares more than 90% homology with genes Y-LB III and B-LB at exons 3 and 4. DNA from eighteen Camperos were analyzed by RFLPs using different restriction endonucleases. Nine and ten different genotypes were detected with *Pst*I and *Pvu*II restriction endonucleases, respectively. Not all chickens with the same polymorphism for one enzyme necessarily showed the same polymorphism with the other. This is not surprising since Y-LB III segregates independently from the B-LB genes. Thus, polymorphism in Camperos seems to be located in both the Y-LB III and B-LB genes.

**B109**

## The Analysis of microsatellite loci in resource family crossed by Large White and Chinese Meishan pig

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In order to detect quantitative trait loci(QTL) responsible for growth, carcass performance, ovulation rate and meat quality, a resource family of pigs has been constructed by using three boars of Large White and seven sows of Chinese Meishan pig as parents. 79 F2 offspring generated by intercrossing with five F1 males and nineteen F1 females had been slaughtered. The traits mentioned above were recorded. Another 90 F2 offspring will be slaughtered next month. The members of the family were genotyped using 31 microsatellite markers, kindly provided by Rothschild as a part of the U.S. Pig Genome Coordination Program, covering six swine chromosomes. Two markers did not yield amplification products for some alleles. Four markers had monomorphic in these parental animals. Twenty-five markers were polymorphic. In our experiments, many alleles sizes of PCR product exceeded the range provided by USDA-MARC map. It seemed that a pig had null allele or more than two alleles amplified by one pair of primers. Some very strong bands were twofold than allele. The reasons of the phenomenon may be the mismatch by Taq DNA polymerase and jumping amplification.

**B110**

## First comprehensive low-density horse linkage map based on two, three-generation, full-sibling, cross-bred horse reference families

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Two, three-generation full-sibling reference families have been produced and form a unique resource for genetic linkage mapping studies in the horse. The F2 generations, now comprising 61 individuals, consist of 28-32 day-old embryos removed non-surgically from two pairs of identical twin mares. The same stallion sired all F2s such that the two full-sibling families are half-sibling with respect to each other. The families are crossbred to maximise levels of heterozygosity and include Arabian, Thoroughbred, Welsh Cob and Icelandic Horse breeds. Milligram quantities of DNA have been isolated from each embryo and from blood samples of the parents and grandparents.

The families have been genotyped with 353 equine microsatellites and 6 biallelic markers, and 42 linkage groups were formed. In addition, the physical location of 85 of the markers is known and this has allowed 37 linkage groups to be anchored to the physical map. The inclusion of dams in the genotyping analysis has allowed the generation of a genetic map of the X chromosome. Markers have been assigned to all 31 autosomes and the X chromosome. The average interval between markers on the map is 10.5 cM and the linkage groups collectively span 1780 cM.

The results demonstrate the benefits for horse linkage mapping studies of genotyping on these unique full-sibling families, which comprise relatively few individuals, by the generation of a comprehensive low-density map of the horse genome.

**B111**

**Characterization of the Beta-2-Microglobulin gene of the HORSE**

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The light chain of the Major Histocompatibility Complex (*MHC*) class I cell surface heterodimer molecule is called *ß-2-microglobulin (ß-2-m).* Coordinate regulation of the *ß-2-m* and *MHC class I* genes in the trophoblast cells of the equine placenta during critical phases of early pregnancy has been indicated by results from our laboratory. This regulation is considered to be important for the generation and maintenance of maternal immunological tolerance of the developing fetus and placenta. This coordinate regulation may be governed by common transcriptional elements shared by these two unlinked genes. Our laboratory has obtained complete sequence of a polymorphic horse *MHC class I* gene, including over 400 bp of its upstream regulatory region during the last year. We have also identified a clone containing the horse *ß-2-m* gene from a horse genomic library produced in pBeloBAC11 (Godard et al. 1998). Characterization of the complete horse *ß-2-m* gene and its promoter region using this clone is underway. Generation of large scale plasmid preps of the clone, restriction enzyme digests, subcloning and sequencing is required for this work. The full cDNA sequence of horse *ß-2-m* is available for these studies (Ellis and Martin 1993). These experiments would allow comparison of the structure and function of the promoter regions of the horse *ß-2-m* and *MHC class I* genes.

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**B112**

## Validation of QTL segregation in Angus half-sib families

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Due to a decline in beef consumption, the need for increased consumer satisfaction has been recognized with tenderness, marbling, palatability, and uniformity of product cited as the most important areas of concern. Unfortunately, these carcass traits are notoriously difficult to predict in live animals, making phenotypic selection generally ineffective. A possible solution is marker-assisted selection which is based upon the existence of linkage disequilibrium between genetic markers such as microsatellites and quantitative trait loci (QTL). Whole genome scans for QTL in a double reciprocal backcross and $F_2$ population produced from Angus and Brahman cattle revealed several chromosomal regions influencing tenderness, marbling, and lean yield (muscling). Eleven QTL affecting Warner-Bratzler Shear Force (WBSF), overall tenderness (as assessed by a taste panel), marbling, and ribeye area are presently being validated for their effects in ten Angus paternal half-sib families of fifty progeny per sire. At least two flanking markers in the region of each QTL will be scored in each animal. Segregation or absence of the QTL will be validated using interval analyses. Additionally, the level of linkage disequilibrium within Angus will be evaluated. Preliminary results reveal that segregation can be detected in these families for all QTL. This will allow implementation of marker-assisted selection within the sire families.

**B113**

**The retrovirus endogenous locus ALVE1 is an associated marker to the development of Rous sarcoma virus-induced tumors in B19 WHIte Leghorn chickens.**

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In B19 White Leghorn chicken line, two divergent sublines were selected with the respect to Rous sarcoma virus (RSV)-induced tumors. Chickens from the progressor subline developed sarcomas after inoculation with virus while chickens from regressor subline were resistant to the development of RSV-induced tumors. Preliminary results have shown that the control of tumor growth in B19 regressor chickens would be mainly due to the host immune response directed against the highly antigenic proteins encoded by the viral replication genes. Then, we have looked for the presence into the two sublines of the endogenous viral genes. The endogenous viral genes represent a group of loci in the chicken genome that are closely related to the RNA of exogenous non defective avian ALSV retroviruses. Three loci have been identified and the presence of one of them, ALVE1, seems to be correlated to tumor development. Several crosses have been done between progressor chickens which carried out the ALVE1 locus and regressor chicken lacking endogenous retroviral sequences. These crosses confirmed that ALVE1 was associated with the progressor phenotype. Nevertheless ALVE1 locus have been described as a non expressed locus and several chickens lacking ALVE1 were able to develop sarcomas. These data suggest that ALVE1 could not be directly responsible for the tumor development but could be a marker associated to the locus reponsible for this phenotype. To confirm this hypothesis, microsatellite markers flanking ALVE1 locus have been used to try to identify the locus actually involved in tumor development.

**B114**

## Development of an $F_2$ Resource Family between Oh-Shamo (Japanese Large Game) and White Leghorn for Chicken QTL Analysis

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We have been developing a chicken $F_2$ resource family to analyse linkage relationship between quantitative trait loci (QTL) and DNA markers. The family is based on Oh-Shamo and White Leghorn breeds. Oh-Shamo is a Japanese native breed for cockfighting and is characterized by yielding large amount of meat and good taste of the meat. In our study, targets are growth-, egg-, and meat-related traits. The growth-related traits contain body weight, shank length, and volume of growth hormone and insulin-like growth factor II in plasma. The egg-related traits comprise egg production rate, egg weight, egg size, eggshell strength, eggshell color, eggshell weight, eggshell thickness, yolk hight, yolk diameter, yolk color, yolk weight, thick albumen size, thick albumen hight, and eggwhite weight. The meat-related traits include carcass weight, meat color, meat pH, protein extractavility, shear value, and volume of myoglobin, inosinic acid, meat components (protein, fat, and water), and 20 kinds of free amino acids. Between the Oh-Shamo and White Leghorn breeds, the traits mentioned above were significantly different in measurements, and approximately 60% of microsatellite-markers tested were heterogeneous. Thus, it can be expected that the QTL analysis using this family will be efficiently performed in the near future. This family will be used also as a reference family to map DNA markers on the chromosomes. At present, we are obtaining a large number of F birds and their quantitative traits data. We are grateful to Dr. H.H. Cheng, USDA, for his kind supply of microsatellite DNA primers to us.

**B115**

**A supplementary resource for linkage mapping in sheep (*Ovis aries*)**

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The ovine linkage map has been developed almost entirely on the Agresearch International Mapping Flock (IMF), and currently contains over 1000 markers. The IMF comprises 9 full-sib families, with 97 offspring in total. While the IMF is, and will remain, the primary reference flock for ovine linkage mapping, difficulties may be encountered in high resolution mapping or in locating weakly polymorphic loci. We have developed an additional set of reference families to supplement the IMF. This set comprises fifteen full-sib families (two sires), with 170 progeny in all, derived by crossing two Romney grand-sires and eight Merino grand-dams. There are, in addition, 230 half-sibs generated by crossing the two parental sires to fine-wool Merinos. While the latter were created primarily to detect QTL for wool production traits, they add additional power for estimating male recombination rates. We have typed 200 polymorphic markers in the full-sib families, and 100 in the half-sibs. The flocks are segregating for the Booroola (*fecB*) and the polled (*Ho*) phenotypes. In addition, the original Romney sires were each homozygous for a Robertsonian translocation, $t_1$ -*rob*(6;24) and $t_2$ - *rob*(9,10) respectively, allowing estimates of centromere positions for the four chromosomes involved. We present linkage data obtained from the above families, compare these with those obtained in the IMF, and discuss the integration of the IMF and CSIRO maps. DNA from these families is available upon request.

**B116**

## A Bayesian multivariate analysis with genome segment mapping: an application for production traits in a Pietrain population

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In the context of the *PigQTech* (a project funded by the European Union), we present a new method aimed at estimating which fraction of the additive variance is contributed by a given set of genome regions (called segments). We make no assumptions about the number or allelic phase of *QTL*s. The method consists of two steps. First, the additive relationship matrix conditional on marker information is computed for each segment using a Monte Carlo method. Second, a Bayesian multivariate approach via the Gibbs sampler is implemented. We have applied the method to the contribution of four genome segments at the genetic variation in a Pietrain pig population for live weight (*LW*) and backfat thickness at 175 days (*BFT*). The pedigree genotyped consisted of 5 sires, 58 dams and 420 offspring. Only offspring had records. The model included systematic effects (sex and batch), a polygenic genetic effect and four segment genetic effects (30 cM.). The following markers were genotyped: *CGA* and *SW1430* (chr. 1); *S0141* and *SW2623* (chr. 2); *SW732, S0206* and *SW2618* (chr. 3), and *S0003* and *SW316* (chr. 6). For *LW*, the heritabilities due to the polygenic background was 0.18, and for segments in chrs. 1, 2, 3, and 6 were 0.08, 0.08, 0,11 and 0.15, respectively. For *BFT*, the heritabilities were 0.19 for the polygenic background and 0.07, 0.15, 0.10, and 0.21 for segments in chrs 1, 2, 3 and 6. Genetic correlation between traits for chr. 1, 3 and 6 was very high (>0.65), but it was low for the polygenic background and chr. 2.

**B117**

**Microdissection of chromosome one and high resolution gene mapping in the pig**

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QTLs for vertebrae number, teat number and birth weight map to the distal part of SSC1q in our resource population. To improve marker resolution in this region of SSC1q we have used chromosome microdissection and radiation hybrid (RH) mapping. Five copies of the telomeric region of SSC1q were microdissected from metaphase spreads and pooled in a 0.5 ml microcentrifuge tube containing 20 ㅟl of recovery solution. The sample was amplified in 30 ㅟl of PCR mixture using a degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR). PCR amplification as well as construction, enrichment and screening of the DNA library was performed as described by Oksana *et al*., 1998. The DNA library had 99% recombinant clones of which greater than 95% had (CA)n repeats. Although the library was highly redundant, oligonuclotide primers were developed for the PCR amplification of 24 new microsatellites. Sixteen of these (67%) amplified a single locus in the INRA-University of Minnesota porcine Radiation Hybrid panel (IMpRH). The RHMAP program assigned 15 of these markers to the SSC1 radiation hybrid map in the distal region of SSC1q. In summary, we have generated 16 new microsatellites that increases the resolution of SSC1q in the region of several known QTLs.

**B118**

**The order of genes on porcine chromosome 12.**

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To isolate the genes of interest it is required for detailed comparative map. According to bidirectional heterologous painting porcine chromosome 12 (*SSC12*) is homologous to entire human chromosome 17 (*HSA17*). It can be supposed that the genes from synteny group of *HSA17* are located on *SSC12*. 17 genes belonging to *HSA17* were roughly located regionally with the use of set of porcine cell hybrid clones. Location of one gene, *NF1*, was made more precise with the use of microdissection of a regions of *SSC12* followed by primer specific PCR. It was found that the genes located in *SSC12p* are presumably assigned to *HSA17q* and *vise versa*. But chromosome region containing *CRYB1, NF1*, and *MCP1* genes was shifted from one chromosome arm to another in pigs relative to humans. To prove it and to order the genes on *SSC12* we used whole genome radiation panel (*ImpRH*) and added 5 genes (*MYL4, TOP2A, THRA, MCP1*, *NF1*) in RH map of *SSC12.* The order of analyzed genes was similar in pigs and humans except of centromer position. In pigs it is between *THRA* and *MCP1-NF1* genes, but in humans – after *NF1* gene.

**B119**

## Cytogenetic mapping in the domestic rabbit (ORYCTOLAGUS CUNICULUS)

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In this study we assigned six Type I loci and two anonymous microsatellite loci to individual rabbit chromosomes by fluorescence in situ hybridization. The Type I loci comprised *CYP2C (cytochrome P450, subfamily IIC)*, *PMP2 (peripheral myelin protein 2)*, *ALOX15 (arachidonate 15-lipoxy-genase)*, *MT1 (metallothionein 1)*, *HK1 (hexokinase 1)* and *GHR (growth hormone receptor).* All these loci, except *HK1* and *GHR*, have been reported to contain microsatellite sequences. The anonymous micro-satellite loci assigned were *Sat13* (Mougel et al., 1997 Anim. Genet. 28, 58) and S*ol33* (Surridge et al., 1997 Anim. Genet. 28, 302). These two loci, as well as the four microsatellite-containing Type I loci, belong to identified linkage groups. DNA from rabbit BAC clones was used as probe for FISH in the case of *CYP2C*, *PMP2*, *ALOX15*, *MT1*, *Sat13* and *Sol33*. For the localization of *HK1* and *GHR*, plasmid clones containing human *HK1* or rabbit *GHR* sequences were used. Localizations were made to six different rabbit chromosomes: OCU 1, 3, 5, 11, 18 and 19. These mapping data add information to the rabbit cytogenetic map, enable the assign-ment of the respective linkage groups to specific chromosomes, and allow for one linkage group alignment along the chromosome.

**B120**

## DNA pooling using dinucleotide microsatellite markers: Chicken extracted DNA, fresh and frozen blood cells

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Costs of linkage mapping of quantitative trait loci (QTL) can be reduced through selective DNA pooling involving densitometric estimates of marker allele frequencies in pooled DNA samples. With poly(TG) microsatellites, such estimates are usually confounded by "shadow" or "stutter" bands, and can be confounded further by differential amplification of alleles. In a previous study with bovine microsatellites, it was shown that the relative intensity of the various shadow bands accompanying an allele main band have a strong positive linear regression on TG repeat number, n, and that the regression equations for the various shadow band orders (i=-1, i=-2, i=-3, i=+1) could be used to correct for the shadow band confounding and accurately estimate allele frequencies. In the present study, the i=-1 regression equation was used to obtain estimates of several chicken microsatellites allele repeat number. Using the allele repeat number estimates in the shadow correction procedure, highly accurate and unbiased allele frequency estimates from pooled DNA samples were obtained, even in the presence of differential amplification. Pooled samples constructed from purified chicken DNA, fresh- and frozen-defrosted chicken blood gave equivalent results. Thus, the use of pooled DNA or blood samples, in conjunction with the regression equations, provides a general technical procedure that can facilitate QTL mapping studies in poultry populations.

**B121**

**Discovery and frequency estimates of single nucleotide polymorphisms (SNP) from pooled DNA**

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Single nucleotide polymorphisms (SNPs) are the most abundant variations in genomes. As such, SNPs are useful for genotyping and high-resolution genetic mapping. Many methods can detect the presence of SNPs in a region of DNA, and many methods can estimate allele frequencies of polymorphisms in a population. Such methods are usually expensive and tedious if many individual animals have to be analysed to obtain allele frequency data. We have adapted an efficient protocol to detect SNP with population allele frequencies in the range of 0.2 to 0.8 based on automated sequencing of PCR product generated from DNA pooled from individuals. Using this protocol, we can readily identify and confirm SNPs for 500bp sequence in the population comprising the pool. Chromatogram peak heights at polymorphic sites vary in proportion to the ratio of the two alleles in the pool. Changes in peak heights from pooled DNA relative to those from individuals, acting as controls, are readily detected by eye in aligned chromatograms. Bi-directional sequencing of the product allows rapid confirmation of the SNP identity. We present some examples of this cost-effective strategy of SNP discovery and frequency estimation in targeted sequences for sheep and cattle.

**B122**

**Two QTLs for growth map to bovine chromosome 14.**

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The Canadian Beef Reference Herd is comprised of only *Bos taurus* breeds, has both purebred and crossbred progeny and is a full sib design (http://skyway.usask.ca/~schmutz). This herd was created to map QTLs that influence production and carcass traits. The growth traits measured were birth weight (BW), weaning weight (WW), yearling weight (YW), average daily gain on pasture (ADG1) and average daily gain in the feedlot (ADG2). The herd was genotyped with 162 microsatellites that were approximately 20 cM apart and had a high level of informativeness. The analysis was a modified identical-by-descent analysis where sib pairs were designated like or unlike genotypically; genetic similarity was tested against the absolute difference of phenotypes using an unpaired, one tailed t-test. When the probability of the sum of the $t^2$ was less than P=0.00156, using a Chi-square distribution with the degrees of freedom equal to the number of informative families, a chromosomal area containing a QTL was identified. A QTL effecting WW, YW, ADG1 but not birth weight was found at BMS2934 (i.e. 67 cM) on chromosome 14. *Corticotrophin releasing hormone (CRH)* maps to 68 cM and is an obvious candidate gene for growth as glucocorticoids are considered to be growth inhibitors. Another QTL that effects BW and ADG2 maps to the centromeric region. Two more candidate genes that are important for growth and metabolism map to this region: *thyroglobulin (TG*) at 7 cM and *cytochrome P450 subfamily X1B polypeptide* 1 *(CYP11B1)*.

**B123**

**Estimate of nucleotide diversity in dogs and other animals using a pool-and-sequence method**

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Nucleotide diversity (π) is a parameter used to determine the amount of sequence variation within a species or population. It is used to gain insights into the genetic structure and history of populations. It can also be used to determine the feasibility of constructing genetic maps based upon single nucleotide polymorphisms (SNPs). Nucleotide diversity has never been estimated in dogs. Segments of twelve canine genes from ten diverse breeds were examined for nucleotide variation by using a pool-and-sequence method. Genomic samples were pooled, PCR-amplified, and sequenced directly. Fourteen SNPs were found in about 5,400 bp of DNA. All of these SNPs were confirmed by restriction enzyme digestion. From these data, canine nucleotide diversity is estimated to be 0.001, a value very similar to that for human populations. Examination of several of these SNPs within individual breeds demonstrated good heterozygosity. These data suggest that it will be relatively easy to develop useful SNP-based maps of the canine genome. The development of SNPs within specific genes will also help to improve comparative mapping strategies for locating genes of interest. Smaller sets of data were also derived for cat, horse, ox and pig that suggest that similar diversity may exist in these species as well.

**B124**

**Polymorphism of *Calpain* locus and relationship with meat tenderness in Piedmont cattle breed**

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Calpain-mediated degradation of myofibrillar proteins is known to be responsible for *post-mortem* meat tenderization. Polymorphism at calpain II regulatory subunit gene (*CAPN*) has been detected in several cattle breeds. Therefore the present investigation was carried out to study the variability of *CAPN* locus in Piedmont breed and to determine whether the polymorphism is related to beef tenderness. 75 subjects were analyzed for *CAPN* genotype, by PCR-RFLP, and for meat tenderness, by Warner-Bratzler shear force (WBs) on LTL at 1, 3, 7 and 11 d *post-mortem*. GLM procedure was used for statistical analysis. The digestion of PCR products with *HhaI* revealed two alleles: $CAPN^{A}$ (900, 620 and 280 bp) and $CAPN^{B}$ (1520 and 280 bp), with frequencies of 0.24 and 0.76 respectively. No significant associations were observed between *CAPN* genotype and Wbs:

| | WBs1 | WBs3 | WBs7 | WBs11 |
|---|---|---|---|---|
| *AA* | 12.20 | 7.99 | 6.82 | 6.27 |
| *AB* | 10.70 | 6.57 | 5.79 | 5.49 |
| *BB* | 9.54 | 7.28 | 6.62 | 5.50 |

The results indicate that this polymorphic site at *CAPN* locus cannot be used to predict beef tenderness.

**B125**

## Genetic variation of Cheju native horses in Korea

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The Cheju native horse is one of the native animals in Korea and has been separated into distinct areas. Therefore, it is a worth study of genetic markers. Random samples of 50 Cheju native horses (CNH) were typed with a set of 12 microsatellites, 7 blood groups, and 9 biochemical polymorphims. The microsatellites loci were amplified by a multiplex procedure. The tests were performed using on ABI 310 automatic prism genetic analyzer. The allele frequencies and exclusion probabilities were estimated. The highest heterozygosity was estimated in microsatellites loci (0.6831). The exclusion probability was estimated using 12 microsatellites, which were larger (PE=0.9972) than 16 systems of blood types (PE=0.9656). The cumulated exclusion probability obtained by microsatellites and blood types was 0.9999. This results indicated that combinations of microsatellites, blood groups, and biochemical polymorphisms could be effective for testing of parentage for the CNH.

**B126**

**Genetic Characterization of Creole Cattle from Argentina, Bolivia and Uruguay.**

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Genetic diversity of seven populations of American Creole cattle adapted to a wide range of environmental conditions (from parallel 50 º to 14 º south), have been characterized by genetic markers. Two populations belonged to Argentina (Patagónico, N=30; and Northwest, N=304), four to Bolivia (Saavedreño, N=198; Yacumeño, N=30; Chaqueño, N=30; and Altiplano, N=11), and one to Uruguay (N=113). Polymorphisms studied by PCR included *BoLA-DYA*, *κ-casein*, $\alpha_{S1}$*-casein*, *β-lactoglobulin*, *PRL*, *GH*, and *F13A*. In addition, blood systems A, B, C, F, J, L, M, S and Z were analyzed by serology methods. Gene and genotype frequencies were estimated by direct counting. The number of alleles within population varied from 1 to 4 for codominant loci, while heterozygosity ranged from 0 to 0.6. Average heterozygosity was similar, varying from 0,343 to 0,385. The $F_{IS}$ index showed that only 5 out of 56 Hardy-Weinberg tests gave significant results. The proposed phenogroups of the B system revealed population and Creole specific alleles. These results provide additional information supporting the existing hypothesis about the historical origin of these breeds. The high degree of genetic diversity observed despite the reduction in population sizes suffered by these breeds during the last century, justifies present and future programs for their conservation.

**B127**

## Preliminary evaluation of the genetic effect of ESR-FSHß genotypes on litter size in pig

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Litter size is one of the most important economical traits in swine and has a major impact on the efficiency of pork production. Phenotypic selection of this trait has been ineffective due to the low heritability of the trait. Finding genes associated with litter size would provide a new and efficient approach for genetic improvement of the litter size trait. Several studies have been conducted to map genes associated with litter size using the candidate gene approach. Estrogen receptor ( ESR ) gene was reported to have a large effect on litter size, approximately 1.5 pigs per litter born and 1 pig born alive (Rothschild et al, 1996 ). More recently follicular stimulating hormone ( FSH ) was found to be a major gene influencing litter size. The average litter size of sows with favourable alleles was 2.53 piglets/litter higher than that of sows with unfavourable alleles. This finding was confirmed in two large populations although the difference between alternative alleles was not as large as originally reported ( Ning Li et al, 1998; Yaofeng Zhao et al, 1999). The purpose of this study was to investigate the effect of genetic combination between ESR gene and FSHß gene. A standard protocol of multiple complex PCR was established to score genotypes of ESR and FSHß genes and was used to genotype 562 sows from seven different breeds (including 3 Chinese native breeds). Two litter size traits, total number born ( TNB ) and the number born alive ( NBA), were analysed using least squares analysis. No significant genetic interaction between ESR locus and FSHß locus was found ( $P > 0.05$ ). The genotype effect on both TNB and NBA was highly significant ( $P < 0.001$ ). Sows with genotype BB-BB has litter sizes 2.1- 3.2 TNB and 2.0-3.0 NBA larger than those of AA-AA genotype. In general, the genetic influence of genotypes in Chinese local breeds is larger than that in European commercial populations. These results imply that selection for the favourable genotype could result in genetic improvement of litter size.

**B128**

**High resolution radiation hybrid map and YAC contig to define a QTL for average daily gain in swine**

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A significant QTL for average daily gain was identified on swine chromosome 1q. This QTL is flanked by two markers (SW373 and SW1301) situated approximately 20 cM apart on the genetic map. In the pursuit of a high-resolution gene map for SSC1q additional markers have been added to the IMpRH map including genes, ESTs, and microsatellites from a microdissected library of the telomeric region of SSC1q. While the initial IMpRH map for this chromosomal region contained only 4 markers, this study provides over 20 new markers within the QTL region. The development of this high-resolution map has subsequently enabled the construction of a partial YAC contig spanning the QTL region. These YACs are also being used to isolate further microsatellites essential for high-resolution linkage mapping in reference and commercial populations. In addition, the combination of physical mapping using YAC and radiation hybrid analysis has enabled a refined estimation of the kb/cR for the IMpRH panel. Mapping two or three markers present in a single YAC on the IMpRH panel has altered this estimate from 70 kb/cR to approximately 15 kb/cR.

**B129**

**GelScore and Genetic Map Maker: Two computer applications essential for radiation hybrid mapping**

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Two computer application have been designed to assist in radiation hybrid mapping. The first of these is GelScore. GelScore is a computer application which allows researchers to accurately and quickly decipher (score) visual data from an agarose gel image. GelScore is able to read/import agarose gel images that are stored in either GIF or JPEG image formats. GelScore automatically overlays a resizable grid onto the displayed gel image. This grid can be interactively resized so that the grid cells align with bands in the image. Initially, each cell is scored as a 0. In order to change this score, the user merely "clicks" (with the mouse button) on any grid cell in order to select the band intensity. The cell will be highlighted at three different levels (0=off (black), 1=on (white), 2=maybe(gray)). Successive clicks will cycle through these three values. The application saves the resulting array of scores to a text file. The gel-scoring application is also able to read a partially scored file so that an image may be partially scored during one session and then re-opened and further scored during another session. This program is an essential tool for radiation hybrid mappers by eliminating scoring errors created by manually entering data. Information regarding this application may be obtained at http://www.ajdcomputing.com/wes/GelScore/.

The second program is Genetic Map Maker. This computer application is designed to graphically display map images using either genetic linkage or radiation hybrid map data. The application reads marker name and map distance data (in Morgans or Rays) and produces an image containing a map of that data. A number of options are available for the creation of the maps including: image format, font face, font size, and map scale. More information about this application is available on the web at http://www.ajdcomputing.com/rachel/mapmaker/.

**B130**

**Interleukin-8 haplotype structure from nucleotide sequence variation in commercial populations of U.S. beef cattle.**

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Interleukin (IL)-8 encodes a proinflammatory cytokine that plays a central role in cell-mediated immunity by attracting and activating neutrophils in the early stages of host defense against bacterial invasion. This report estimates the genetic structure of commercial cattle populations for the IL8 locus, a requisite for studies designed to test whether IL8 alleles are correlated with infection phenotypes. Five previously unknown single nucleotide polymorphism (SNP) markers were identified by electrophoretic DNA sequencing of two IL8 introns that were amplified from a novel collection of 96 individuals representing 17 popular cattle breeds. Assays for automated genotype scoring by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were developed and used to independently verify the five new SNP alleles and two previously known SNPs. These MALDI-TOF MS assays were used to determine the allele and genotype frequencies of breed groups in the SNP discovery panel. Three haplotypes for the set of seven SNPs were assigned and confirmed by analyzing segregation in 313 individuals from MARC reference population. Two additional haplotypes were unambiguously deduced from a homozygous sire and an allele cloned from a heterozygous sire, respectively. A sixth haplotype was identified in a related species, American bison, but was not present in the group of cattle analyzed. The identification of IL8 haplotype structures from commercial populations and the development of robust automated genotype assays provides a means for efficiently using IL8 markers in a variety of genetic studies in production environments.

**B131**

**Identification of QTL for production traits in poultry**

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Poultry meat and eggs continue to be very important agricultural commodities not only in the United States, but worldwide. In order for the poultry industry to continue to flourish, advances in poultry genetics are essential and the identification of QTL, especially for production traits, has to be pursued. In order to achieve this, a meat-type x egg-type resource population was developed using two partially inbred poultry lines (Agriculture Canada Strains 21 and WG) and a large number of economically important traits (growth, reproduction, and mortality) were measured in this population. Using 64 evenly spaced microsatellite markers providing approximately 50% genome coverage at 30 cM marker spacing, 3 sires, 19 dams, 73 F1 individuals and a selected subset of the 586 F2 progeny were genotyped. Initial marker association analysis showed that suggestive markers ($P<0.05$) could be identified on 19 chromosomes or linkage groups with an average of ~10 per trait. In the future, the areas containing these putative QTL will be saturated with additional markers and the remaining F2 progeny will be genotyped and analyzed. Finally, all identified QTL will be assessed as to their existence and segregation in two within-type resource populations. It is hoped that this work will lay the groundwork for the use of linked markers, along with those already identified for disease resistance, in marker assisted selection in poultry.

**B132**

**On criteria of marker-informativeness in an $F_2$/outbred cross context**

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Availability of dense marker maps emphasizes predictors of marker-informativeness (MI) as criteria for marker-selection in the context of different experimental designs. The Polymorphism Information Content (PIC) statistic was developed for a specific 2-generation model. Generalization of PIC to represent MI for a 3-generation $F_2$ cross requires that two additional sources of non-informativeness be added to the PIC formula to account for loss of information: matings between like-heterozygous $F_1$ individuals, one of which is non-informative; and matings between like-heterozygous $F_1$ individuals, which are both fully informative but where line of origin of the same alleles is reciprocal. These *non-additive* terms were added to the PIC formula to yield a general representation of $F_2$ MI. Two computer programs were developed for an $F_2$ cross between divergent selection lines of mice (F=~0.2). A total of 403 markers had been genotyped for $F_0$ grandparents (n=31), and 16 markers had been genotyped in the complete pedigree (n=559 $F_2$). One program (RM) was based on assumptions of random-mating, while the other (SM) took into consideration the pedigreed mating structure. For the 403 markers, average deviation between RM and SM was .007. Correlations between predicted and actual MI for 16 markers were .97 for SM and .92 for RM, while averages of the deviations between predicted and actual values were .01 and .05 for SM and RM, respectively. Corresponding deviations from realized MI never exceeded .09 and .19 for SM and RM, respectively. A computer program to optimize mating system with respect to MI is in preparation.

How to Search

**C001**

**Generation and mapping of expressed sequence tags from virally infected swine cells**

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In an effort to identify genes that are involved in swine alveolar macrophage responses during viral infection, we employed differential display reverse transcription (DDRT) - PCR to identify 78 expressed sequence tags (ESTs) regulated in cultures infected by porcine reproductive and respiratory syndrome virus (PRRSV). Sequence analyses showed that these of ESTs, 13% (10/78) had significant similarity (>93%) to known pig ESTs or genes, and 46% (36/78) matched ESTs or genes from other species with homology > 80%. To determine chromosomal localization in the swine genome, primer pairs were designed to amplify 100 – 300 bp amplicons and PCR-based mapping was performed across a swine somatic cell hybrid or radiation hybrid mapping panel under optimized annealing temperatures. A total of 14 novel porcine ESTs were mapped via the swine somatic cell map, and 43 porcine ESTs were mapped using the swine radiation hybrid map (LOD > 4.8). The swine 2'-5' oligoadenylate synthetase gene, which is considerably downregulated by PRRSV infection at 24 hours post infection, was linked to SW1321 on swine chromosome 14 with LOD = 12.14. These PRRSV-associated porcine ESTs represent good candidates for dissecting host genes which may have major effect on disease resistance and for understanding PRRSV pathogenesis.

**C002**

**Characterization of hepatic gene expression pattern using mRNA differential display of farm animals with differing metabolic types fed with different protein diets**

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The subject of the present study was the identification of DNA sequences differentially expressed in livers of lactating cows from different metabolic types (milk, meat/milk and meat types) and of pigs fed different protein diets (soy protein vs. casein). The technique of differential display of messenger RNA molecules was applied to characterize expression patterns. cDNA bands differentially displayed in silver-stained polyacrylamide gels were cloned, characterized and used to establish cDNA arrays. In cows of different metabolic types, 74.3% of the 737 cDNA bands analyzed were displayed differentially. A database search revealed that 195 of the 295 isolated and characterized sequences exhibited homology to database entries. Most differentially displayed sequences were homologous to known genes of unspecific immune response such as lysozyme, TNF-R, transcriptional factors and signal peptides (each 10-15%). In pigs fed different protein diets, 27 of the 47 differentially displayed sequences exhibited homology to known genes. Of these, about one fourth represent signal peptides such as proton-ATPase-like protein, phosphate-carrier-protein, transmembrane 4 protein and about 10% were molecules of respiratory chain and blood proteins. Differentially displayed sequences were isolated, amplified and used to contrast cDNA arrays. Expression patterns specific for metabolic types in cows and pigs fed two protein diets were identified by cDNA array hybridization.

**C003**

**Microsatellite markers of antibody response in adult chickens**

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A genomic scan approach used microsatellites to investigate quantitative trait loci affecting immune response in the chicken. Highly inbred (> 99%) males of two MHC-congenic Fayoumi lines were mated with G-B1 Leghorn hens. Therefore, the $F_2$ population was essentially a full-sib design with sire line reflecting MHC effect. Adult $F_2$ hens (n=158) were injected twice with sheep erythrocytes and whole fixed *Brucella abortus* (BA). Agglutinating antibody titer at seven days, after primary immunization, and mean titer of the final three samples were used as parameters for primary and equilibrium phases, respectively. Secondary phase parameters of maximum titers and time needed to achieve maximum titers were estimated from seven post-secondary titers using a non-linear regression model. The 20% high and low phenotype birds for each trait formed 16 DNA pools. GeneScan™ peak heights were used to estimate DNA pool allele frequencies of 66 microsatellites. A total of 35 suggestive marker-trait associations (frequency differences >0.15 between high and low pools) were found. Several markers were consistently associated with similar traits (antigen or response phase). Selective or whole population individual typing was used for a few suggestive markers. The ADL0201 and MCW0294 were significantly associated ($p<0.05$, GLM) with equilibrium phase antibody response to BA, and ADL0023 with primary immune response to BA. The results suggest that regions on chromosomes 3, 5, and Z may affect antibody response in the adult chicken.

**C004**

## Polymorphisms in the 5'-flanking region of the bovine β-lactoglobulin gene with possible effects on gene expression

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β-lactoglobulin, the major whey protein in bovine milk, exists in a number of genetic variants of which the most common are A and B. It is well known that the A variant shows a higher expression in milk whereby more β-lactoglobulin is being produced compared to the B variant. The difference in expression is likely to be caused by polymorphism in the 5'-flanking region of the β-lactoglobulin gene. To test this hypothesis, variation was studied in positions -462 (R11) and –435 (R10) relative to the transcription start point in the 5'-flanking region of the bovine β-lactoglobulin gene. Also, the degree of linkage disequilibrium between the alleles at these positions and the protein variant in the coding part of the gene was analysed. The protein variant, A or B, was determined based on the nucleotide sequence coding for the 64$^{th}$ amino acid of the mature protein. The study included 359 cows, 298 of the Swedish Red and White breed and 61 of the Swedish Holstein breed, from the two university experimental herds in Uppsala. Genotyping was based on the Allele Discrimination by Primer Length (ADPL) method. The results show that variation exists in the positions R11 and R10, with intermediate frequencies of the two existing alleles in each position. The same was also found for the protein variants in the coding part of the gene. The linkage disequilibrium between the alleles at the positions R11 and R10, as well as between these alleles and the protein variants in the coding part of the gene, was complete. Thus, in the present material the positions R11 and R10 do not give any additional contribution as regards the variation in expression of the β-lactoglobulin gene.

**C005**

## Fine mapping of QTL for health and fertility in Nordic red cattle

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The reliable recording and breeding evaluation of numerous dairy, beef, health and reproduction traits in the Nordic cattle breeds offer unique possibilities for QTL mapping according to the granddaughter design. Genome scans using this design are performed in Norway, Finland and Sweden. Putative QTL for functional traits have been identified, but the QTL alleles segregate at low frequencies and the mapping resolution in each of the studies is low. However, the populations are related through an extensive historical and current exchange of semen and some of the QTL-results are found in several countries. Under the assumption that the QTL are genetically homogenous, it is possible to increase the mapping resolution by the use of mapping across populations. Such a joint fine mapping project has been initiated, where the main focus is put on five chromosome regions with putative QTL for clinical mastitis. The project relies on a close co-operation with the farmer-owned breeding companies in the respective countries. Currently, new families segregating for the QTL are being selected and high-resolution genetic maps for the actual regions are being developed. This project, which utilizes the pedigree links and comparable trait evaluations between populations, will provide good opportunities to increase the mapping resolution for the QTL and ultimately for the positional cloning of the functional genes.

**C006**

## Characterization of beta-defensins - a family of peptide antibiotics also expressed in the epithelium of the bovine mammary gland

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It is well known that the heritability of mastitis is low and that management and high milk production play a major role in the pathogenesis. Further, the natural defense of the bovine udder is complex and the individual components may play only minor roles. It is however also obvious that naturally occuring variations in general immunity may be relevant to mastitis resistance. A first step to qualify the defensins as candidate genes for udder health is to verify their expression in the bovine mammary gland tissue. We designed oligonucleotides from published bovine defensins. Subsequent PCR analysis of cDNA derived from samples of udder tissue with chronical mastitis revealed expression of genes with very high similarity to the three published bovine epithelial beta-defensins: tracheal antimicrobial peptide (TAP), lingual antimicrobial peptide (LAP), and enteric beta-defensin (EBD). Their translation into amino acids yielded three sequences that are identical with TAP, LAP and EBD, respectively. To test whether the beta-defensin expression is enhanced or its pattern is altered by infection we collected samples from healthy and inflamed udder quarters that are presently being investigated using RT-PCR. In order to characterize the genomic organization of the bovine beta-defensin genes we have isolated four bovine BAC clones that were positive at PCR analysis using the beta-defensin primers. We are sequencing the inserts of these clones using BAC end sequencing and a set of different beta-defensin primers.

**C007**

**Utility of a molecular-based MHC class II typing system to identify broadly-recognized, conserved CD4+ Th1 epitopes for an Equine lentivirus**

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Equine Infectious Anemia Virus (EIAV) is a horse lentivirus that results in a lifelong, persistent infection. CD8+ cytotoxic T-lymphocytes (CTL) may play a direct role in terminating plasma viremia during the acute infection phase. CD4+ type-1 helper T-lymphocytes (Th1) that secrete interferon-γ (IFN-γ) appear to promote CTL activity and help maintain memory CTL. Thus, identifying conserved epitopes broadly recognized by EIAV-specific CD4+ Th1 would contribute significantly to vaccine design strategies. To this end, seven long-term EIAV-infected horses were typed at the MHC class II *DQA, DRA* and *DRB* loci to determine the extent of allelic variation in this group. Peripheral blood mononuclear cells were tested for recognition of EIAV-specific peptides from the Gag p26 capsid region, and a portion of Pol, in standard T-lymphocyte proliferation assays. Both regions are highly conserved among EIAV isolates, and this Pol region has 51-63% amino acid homology to other lentiviral Pol proteins. We identified three peptides recognized by either four or five horses, and all but one horse responded to at least one of the peptides. These peptides were further tested for their ability to induce IFN-γ production in responding T-lymphocytes, and the responsive cell phenotype was confirmed by flow cytometry.

**C008**

**The use of blood groups under creation of the new swine type in the white big breed**

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IF produces 10,000-11,000 tons of pork per year. The IF breeding farm includes 550 sows and 40 boars grows and produces replacement gilts for the breeding and production farms (300 and at least 3000, respectively). The replacement gilts are transferred to the production farm at 7 months of age and 120 kg body weight. The IF breeding farm was formed in 1986 to enable implementation of routine parentage control and verification system based on blood groups. The selection procedure bawed on genetic type of animal was applied since. The selection of breeding boars was based on the medium and increased homozygosity in the hyplotype of 10 genes. Respectively, sows with the medium and partial homozygosity for the hyplotype were also selected. Boars with high immunogen blood groups: *Bb, Ea, Eb* and *Ga* were excluded from the breeding program. The greatest change in allele frequencies of the *A-and-K* loci was carried out. Current frequency of the *AO-and-Kb* genotype equals 1 at the herd level. Average litter size has increased to 12.5 piglets farrowed and more than 13 piglets for the most mature sows. The piglet mortality rate was improved. The high herd uniformity for carcass traits was achieved with distinct type within the Large White breed. The new pig type grows to 100 kg within 190-200 days, with a fat thickness of 26 mm and ham weight of 10.8 kg. Sows have 7 pairs of developed teats. The created population is used at a maternal line in crossing with animals of the Duroc breed.

**C009**

**PCR-RFLP of bovine MHC class I genes**

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In studies of variation in disease susceptibility, the major histocompatibility complex (MHC) is of particular interest. Polymorphism in these genes has direct functional consequences, since the antigen binding sites of the molecules are encoded by the most polymorphic exons. Thus, the ability to characterise MHC polymorphism is important in our understanding of the genetics of immune responsiveness. In the present study we have developed methods for the PCR amplification of bovine MHC class I gene fragments, and for the analysis of polymorphic exons of these genes. Initially, class I gene fragments were amplified from genomic DNA samples from animals that had been typed in the 5th BoLA workshop and from a genomic class I clone encoding BoLA-A11 specificity. A fragment of 700bp comprising exon 2, intron 2 and exon 3 was amplified from genomic DNA of BoLA-typed animals. The amplified fragments were analysed using a panel of restriction enzymes that were expected to produce polymorphic patterns. The results were interpreted by correlating the DNA polymorphisms with the known serological types of the animals used. A few bands in digestions with *Taq*I and *Dde*I could be associated with specific BoLA-A types. However, as more BoLA types were added to the analysis, less discrimination was apparent, i.e. sharing of bands between BoLA types was seen. The application of this approach to the analysis of BoLA class I polymorphism in European and Indian cattle breeds will be discussed.

**C010**

## Sequence divergence at the 3'-end of *BoLA-DQB* genes suggests multiple allelic lineages

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The MHC *DQ* genes of cattle appear to have evolved by gene duplication and divergence events. Each cattle class II haplotype expresses one or more DQ products due to duplication of the *DQ* genes in about half of the common class II haplotypes. This is functionally significant since the polymorphism of both *DQA* and *DQB* genes, combined with duplication, has the potential to markedly increase the variety of class II molecules at the cell surface by different pairings of alpha and beta chains. While almost 40 alleles of the *DQA* and *DQB* genes have been characterised by sequencing of the polymorphic second exon, these data are insufficient to clearly assign all of the *DQA* and *DQB* alleles to specific loci, and additional haplotype data are generally lacking. Analysis of full-length sequences from known haplotypes may provide a clearer picture of *DQ* gene evolution. Full-length *DQB* sequences were amplified by RT-PCR from bovine class II haplotypes with both duplicated and unduplicated *DQ* genes. Sequencing of clones derived using specific 3'-end primers and 3'-RACE demonstrated both length and sequence differences in the 3'-untranslated regions, which may be characteristic of different *DQB* gene lineages.

**C011**

**Relationship between polymorphism in DRB1 gene and nematode fecal egg count in sheep**

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The study aimed at determining the effects of ovine major histocompatibility complex (OLA) class II alleles on resistance to parasite infection in sheep. Two generations (592 animals in total) of Polish Heath Sheep (Romanov type) were examined over a period of four years (1995-1998). Gastrointestinal fecal egg count (FEC), an indicator trait of resistance to GI nematodes, was determined using McMaster's method four (in lambs) to six (in ewes) times during pasture season each year. Molecular analysis of polymorphism in OLA class II DRB1 gene was performed using PCR method. According to published data (Ammer et al., 1992 Immunogenet. 35, 332; Ellegren et al., 1993 Anim. Genet. 24, 269; Schweiger et al., 1993 Molec.Ecol. 2, 55 also J. Mol. Evol. 37, 260) the analysed DRB1 gene fragment contains exon 2 and microsatellite $(gt)_n(ga)_m$ in intron 2. The length of amplified DRB1 gene fragment was determined with the help of DNA sequencer and then used for allele identification. In total, 20 alleles (454 bp to 576 bp) were found and the most frequent were 488 bp and 506 bp (0.163 and 0.181, respectively). Association between FEC and polymorphism in DRB1 gene was evaluated using GLM procedure (SAS Institute, Cary, NC 1990). A statistically significant effect was found of genotype in DRB1 locus on nematode egg counts in feces of examined sheep. Comparison of the frequency of DRB1 alelles in two groups of animals with FEC of lower and higher than average value indicates that 488 bp and 530 bp alleles are potential markers for resistance to gastrointestinal nematode infection in sheep.

**C012**

**An immunogenetic investigation of *B* haplotypes of the *Mhc* in northern bobwhite/ masked bobwhite hybrid quail**

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The evolutionary role of the *Mhc* in protecting avian populations from certain disease organisms suggests that natural diversity of haplotypes may be critical in the preservation of endangered populations. To obtain information on *Mhc* haplotype diversity in the endangered masked bobwhite quail (*Colinus ridgwayi*), we are utilizing a captive population of a non-endangered subspecies, the northern bobwhite quail *(Colinus virginianus)*. In addition to immunogenetic analysis of pedigreed families within the quail species, we have produced two large families of hybrids by crossing two masked males individually to northern females. Several antisera produced within the families proved to be specific for *B* haplotypes segregating in the hybrid families. To obtain antisera in greater quantities for typing the masked quail, xenogenic antisera were produced in ring-necked pheasants. Donor erythrocytes from a masked bobwhite sire and one of his hybrid sons were used to individually immunize ring-necked pheasants. Both antisera proved to be specific for haplotype *B8* in the pheasant. An additional source of antisera in large quantities is chicken anti-chicken antisera specific for B-F or B-G subregional antigens of the chicken. By appropriate absorptions with selected quail erythrocytes, such antisera prove to be specific for particular quail *B* haplotypes. With the utilization of antisera produced within and between selected species, the objective of monitoring the *Mhc* haplotype frequencies in the colony of masked bobwhite breeders maintained by the U.S. Fish and Wildlife Service appears reasonable.

**C013**

**Facilitating detection of physiological effects of polymorphic gene systems through mating design**

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Parent lines transmitting alleles of 11 polymorphic gene systems are being utilized for producing progeny of appropriate genotype combinations for physiological evaluation. Two parent lines, derived originally from White Leghorn and Ancona stocks, have been synthesized utilizing *B* system haplotypes of the *Mhc* with established physiological effects as the basis for evaluating genotypes resulting from simultaneous segregation of alleles of the other 10 genetic systems, including *Rfp-Y*, the second cluster of *Mhc* genes in the chicken. Presently the repertoire of haplotypes of the two *Mhc* regions includes *B2, B5, B19* and *B21*, and *Rfp-Y 1.1*, *Rfp-Y1.2*, *Rfp-Y2*, *Rfp-Y3* and *Rfp-Y6*. Superimposed on the *Mhc* haplotypes are simultaneously segregating alleles of nine alloantigen systems: *A1, A2, A3* and *A8*; *C2, C3* and *C5*; *D1, D2* and *D3*; *E1, E2, E3* and *E5*; *H1* and *H2*; *I2* and *I8*; *K2* and *K3*; *L1* and *L2*; and *P1* and *P4*. The production of progeny for evaluation in pedigreed families allows for full genetic analysis of direct effects as well as interaction between system genotypes. Progeny with segregating haplotypes have demonstrated significant effects of one or more systems for immune response against virus induced tumors and/or macrophage function. Progeny of the Northern Illinois University parent lines are made available to interested investigators through collaborative research.

**C014**

## BoLA-DRB/DQB haplotypes as molecular markers of genetic susceptibility and resistance to bovine dermatophilosis

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Bovine dermatophilosis is a severe skin infection inducing a loss in productivity and a 15% mortality rate. This disease is associated with the tick *Amblyomma variegatum*. Currently, no vaccine is expected and chemoresistance phenomena decrease the means of control (acaricides and antibiotics). Breeders' observed that the disease seemed to be controlled by genetic determinism. Based on an 8 year-long ecopathological survey of 568 zebu Brahman cattle from several herds located in Martinique Island (FWI), we classified into two extreme groups 123 unrelated animals of both sexes, reared in the same environmental conditions. The most resistant individuals (n=61) were never infected whereas the susceptible individuals (n=62) showed severe clinical signs and later died. Using a candidate gene approach we studied the DNA polymorphisms of BoLA-DRB3 and DQB genes encoding molecules involved in the pathogen/host interface mechanisms. Several BoLA-DRB3 and DQB alleles seem strongly linked in particular haplotypes. The DRB3*09 (fda) PCR-RFLP allele linked to the DQB*1804 allele constitutes a highly significant marker for susceptibility ($P<0.001$). Eugenic selection was developed in the field by eliminating the animals with this haplotype for susceptibility, and the disease prevalence was reduced from 0.76 to 0.10 over 4 years. On the other hand, the BoLA-DRB3.2*4201(gaa) linked to the DQB*1805 constitutes a haplotype correlating with the resistance character ($P<0.001$). These identified markers were validated in other cattle breeds in Africa (Gudali zebu) and Madagascar (Brahman). An F1 crossbreeding plan to study the transmission of the genotypic and phenotypic characters of dermatophilosis resistance and susceptibility based on these BoLA DRB3/DQB haplotypes is in progress.

**C015**

## Association between equine leukocyte antigens and allergen-specific serum immunoglobulin E levels in Lipizzan horses

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IgE plays a key role in the pathogenesis of atopy, a subgroup of allergic diseases with a genetic predisposition to generate IgE against innocuous environmental allergens. Additionally to genetic factors that influence a general predisposition for high IgE responses, the MHC influences the ability to produce specific IgE against small, well-defined allergens. The aim of the presented study was to investigate whether an association between ELA class I alleles and specific serum IgE levels can be demonstrated in the horse. For that purpose, IgE levels against *Alternaria alternata* (Alt a) and *Aspergillus fumigatus* (Asp f*)* extracts and against recombinant (r) Alt a 1 and rAsp f 7 and 8 were determined in sera from 427 Lipizzans from six studs. Serological ELA-typing was performed according to Lazary et al. (1988, Animal Genetics 19, 447). Effects of stud, sex and age on allergen-specific IgE levels were included in the gene substitution models used to test effects of ELA class I alleles on specific IgE levels. A significant ($p<0.05$) positive association between ELA-A1 and rAsp f 7 –specific IgE, and significant ($p<0.01$) negative associations between ELA-A8 and rAlt a 1, rAsp f 7 and rAsp f 8 –specific IgE were demonstrated. Furthermore, ELA-A1 was associated with higher IgE levels against Asp f extract ($p<0.05$) and ELA-A14 with lower IgE titres against Alt a and Asp f extracts ($p<0.05$). further studies like segregation studies in families and determination of ELA class II alleles are needed to better characterise the MHC associations identified in this investigation.

**C016**

**Analysis of molecular factors affecting variability in BSE and scrapie susceptibility**

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The susceptibility or incubation period of scrapie in sheep is influenced by a number of variations within the PrP coding region (codons 136 A/V,154 Q/R and 171 Q/R/H), however these variants are not sufficient to cause disease. In cattle there is less variation in the PrP coding region; this is limited to differences in the number of octapeptide repeats (5, 6 or 7) and two silent single nucleotide polymorphisms (SNP). Linkage studies have not associated these polymorphisms with incidence of disease. The 6-octapeptide repeat allele is the most common and subdivided by an internal SNP (A and C alleles; 5-repeat allele termed B). BSE-affected animals and their relatives are found to be more likely to be of the AA genotype. The cattle studies suggest that there may be other polymorphisms outside the PrP coding region that may influence gene expression. We have determined the sequence of the bovine PrP gene. An strategy of overlapping primers has been implemented that allows direct sequence determination of the entire 22kb PrP gene. New polymorphisms identified so far include 4 insertions or deletions and 15 SNPs. These variants will be tested in association studies on 4 large half-sib families in which BSE occurred. This approach is also being used in a study of the sheep sheep PrP gene using 16 Norwegian sheep DNA samples that carry all known PrP ORF variants. We are also scanning the cattle genome for other genes that may affect BSE susceptibility, using both microsatellite and AFLP markers. The case and control samples from large half-sib families will be screened with 146 microsatellite markers that are polymorphic in the sires. The AFLP technique is being applied to pooled groups and individual DNA samples from these families. Comparison of animals with BSE with negative control cases may identify polymorphisms that are linked to the disease states.

**C017**

## The relationship between polymorphism of the *BoLA-DRB3* gene and resistance or susceptibility to bovine leukemia virus-induced lymphoma

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For the characterization of bovine leukemia virus (BLV)-induced leukemogenesis, we investigated the yet unknown association between polymorphism of *BoLA-DRB3* gene and BLV-induced lymphoma. The nucleotide sequencing of exon 2 of the *BoLA-DRB3* gene, which is highly polymorphic, of 81 BLV-infected animals with 3 independent stages such as an aleukemic healthy, persistent lymphocytosis (PL) and lymphoma was determined by PCR-sequenced-based typing. We identified 23 distinct *BoLA-DRB3* alleles, including 3 new alleles. The population of healthy cattle positive for *BoLA-DRB3*1401* was higher than the proportion-bearing individuals in 200 control cases that were positive for the same allele. By contrast, the *BoLA-DRB3*1601* allele was found most frequently in cattle with PL and lymphoma. Sequence analysis revealed that, approximately 56% of 43 BLV-infected but healthy cattle carry at least one *BoLA-DRB3* allele encoding $Arg^{71}$ or $Lys^{71}$, $Glu^{74}$, $Arg^{77}$ and $Val^{78}$ of 1 domain of DR molecule, which suggested that alleles encoding the KERV and RERV motifs might protect against tumor development. By contrast, approximately 70% of 23 BLV-infected cattle with lymphoma carry two alleles encoding $Ala^{74}$, $Thr^{77}$ and $Tyr^{78}$, indicating that the ATY/ ATY genotype might be associated with susceptibility to lymphoma. Animals carry alleles encoding KERV or RERV were distinguished with the combination of *Psr*I and *Dra*III digestion of the PCR products. Such, these results suggest that the existence of alleles associated with resistance and susceptibility to BLV-induced leukemogenesis.

**C018**

## Gene frequency of κ-casein *E* in Swedish Red and White breeding bulls

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A continuous decrease in cheese yield from milk has been observed by the dairy industry in both Sweden and Finland. This coincides with an observed increase of the genetic variant *E* of κ-casein. This variant results from a single nucleotide substitution that leads to an amino acid exchange from serine to glycine. Because serine plays an important role in the κ-casein molecule through its binding of calcium, the loss of a the serine molecule is likely to affect casein micelle stability and thereby the firmness of the milk coagulum. The *E*-variant has in several studies been associated with poor coagulation properties of milk, which is why means to control the occurrence of this allele in dairy cattle breeds is likely to be of interest to the dairy industry. A recent Finnish study reported an unexpectedly high frequency of the *E*-variant (0.307) in the Finnish Ayrshire breed (FAy). Because of the large influx of genetic material from FAy to the Swedish Red and White breed (SRB) during the last decades, it was considered urgent to get an estimate of the current distribution of this allele among SRB breeding bulls. Altogether, 300 proven and unproven bulls were genotyped for the *κ-casein* locus. DNA was extracted from sperm and genotyping was performed using the Allele Discrimination by Primer Length method (Lindersson et al., 1995 Anim. Genet. 26, 67). The observed frequencies of the *A*, *B*, and *E* variants of *κ-casein* were 0.647, 0.133, and 0.220, respectively. In an international perspective, the frequency of the *E*-variant in the sample of SRB breeding bulls is high. We are currently investigating the coagulation properties of κ-casein E, relative to the *A* and *B* variants of the protein.

**C019**

**Utilization of illegitimate transcription for the analysis of bovine follicle-stimulating hormone receptor gene**

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The synthesis of *mRNA* of various tissues-specific genes in blood cells, denominated as illegitimate transcription, was recently reported. The literature has demonstrated the possibility of identifying mutations in the human *follicle-stimulating hormone receptor* gene (*FSHR*) by exploring this phenomenon. This work aimed to: i) demonstrate the occurrence of this type of transcription in cattle and ii) to evaluate the feasibility of using this approach for the search of molecular markers. Leukocyte *mRNA* was purified from bovine whole blood. *cDNA* was synthesized by reverse transcription and amplified by the polymerase chain reaction (*PCR*) and *nested-PCR* with specific primers for the bovine *FSHR* gene. The amplified *DNA* fragment was digested with *Hind III* and the products were analyzed in silver-stained polyacrylamide gel electrophoresis. The results indicate the synthesis of *mRNA* corresponding to this gene in bovine blood cells. The non-tissue-specific transcription observed corresponds to the phenomenon described in humans where basal transcription of any gene occurs in any cellular type. The feasibility of the amplification by *RT-PCR* showed the possibility of using this approach as a tool for the analysis of the transcription of any specific gene in blood cells.

**C020**

**Transcript profiling of adult and fetal spleen using a cattle microarray**

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The large scale sequencing of cattle ESTs has created invaluable resources for functional genomics. We have created a 768 gene microarray on glass slides using PCR products derived from cDNA clones sequenced from cattle spleen and normalized placenta libraries. Hybridization experiments with total RNA samples extracted from adult and fetal spleen resulted in the identification of fifteen sets of spots representing twelve unique genes with significant two-fold or greater differential expression between samples. Among the genes differentially expressed, three are involved in immunological function (*IgM heavy chain, Ig J chain* and *cathepsin S*), two are genes associated with cell structure (*Collagen 1a2* and *Actin alpha 2*), and four are genes of unknown function. These results demonstrate the power of our prototype microarray for revealing developmental changes in gene expression related to immune function. As the number of genes on our microarrays increases transcript profiling will become an increasingly powerful tool for deciphering the regulatory pathways that govern cell and animal physiology, including lactation, immunity, reproduction and nutrition.

**C021**

**Differential expression of the *GTL2* gene from the *callipyge* region of ovine chromosome 18**

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The inheritance pattern of the skeletal muscle hypertrophy phenotype due to the *callipyge* gene has been characterized as polar overdominance. We hypothesized that this trait must be due to a gain or loss of expression of a gene due to the reversible nature of the gene in paternal versus maternal inheritance. Therefore, suppression subtraction cDNA probes were made from skeletal muscle mRNA from normal (NN) and *callipyge* ($C^{Pat}N^{Mat}$) lambs at 14 and 56 days of age. The subtraction probes (NN-CN and CN-NN) were hybridized to Southern blots containing 35 bovine and ovine bacterial artificial chromosomes (BAC) that comprise a physical contig of the callipyge region. The CN-NN probes hybridized to restriction enzyme fragments from two ovine and seven bovine BAC. Sequence analysis of the subcloned genomic DNA and partial cDNA clones, isolated by hybrid selection, indicates short regions of similarity to mouse *gtl2*. Northern blots of RNA from three muscles that undergo hypertrophy in *callipyge* animals were probed with the ovine *GTL2* cDNA. A population of *GTL2* mRNA centered around 2,400 nt were abundantly expressed in 14 day prenatal NN and $C^{Pat}N^{Mat}$ lambs but were down regulated in 14 day and 56 day postnatal NN lambs. The expression of *GTL2* remained elevated in 14 and 56 day old $C^{Pat}N^{Mat}$ lambs as well as in 56 day old $N^{Pat}C^{Mat}$ and CC lambs. Expression of *GTL2* in the supraspinatus, which does not undergo hypertrophy, was very low for all genotypes and ages. Ovine *GTL2* is an excellent candidate for the *callipyge* gene due to its chromosomal position and altered postnatal expression in muscles that undergo hypertrophy in lambs carrying the C allele.

**C022**

**Identification and characterization of genes involved in female cattle reproduction by use of representational differential analysis (RDA)**

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RDA is a PCR based subtraction method developed both for identification of differences between genomic DNA sequences and for detection of differential gene expression. We have used this technique for isolation of genes expressed in the corpus luteum of the ovary in cattle. So far very few expressed sequence tags (EST) have been published in cattle, and there is a strong interest for identification and mapping of genes related to reproductive traits. The corpus luteum, which is formed in the ovary after ovulation, function as a secretory gland during the luteal phase and, if initiated, throughout pregnancy. In the first experiments skeletal muscle RNA was used as the subtracting agent, for the isolation of specific transcripts from the *corpus luteum*. Sequences from previously known bovine genes, sequences with homology to known human genes as well as unknown sequences from putative new genes have been identified. Hybridization of identified genes to northern blots of RNA from *corpus luteum* and muscle reveals the strength of the RDA technique for this application. Further characterization of isolated genes includes genome mapping and analysis of tissue specific expression levels.

**C023**

**Identification of genes associated with genetic variability of hepatic lipid metabolism in Chickens by mRNA differental display analysis.**

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The hepatic lipid metabolism plays a major role in the chicken fat deposition and fatness variability. In order to identify some of the genes involved in the regulation of this process an anonymous approach was performed by mRNA differential display analysis. Five individuals were selected from lean and fat chicken lines according respectively to low and high mRNA levels of gene coding for Stearoyl-CoA desaturase, Apolipoprotein A1, ATP citrate lyase, C/EBPα and Malic Enzyme. In the aim of reducing the amount of false positive results, some modifications were added to the original differential display technique. The first one was to use longer primers associated with more stringent PCR conditions. Then, in order to free from contaminations when removing the cDNA bands of interest from differential display analysis, single strand conformation polymorphism gel electrophoresis were performed, allowing better recovering of this bands. Finally, the screening procedure was lightened by using a reverse northern blot procedure. After reamplification, the cDNAs were "dot blotted" onto duplicate filters and hybridized separatly with labeled cDNA probe synthetised from lean and fat chicken mRNA samples. These studies allowed to isolate 56 cDNA, with potentially different expression levels between fat and lean chicken, some of them presenting similarity with sequenced genes or EST present in the Genbank database. The identification and characterisation of these differentially expressed genes will point out those which are playing a role in fattening.

**C024**

## QTLs for total and differential leukocyte numbers in the pig

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QTLs with significant effects on porcine immune capacity traits have been identified in a reference pedigree (European Wild Boar x Yorkshire). The purpose of the present study was to further analyse the QTLs for total and differential leukocyte numbers. Two $F_2$ sows were back-crossed to Yorkshire boars. One of the resulting $F_3$ boars was mated to 5 Yorkshire sows and produced the 47 piglets used in the present study. Total numbers of peripheral white blood cells (WBC), proportion and numbers of polymorphonuclear leukocytes (neutrophils, eosinophils, basophils) and mononuclear cells (total, $IgM^+$, $CD2^+$, $CD4^+$, $CD8^+$, MHC class $II^+$, $N1c^+$ cells) were determined by conventional heamatology and immunolabelling combined with flow cytometry. In addition, blood haemoglobin (HB) and hematocrit (HEM) levels were recorded. All individuals were genotyped for the microsatellite markers S0082, Sw373 and Sw974 on chr 1, and S0069 and S0086 on chr 8. The PCR amplified products were separated in standard sequencing gel and visualised by autoradiography. Pigs heterozygous for Wild boar/Yorkshire alleles at the chr 1 loci had slightly higher numbers of WBC ($p< 0.10$), band formed neutrophils ($p< 0.001$) and $CD8^+$ cells ($p< 0.001$) compared to pigs with only Yorkshire alleles. Wild boar alleles at the chr 8 loci conferred higher HB and HEM levels ($p< 0.01$), slightly higher numbers of mature neutrophils ($p< 0.10$), but fewer $CD4^+$ cells ($p< 0.05$) compared to Sw Yorkshire alleles. Thus, the results confirm that the chromosome regions have an impact on number of peripheral blood cells.

**C025**

**Tandem duplications in bovine mitochondrial DNA disrupting a D-loop control element**

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Mitochondrial genes are essential for cellular metabolism and numerous mitochondrial DNA (mtDNA) rearrangements have been associated with clinical phenotypes in humans. During routine PCR-amplification of bovine mtDNA control regions (CRs), we observed a 'fuzzy' band, indicating heteroplasmic length variability in an individual. Repeated amplifications using this DNA sample and several controls yielded identical results. The PCR product with suspected length variation was therefore isolated and cloned in a plasmid vector. Gel analysis of cloned inserts revealed considerable length variation between individual clones, ranging from ~ 25 to ~200 bp. Seven clones selected for sequencing revealed tandem duplications of a 22 bp element, corresponding to nucleotide positions 15968-15989 of the bovine reference sequence, as cause of the observed CR length heterogeneity. From one (wild type) to nine copies of this sequence were detected. Other 5' and 3' flanking sequences were completely identical in these clones. Secondary structure analyses of the repeated sequence and its flanking regions showed that the duplications disrupt a stem-loop structure that has been suggested as recognition site for the arrest of H-strand synthesis. Possible adverse phenotypic effects of these insertions are therefore currently being investigated.

**C026**

**Development of a real-time PCR detection method for the quantitation of MPO transcripts in Porcine tissues.**

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*Myeloperoxidase* (MPO) is a hemoprotein present in azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. It catalyzes the oxidation of halide ions to their respective hypohalous acids, which are used for microbial killing by phagocytic cells. Measurement of MPO activity is often used as a marker of neutrophil infiltration into tissues. We have designed a quantitative RT-PCR detection method for porcine MPO transcripts using TaqMan real-time PCR technology. Forward and reverse primers plus a flourescent probe were developed for MPO and the housekeeping gene *Beta-Actin* (ACTB*).* Total RNA was isolated from lung and spleen tissue collected 7 days post-intranasal inoculation with *Salmonella choleraesuis* (n=4) or saline (n=4). The lung and spleen samples were pooled before RNA isolation to create negative control and infected RNA's for each tissue. MPO expression was normalized for ACTB expression, and reported using relative units (RU) in reference to the negative control lung (1.00 ± 0.146 RU). Expression of MPO mRNA was highest in infected spleen (12.54 ± 2.847 RU), followed by the control spleen sample (2.91 ± 1.499 RU). There was no difference in MPO expression between control and infected lung samples. In conclusion, levels of MPO mRNA expression in porcine spleen and lung indicate a differential response to infection between the 2 tissues. This difference may be associated with bacterial-host adaptation of *S. choleraesuis*. The TaqMan assay for MPO can also be used to discover tissue-specific responses between individuals or groups of pigs exhibiting distinct phenotypic responses to infection.

**C027**

## Association between Leptin (LEP) / Leptin receptor (LEPR) polymorphisms and fatness related traits in a porcine resource family

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Leptin (LEP) with its receptor (LEPR) is a key hormone for the regulation of energy balance in mammals. Mutations in the LEP and LEPR are causative for the morbid obesity in ob- and db- mouse strains. In human families genetic variants of both genes are associated to obesity related phenotypes. A polymorphism in the LEP and two polymorphisms in the LEPR (Stratil et al., 1997, 1998) were typed in 390 $F_2$ pigs of a resource family derived from a cross of the Berlin Miniature Pig and Duroc. Least square means for quantitative traits including growth, feed efficiency, back fat measurements, lean meat content and meat quality were calculated for each genotype and the genotype combination of the two LEPR polymorphisms. Compared to commercial breeds the pigs of the resource family showed a high degree of fatness (meat to fat ratio 1.25; back fat 37.9 mm). Both the LEP-genotypes and the combination of the LEPR-genotypes were significantly associated with the lean meat content (LEP:p=0.002; LEPR:p=0.0001), back fat (LEP:p=0.002; LEPR:p=0.0006) and the meat to fat ratio (LEP:p=0.0006; LEPR:p=0.0001). The phenotypic differences in backfat measurements between the LEP-genotypes were up to 5 mm and for the LEPR-genotypes up to 9 mm. A significant interaction was found due to the fact that the combination of the LEPR-genotypes with the biggest effects on body fatness were detected within a single LEP-genotype. It is not clear whether the typed polymorphisms are functional or in linkage disequilibrium with causal mutations influencing body fatness in pigs.

**C028**

## A comparison of the delta 6 desaturase genes of marine and fresh water teleosts

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Our objective is to determine the genetic and molecular basis for the fact that marine teleosts, unlike their fresh water counterparts, have a repressed ability to synthesise long chain highly unsaturated fatty acids (HUFA). These include the omega-3 HUFA's eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) which, in non-marine species, are synthesised from the 18-carbon precursor linolenic acid (18:3n-3). The deficiency in HUFA biosynthesis in marine fish is of considerable practical significance because, in consequence, farmed marine species require a dietary source of presynthesised HUFA. This is provided by processed products of "feed" species of marine fish. These include sand eels and other "industrial" fish, which themselves obtain HUFA through the marine food chain. Indicators suggest that the wild fishery supporting the aquaculture feed industry is unsustainable at current levels of exploitation. Consideration of the complex HUFA synthetic pathway indicates several steps which could be compromised in marine fish. However, we have chosen to examine first the delta 6 desaturation step as it is known to be rate limiting in mammals. To this end, we have cloned and compared the delta 6 desaturase genes of representative marine and freshwater teleost species. The genes are being compared with a view to relating structural and potential functional differences with different HUFA synthesis phenotypes.

**C029**

**Detection of QTL influencing conformation traits in Holstein-Friesian cattle**

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An extension of our previous genome scan (Heyen et al. *Physiological Genomics* 1:165-175) was conducted in the North American Holstein-Friesian population for quantitative trait loci (QTL) affecting conformation traits using a granddaughter design. Resource families consisted of 1011 sons of eight elite sires. Genome coverage was estimated to be 2551 cM (85%) for 174 markers. Each marker was tested for effects on 22 type traits including body, udder, feet and legs, and dairy conformation using analysis of variance. Joint analysis of all families identified marker effects on 6 chromosomes (BTA1, 5, 6, 7, 10, 11) that exceeded the suggestive threshold for QTL effects. Marker effects on the predicted transmitted ability for overall type (genomewide significance) and front teat placement (suggestive significance) were found on BTA5 in family 2. A multimarker regression analysis was performed to refine the map position of this QTL on BTA5. Confidence intervals for this QTL overlap with a previously identified QTL for somatic cell score. These data suggest the presence of a single gene or closely linked genes influencing phenotypically correlated traits. The QTL identified in this study maybe useful for marker-assisted selection and for selection of a refined set of candidate genes affecting these traits.

**C030**

## Use of a genetic map from the SALMAP project to localise the *NRAMP*-ß gene on the rainbow trout (*Oncorhynchus mykiss*) genetic linkage map

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Apart from serving a function as carriers of divalent cations, the *NRAMP* genes appears to be involved in the general resistance to infectious pathogens. Genetic variation in the *NRAMP* genes has been found to be associated with resistance to intracellular parasites in different species. Aquacultural species are exposed to a large variety of pathogens in their environment and the *NRAMP* genes are therefore interesting as potential candidate genes for QTLs for genetic resistance towards infections. The genetic localisation of these genes in the present genetic maps of the species makes it possible to study the effect of the genes in resource families. A PCR primer set spanning an intron of the *NRAMP*-ß gene was designed based on the available cDNA sequence of the gene in rainbow trout and identification of intron-exon boundaries by comparison to homologous human sequences. A single nucleotide polymorphism (SNP) was detected by direct sequencing of the PCR product from 6 individuals. An allele specific PCR was designed to genotype the SNP. The SALMAP reference family lot 25 was genotyped for the SNP and linkage analysis was performed against the approx. 300 primarily anonymous markers in the current map (Sakamoto et al. in press. Genetics) generated within the EU-funded SALMAP project. This made it possible to locate the *NRAMP*-ß gene within the genetic map of rainbow trout. The inclusion of a potential candidate gene for a QTL of considerable interest for the aquaculture industry along with future incorporation of additional genes will greatly improve the value of the genetic map of the rainbow trout, when utilising the information for conducting genome scans for QTL studies and genetic mapping of single gene effects.

**C031**

## Genetic variations at the type I and type II markers loci in different Chinese local and Western commercial pig breeds

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Various type I and type II markers in 31 Chinese local pig breeds (strains) distributed in Central and Eastern China and 4 commonly used Western commercial pig breeds imported to China have been being studied during the near 10 years. For the type II markers, 11 blood group loci, 6 protein loci, 120 RAPD primers and 10 microsatellite loci were tested. The results show that the $E^{aeg}$ (0.34-1.0 vs 0.001-0.28), $F^{a}$ (0.50-0.79 vs 0.0005-0.09) and $Tf^{C}$ (0.13-0.37 vs 0.001-0.01) alleles may be considered as three genetic markers which differed the Chinese native pigs from the Western pigs. In the RAPD test, 20% of the studied 120 primers proved to be polymorphism. Typical RAPD band was found in some Chinese local pigs by primer I07. The 10 microsatellite loci test were carried out by ABI 310, all the 10 tested loci in almost all 20 tested Chinese local pig breeds have been found with new allele sizes additional to the FAO-MoDAD suggested ones, especially at the loci S0101 and S0090. For the type I markers, researches have been carrying on in the typical Chinese native pig breeds Taihu (170), Chinese minipigs (214) and 20 other Chinese local and Western pig breeds and 2 of their synthetic new pig breeds. Candidate genes EsR, FSHB, PRLR for pig reproductivity, Hal, HSL, H-FABP, Myogenin, GDF-8 for pork quality and quantity, GH,IGF-1,PIT-1 for growth, Kit,MC1R for coat color and NRAMP1 for general bacteria resistance (diarrhoea) have been being tested in the above pig populations by PCR-RFLP or/and PCR-SSCP. Tests and analysis on the EsR, FSHB, Hal and HSL have been finished, the others are going to be completed by the end of July, 2001. The EsR and FSHB loci have both demonstrated large genetic polymorphism. Their effect on six pig reproductive traits were analyzed in Yorkshire (350), Duroc (76), Landrace(152), Erhualian (108), Xian pig (129) and Wuzhishan pig (85) breeds. The least square mean analysis show that sows of BB genotype at EsR and FSHB locus respectively produces 0.63-3.58, 0.55-2.21 NBA (number born alive) per parity more than those of AA genotype. The frequency of $Hal^{n}$ gene in the tested Western pigs are 0.014 - 0.583 while no $Hal^{n}$ was found in the 20 studied Chinese pig breeds. The differences of $HSL^{B}$ gene frequency between the Chinese and Western pigs are also statistically significant ( 0.093-0.2727 vs 0.5-0.9794). Relationship studies show no significant differences between various HSL genotypes and pig growth and carcass performances, although the HSL AB heterozygote presents better performances at the back fat thickness, average daily gain and feed efficiency in both Western and Chinese-Western synthetic pig populations. The large genetic differentiation between the Chinese local and the Western commercial pigs at both type I and II markers loci proved by the present study show that the wide biodiversity in the Chinese native pigs is still an irreplaceable resource for the future pig industry development.

**C032**

**Association between the prolactin receptor gene and reproductive components in swine**

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Previous studies by Iowa State University and PIC have shown the *prolactin receptor* (*PRLR*) gene to be positively associated with litter traits in swine. In the current study, the *PRLR* was investigated as a potential candidate gene influencing reproductive components in swine. A total of 46 Yorkshire, 27 Large White, and 69 crossbred females were genotyped at the *PRLR* locus and classified as genotype 11, 12, or 22. Females were mated to Hampshire boars and slaughtered at approximately 75 days of gestation. Data collected from gravid uterine tracts included ovulation rate, uterine weight, uterine horn length, number of fetuses, total fetal weight, average fetal weight, number of mummies, fetal space, and fetal survival. Data were analyzed using a model that included the fixed effects of *PRLR* genotype, parity, breed, and all significant two-way interactions. For several traits, fixed effect of horn was added to the model to determine the presence of between horn effects. *PRLR* genotype was found to influence ($P < 0.1$) number of fetuses per horn, average fetal weight, and total fetal weight. For each of these traits, allele 2 conferred a performance advantage over allele 1. Animals with the 22 genotype had a larger ($P < 0.1$) average fetal weight per horn and number of fetuses per horn (369.7 ± 6.7g, 5.41 ± 0.2) than animals with the 11 genotype (347.7 ± 9.7g, 4.81 ± 0.3). *PRLR* genotype also displayed a favorable, but statistically nonsignificant, trend with respect to fetal survival. The *PRLR* gene is favorably associated with several reproductive tract traits. A patent has been issued for the use of this gene to improve reproductive traits.

**C033**

## Optimisation of the bioartificial liver based on microarray

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Microarray technology is a powerful tool to investigate differential gene expession. We are developing microarrays to conduct an expression analysis of transcriptionally active genes in porcine hepatocytes following deposition into a hollow fiber bioreactor developed to support patients with acute hepatic failure. To date the bioartificial liver (BAL) system can only be used for a limited period because the hepatocytes loose liver specific functions and finally die. Use of the BAL system for prolonged periods of time makes it essential to determine the factors that contribute to the loss of liver specific functions and hepatocyte cell death. We are arraying porcine expressed sequence tags (ESTs) isolated from normalised and suppression subtracted (SSH) libraries to identify genes responsible for specific changes in cell cycle and key hepatic enzymes in metabolic pathways.The investigation and identification of expression patterns of such metabolically relevant genes should enable us to optimise the BAL system by modifying extrahepatic nutrient flux prior to BAL use in acute hepatic failure.

**C034**

## QTL mapping of functional traits in the German dairy cattle population

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In dairy cattle populations an increasing amount of knowledge is elucidated about quantitative trait loci (QTL) influencing milk production traits. However, only very limited data are available on the genetic background of health traits and functional traits like fertility or longevity in spite of their economical impact especially in modern production systems.

Therefore, we set up a whole-genome scan to map QTL for health traits and functional traits in a granddaughter design comprising three German cattle breeds (Holsteins, Simmental, Brown Swiss). 248 genetic markers (236 microsatellite markers, eight SSCP, five blood group polymorphisms and four polymorphic proteins/enzymes) covering all autosomes and the pseudautosomal region of the sex chromosomes were included. Deregressed proofs of sons for somatic cell score, an indicator of udder health, for stillbirth (paternal and maternal), dystocia (paternal and maternal) and fertility (maternal and paternal non-return rates) were investigated. Statistical analysis was performed in a regression analysis across families with permutation tests to determine levels of significance. QTL for somatic cell score, for stillbirth and for dystocia were found with a 5 % chromosomewise significance. Although these results offer a chance to include these traits in Marker assisted selection (MAS) prior to application further confirmational studies are necessary.

**C035**

## Use of macroarrays enriched for ruminant mammary cDNAs to study the mechanisms underlying the mammary function

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The mammary gland is a dynamic organ, subject to hormonally induced development, differentiation and involution. During lactation, mammary epithelial cells are the scene of intensive activity which leads to biosynthesis and secretion of milk components. To help define the mammary specific mechanisms underlying the mammary function, we have used high-throughput gene expression analysis tools. A macroarray with 400 cDNA mainly isolated from the mammary gland of a lactating goat was constructed. Gene expression profiles of i) different physiological status (pregnancy *vs* lactation) and for ii) different phenotypes such as αs1-casein content (high *vs* low) in milk, were compared to identify genes whose regulation is associated with these processes. Arrays were hybridized with cDNA complex probes derived from goat mammary mRNA extracted from 3 different physiological stages (mid- and late pregnant and lactating) and 3 different αs1-casein genotype (high = αs1-CasA/A *vs* low = αs1-CasF/F and Null = αs1-CasO/O). The comparison of the obtained profiles has allowed to evaluate the expected variability in expression of known genes (such as those encoding caseins) in ruminant mammary tissues and also to identify up- or down-regulated mammary novel genes. Currently, an extended cDNA macroarray with thousands of genes is in progress. Gene expression profiling in the mammary gland will facilitate our understanding of its biology as well as providing candidate genes for milk production traits.

**C036**

## Predicting Heterosis Using Biochemical and RAPD Markers in Animal Species

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The animal industry has a history of using breed crosses and/or strain crosses in commercial production, mainly to take advantage of heterosis. Association of heterosis of economic traits with biochemical and RAPD markers were therefore investigated, which involved 23 alleles at 8 polymorphic biochemical loci in poultry populations and 5 RAPD markers amplified by 2 random primers in swine stocks. Genetic distance between parental populations based on markers of polymorphic blood groups and isozymes were favorably associated with percent heterosis in poultry. However, neither RAPD-based parental difference nor average band sharing was predictive of the level of heterosis in swine. The result was not an evidence that RAPD markers were not promising for predicting heterosis, but rather that choosing proper markers and populations were more important than choosing types of markers. Sequent random sampling experiments on choosing biochemical markers applied in predicting heterosis supported the hypothesis that association of heterosis with markers might varied greatly with markers employed. Therefore, improper use of markers in predicting heterosis was likely to result in deviation.

**C037**

**Characterization of naturally processed peptides presented by the bovine major histocompatibility complex class II DR molecule**

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The objective of this study was to determine the peptide binding motif of a bovine major histocompatibility complex DRB3 allele associated with occurrence of mastitis (*BoLA-DRB3.2*23*) in addition to that of a control allele (*BoLA-DRB3.2*8*). These alleles were cloned and expressed in mouse L cells. BoLA-DR molecules were immunoaffinity purified from transfected cells. Subsequently, the sequence of naturally processed peptides presented by these alleles was determined by HPLC-tandem mass spectrometry (Keck Lab, UoVirginia). Eluted peptides were between 14-20 amino acid long and had ragged N- and C-terminals. While some of the peptides had an exogenous source, most originated from endogenous sources. Among endogenous peptides, two endoplasmic reticulum-dervied peptides were noticed, indicating a degree of overlap between MHC-I and class II antigen presentation pathways. Finally, a putative peptide binding motif was assigned to allele **23*, which comprised an aromatic or a hydrophobic residue at relative position 1, a hydrophobic residue at position 4 and a small residue at position 6.

**C038**

**Hypothalamus-pituitary-gonadal axis genes as candidates for early puberty phenotype in Bos primigenius indicus**

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It is known that the endocrine system controls the reproductive function which is regulated by the hypothalamus-pituitary-gonadal axis and their interactions. *FSH* and *LH* receptor genes are expressed in the gonads and *GnRH* receptor gene is expressed in the anterior pituitary gland. The signaling of these receptors is essential for normal reproductive function, for the initiation and maintenance of spermatogenesis and for follicle development. They belong to the G-protein-coupled receptor family and are highly homologous to other glycoprotein hormone, and their activation triggers the *AMPc* pathway. Missense mutations of the *FSH, LH* or *GnRH* receptors that activate or inactivate their function would be helpful to understand the role of these gonadotropins in gametogenesis. Exon 10 of the *FSHR* gene, exon 11 of the *LHR* gene and exon 1, 2 and 3 of the *GnRHR* gene were *PCR* amplified from genomic Bos primigenius indicus *DNA*, screened by single-stranded conformation polymorphism (*SSCP*) gel electrophoresis and sequenced. Polymorphisms were identified by *SSCP* (5 in *FSHR*, 23 in *LHR* and 7 in *GnRHR*) and part of them were already characterized by DNA sequencing. These results are under analysis and could be important tools for *DNA* marker identification.

**C039**

**Identification of differentially expressed genes in response to an anabolic compound in bovine skeletal muscle.**

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A novel compound that stimulates anabolic activity in cattle has been identified. To better understand the compound's mechanism of action, this research was conducted to identify genes differentially expressed in skeletal muscle in response to the compound. Five steers were administered the compound and tissue biopsies were taken from the *longissimus dorsi* muscle prior to, and 24 hours following treatment. Total RNA was extracted from each biopsy and equal amounts of RNA from each steer were pooled within treatment. Reverse transcription and differential display PCR were performed in duplicate using 240 primer combinations. PCR products were separated on polyacrylamide gels and visualized by autoradiography. A total of 118 potentially differentially expressed products were identified. Eighty-two bands were excised, re-amplified by PCR, cloned, and sequenced. Confirmation of differential expression was achieved for six of 27 sequences by duplex RT-PCR using gene specific and beta-actin primers. A 156 bp region of a 534 bp differentially expressed clone was 90% identical to a region of the human gene *CGI-18*. A probe spanning this region was used to isolate two clones from a bovine skeletal muscle cDNA library (Stratagene). Using sequence data from the larger cDNA clone, PCR primers were designed and used to obtain a 3 kb genomic clone (Genome Walker kit, Clontech). Homologous sequence of the human *CGI-18* gene is included within this clone. In conclusion, a genomic clone of a novel bovine gene that is up-regulated in skeletal muscle in response to an anabolic compound was identified.

**C040**

**Molecular Characterization of Chicken Myostatin Gene and Expression Patterns in Different Tissues**

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A new murine TGF-ß family member, growth/differentiation factor-8 *(myostatin),* is expressed specifically in developing and adult skeletal muscle and may be a negative regulator of skeletal muscle development. This study aims at characterization and identification of genomic organization of chicken *myostatin* gene. To define the genomic structure, chicken genomic *myostatin* gene was amplified by PCR using the forward primer and reverse primer that were designed according to chicken *myostatin* cDNA sequence. Sequencing of genomic DNA was performed by using primer-walking method. The *myostatin* mRNA expression patterns were examined in chick embryos with RT-PCR and different tissues prepared from adult White Leghorn chicken (34-week-old after parturition) with Northern blot analysis, respectively.

In this study, we identified the genomic organization and sequence of chicken *myostatin* gene. RT-PCR and Northern blots results of various tissues showed different mRNA expression levels in developmental stages of chick embryos and demonstrated strong expression of *myostatin* mRNA in skeletal muscle. But expression of chicken *myostatin* mRNA was not restricted to skeletal muscle. These facts suggest that chicken *myostatin* gene would play an important role not only in skeletal muscle cell but also in different cell.

**C041**

**Transferrin variants among foals succumbing to *Rhodococcus equi* infection**.

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Transferrin is an iron transport plasma protein with 13 recognized electrophoretic variants in the equine population. Since transferrin sequesters iron, it is a bacteriostatic agent. Polymorphism of transferrin may play a role in resistance or susceptibility to bacterial pathogens. Therefore, we investigated the transferrin types among foals that died as a result of infection with the bacteria, *Rhodococcus equi*. Transferrin can be typed by sequencing exons 13 and 15 to identify SNPs associated with the different transferrin electrophoretic variants (Brandon et al., 1999 Anim. Genet. 30, 439). DNA was extracted from paraffin embedded tissues of 17 foals (15 Thoroughbred, 1 Rocky Mountain Horse and 1 American Saddlebred) that succumbed to *Rhodococcus equi*. All of the foals had the same type for exon 13; however due to limitations of SNP testing we were unable to distinguish the $F_1$, $F_2$, F* and D transferrin variants. Indeed, the horses could be heterozygous for the different variants. In future work we will sequence additional samples for exon 13 and exon 15 to increase the resolution of variant typing and compare these transferrin gene frequencies to the frequencies of the corresponding breeds.

**C042**

**Leptin expression in pigs selected for high and low cortisol levels.**

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The hypothalamo-pituitary-adrenal axis (HPA-axis) plays a significant role in the control of energy metabolism in vertebrates. The effect of cortisol on obesity and insulin resistance is well established. Since the discovery of leptin evidence has emerged, that interdependencies also exist with this adipose tissue specific hormone. The findings in the literature, however, are contradictory. We have therefore carried out a selection of young pigs of the Large White breed for high and low cortisol levels, based on their 24h urinary cortisol excretion. Differences in renal excretion rates were corrected by expressing cortisol concentration per milligram of urinary creatinine. The heritability was estimated by the restricted maximum likelihood procedure for the animal model. The estimated heritability was 0.69 for the urinary cortisol / creatinine ratio and 0.37 for urinary cortisol concentration. In order to quantify leptin gene expression, we developed a RNase protection assay (RPA) in solution. Animals of the high cortisol group showed significant higher backfat thickness, higher fasting insulin levels and significant lower leptin expression in the peritoneal adipose tissue (lamina subserosa). Despite the existence of a glucocorticoid response element in the mouse, rat and human leptin promotor, we observed a negative correlation between cortisol and leptin mRNA. This may be explained by an overriding role of leptin, exerting an inhibitory effect on the HPA-axis.

**C043**

**A deletion of *PCLN-1* gene is responsible for renal tubular dysplasia in Japanese black cattle**

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Renal tubular dysplasia is a hereditary disease of Japanese black cattle showing renal failure and growth retardation with an autosomal recessive trait. We have mapped the locus responsible for the disease (*RTD*) in a 4 cM region between microsatellite markers *BMS4003* and *INRA119*on bovine chromosome 1 by homozygosity mapping using an inbred pedigree. In the present study, we found that a genomic segment of bovine chromosome 1 including the microsatellite marker *BMS4009* was deleted in the affected animals. Construction of a BAC contig covering this region and comparison of the nucleotide sequences of this region between normal and affected animals revealed that a large genomic region including exons 1 to 4 of the bovine paracellin-1 (*PCLN-1*) gene was deleted in the affected animals. The *PCLN-1*gene, which is responsible for human renal hypomagnesemia with hypercaciuria and nephrocalcinosis, encodes a tight junction protein of renal epithelial cells. We concluded that the deletion of the *PCLN-1*gene is responsible for renal tubular dysplasia of cattle.

**C044**

**Identification of differentially expressed genes in hypertophying skeletal muscle.**

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The stretching and/or work overloading of skeletal muscle induces hypertrophy of myofibers and myotubes *in vitro* and *in vivo.* The cellular mechanisms contributing to an increase in muscle mass in response to stretch/work overload include increased protein synthesis and satellite cell proliferation. While there is a body of knowledge that suggests integrins and autocrine growth factors are involved in mechanotransduction, it is still unclear what the global changes in gene expression pattern are. The objective of this experiment was to identify differentially-expressed genes in hypertrophying skeletal muscle using suppression subtractive hybridization (SSH). The soleus and plantaris muscles were collected from two gastrocnemius ablated and two sham operated rats, three days after surgery. For SSH analysis, total RNA was extracted and pooled by muscle source and treatment. Subtraction of soleus and plantaris cDNA was performed with the PCR-Select cDNA Subtraction Kit (Clontech). Differentially-expressed cDNAs were amplified by two suppression PCR amplifications with the Advantage cDNA PCR Kit (Clontech). Subtracted PCR products were ligated into the T/A cloning plasmid vector pCRII (Invitrogen) and sequenced to determine their identity. Of the first 85 Rat ESTs sequenced, 68% of the ESTs were identified with homologies to known genes. Whereas, 32% of the ESTs were novel or had homologies to an EST in Genbank. These results provide new information concerning changes in gene expression associated with skeletal muscle hypertrophy and indicated that SSH is an efficient method for identifying differentially-expressed genes.

**C045**

## A polymorphism in the putative start codon of *alpha mannosidase 2B2* does not appear to affect ovulation rate in swine

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*Alpha mannosidase 2B2* is being investigated as a potential candidate gene for ovulation rate in swine located on chromosome 8. Exon 1 was sequenced from the 20 grandparents of the MARC resource population. Four polymorphisms were detected; three of which were in the 5' UTR. The fourth polymorphism was a C/T transition in the putative start codon as reported in GenBank Accession number D28521. Another ATG, which could serve as a start codon, is located in the same open reading frame 33 bp downstream. The predominant allele contained a C at this position indicating that the latter start codon is the most frequently used. Three of the grandparents (two Meishan and one White Composite) were C/T heterozygotes. The remaining animals were homozygous for the C allele. An assay to detect this polymorphism was design by microsequencing the region and determining the alleles via MALDI-TOF mass spectrometry. Selected animals from the third through fifth generations of the MARC resource population were genotyped. The mean ovulation rate was 14.19 ± 2.52 for the C/T heterozygote (n = 36) and 14.48 ± 2.58 for the C/C homozygote (n = 312). Only one animal was determined to be homozygous for the T allele due to the low frequency in our population and no phenotype was available. The polymorphism in the putative start codon of *alpha mannosidase 2B2* does not appear to be influencing ovulation rate.

**C046**

**Monitoring gene expression throughout skeletal muscle development in swine**

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Skeletal muscle or pork is the major product of swine production. Therefore, identifying and analyzing the expression of genes involved in the control of muscle development may be particularly useful for improving the quality and quantity of pork produced. To monitor gene expression throughout skeletal muscle development, muscle tissue from several different gestational and postnatal stages was collected. Skeletal muscle tissue was harvested at days 29, 35, 43, 49, 56, 64, 70, 78, 84, 93, 99 and 106 of gestation, 14 and 160 days postnatal, and from 2-4 year old adults. From these tissues mRNAs were successfully isolated for the construction of cDNA libraries. Four libraries are being constructed including early gestation (days 29, 35, and 43), late gestation (days 78, 84 and 93), postnatal, and adult stages of development. Clones from the libraries will be used to construct filter arrays and expression profiles will be characterized by analyzing hybridization signatures using labeled mRNA from the various developmental stages. This cDNA array will allow us to study expression patterns throughout skeletal muscle differentiation and development in swine. Subsequently, differentially expressed clones will be sequenced and analyzed for the potential involvement in muscle development pathways. Ultimately, the utility of this genome-based approach is to study complex genetic networks that may contribute to quantitative variation in pork meat production and quality.

**C047**

## A missense mutation in *LYST* gene is responsible for Chediak-Higashi Syndrome of Japanese black cattle

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Chediak-Higashi Syndrome (CHS) of Japanese black cattle is a hereditary disease with prolonged bleeding time and partial albinism. In the present study, we mapped the locus for this disease on bovine chromosome 28 by linkage analysis and assigned the bovine *LYST* gene, the homologue of the gene for human CHS, to the same chromosome using a somatic cell hybrid panel. These findings suggested that a mutation in this gene is responsible for the cattle disease. We, therefore, isolated cDNAs encoding bovine *LYST* from a bovine brain cDNA library and compared the nucleotide sequence of this gene between normal and affected animals. Notably, a nucleotide substitution of G to A transition, resulting in an amino acid substitution of histidine to arginine (H2015R) was identified in the affected animal. The substitution was completely corresponding with the CHS phenotype in the pedigree. We concluded that H2015R is the causative mutation in CHS of Japanese black cattle.

**C048**

**A single amino acid change in type X collagen causes dwarfism and metaphyseal chondrodysplasia in pigs**

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Chondrodysplasias are a genetically heterogeneous group of skeletal disorders caused by mutations either in genes encoding extracellular matrix components such as collagens or in genes expressed during the complex developmental program of skeletogenesis. We have identified a naturally occurring dominant mutation in *COL10A1* in pigs. The mutation changes a single amino acid in the carboxyl terminus of type X collagen, which makes the mutated collagen molecule unable to assemble into a triple helix. The phenotypic consequence is abnormal function of the growth plates in the long bones, resulting in dwarfism and metaphyseal chondrodysplasia. An amino acid substitution at the equivalent position in human type X collagen has been associated with the clinical phenotype Schmid metaphyseal chondrodysplasia (SMCD). This work establishes that the dwarf pigs by genetic, biochemical, and histological criteria provide a new animal model of human SMCD.

**C049**

## Estimation of SNP frequencies in European chicken populations

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To estimate the possibilities of using the SNP as markers for genetic diversity studies in chicken, we undertook the detailed analysis of several non-coding loci chosen from independent locations on the genetic map. Primers were designed for PCR and products of an average size of 500 bp were sequenced directly. Ten different chicken populations sampled in the EC AVIANDIV project were chosen for the analysis. This set consisted of wild-type populations, indigenous breeds, standardised breeds, and lines selected for quantitative traits. They displayed considerable genetic diversity based on microsatellite typing in DNA pools. For each population, 10 individuals were sequenced. Our first results with 6 fragments analysed to date, covering over 3 kb of DNA, indicate a very high level of single nucleotide polymorphism in chicken, with the number of SNPs detected ranging from 1 to 17 per fragment and an average of one SNP per 65 bp of DNA. The different SNP alleles at a given locus combine into numerous haplotypes found in varying frequencies. A high number of SNPs at a single sequenced locus within some populations combine only into a small number of haplotypes, indicative of a loss of alleles over time. The high level of SNP polymorphism that can be found in chicken will facilitate the development of DNA chips or related technologies for large-scale genotyping in the future.

**C050**

## A polymorphic genotype in the Bovine *Lipoprotein lipase* gene is associated with intramuscular fat contents

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Lipoprotein lipase (*LPL*) plays a major role in the regulation of lipid metabolism by hydrolyzing dietary or endogenous triglycerides of circulating chylomicrons and very low density lipoproteins (VLDL) to monoglycerides and free fatty acids, which are taken up by cells for oxidation (muscles) or storage (adipose tissue). *LPL* gene could be considered as one of the candidates, which account for the variation in beef meat quality. We investigated this possibility in Hanwoo (Korean cattle) and 4 other cattle breeds. Direct cycle sequencing and PCR-RFLP were performed with primers designed on the exon 5 ~ 7 of the bovine *LPL* cDNA sequences. Direct cycle sequencing of the resulting products from five unrelated Hanwoo was identified the expected exon regions, and detected several single nucleotide polymorphisms. Three PCR-RFLPs were found in the intron 5 by digestion with *Alu*I, *Rsa*I and *Hae*III, and one PCR-RFLP in the intron 6 with *Pst*I. In *Pst*I digestion of 106 Hanwoo, 77 Holstein, 22 Angus, 32 Charolais and 20 Hereford, no significant difference was detected in allele frequency among the different cattle breeds, and the frequency of A allele was higher than the frequency of B allele. The effects of this polymorphism on back fat thickness, marbling score and intramuscular fat contents were examined in 106 Hanwoo using least square methods. The intramuscular fat contents of AB genotype was significantly higher than those of AA and BB genotypes ($P<0.05$).

**C051**

**BoLA DR peptide binding groove charge residue polymorphisms and immune response to *M.bovis* antigens.**

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The importance to relate the MHC – disease associations in terms of similar structure within the MHC peptide-binding groove has been recently recognised. Molecular structure of HLA-DR shows subregions in the DR binding groove, which play a major role on antigenic peptide binding and T-cell recognition. Particularly physico-chemical polymorphisms of few aminoacid residues in human DRβ chain pocket '4', influence the immune response patterns.

Physico-chemical polymorphisms in peptide sequences corresponding to homologous HLA DRβ chain pocket '4' have been analysed in 47 not related calves. Animals were *M.bovis* sensitised, MHC class I/II typed and sequenced, tested by linfoproliferation test (LP) and indirect ELISA with bovine Purified Protein Derivative to evaluate the humoral and cell mediate immune response. Considering the 10 higher and the 10 lower responders for each immune test, aminoacid electric polymorphism effect on immune response has been statistically estimate. Presence of positively charged aminoacids at position β62 (corresponding to human β70) showed a significant positive effect on LP (P>T=0.05; Adj $R^2$ 0.38; P>F 0.006). On the reverse, presence of negatively charged aminoacids at the same position was correlate to a positive effect on antibody production. Present results confirm the importance of electric charge polymorphism of DR β residues on antigen binding-T cell recognition and their selective effect on Th priming, inducing Th1 or Th2 development and thus a cell mediated or humoral type response.

**C052**

**Genetic characterization of the South American camelids using microsatellite markers**

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The South American camelid (SAC) species are not completely reproductively isolated, as fertile hybrids of any combination of taxa can be produced. This has led to the general belief that in this group a process of speciation is still in progress, being also the origin of domestic species under debate. The main aim of this work was to help to define the genetic diversity and relationships among populations representing these species, alpaca (*Lama pacos*), llama (*L. glama*), guanaco (*L. guanicoe*) and vicuña (*Vicugna vicugna*). This work was done using a set of 23 microsatellite markers developed in our laboratory.

Phenetic analysis based on 38 alpacas, 14 llamas, 16 guanacos and 16 vicuñas showed that each species are clustered separately, with the only exception of some alpacas that were excluded of the alpaca group; these animals correspond morphologically to hybrids ("guarizos"). Interestingly, alpaca and vicuña clusters formed a major group, clearly separated from the llama and guanaco clusters, which were closer among them. This comes to support new evidence based on mithocondial DNA analysis, that suggests the alpacas as descendants from vicuñas (E. Palma, personal communication). Finally, principal component analysis (PCA) based on 193 polymorphic alleles showed that wild species (guanaco and vicuña) had more narrow genetic background than domestic species (alpacas and llamas). The domestic SAC clusters are more heterogeneous, what could be explained by the management of mixed herds, facilitating the generation of guarizos.

**C053**

**A relationship between *PIT1* gene polymorphism and production traits in pig.**

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The objective of this study was to evaluate an association between porcine *PIT1* gene polymorphism and growth rate and carcass quality in F2 animals (Zlotnicka Spotted boars and Polish Large White sows were used as grandparents) – a part of experimental material arranged for QTL mapping project. A total of 88 F2 offspring from 13 males and 67 females F1 have been analyzed. The *Rsa*I PCR/RFLP polymorphism of *PIT1* gene was identified according to Yu et al.,1994, Anim.Genet.25, 229. A least square method was used for evaluation of significance of *PIT1* gene polymorphism effect on a value of production traits. Three *PIT1* genotypes were identified: EE (n=29), EF (n=28), FF (n=30). Including half carcass weight as a covariate a significant effect of *PIT1* genotype on the following traits was observed: meat content in ham, average backfat depth, meat content in carcass, meat weight in half of carcass. Including age of porkers at slaughter a significant effect on weight of ham muscles, fat thickness over the loin and meat content in carcass was observed between homozygotes. These results suggest that *PIT1* gene polymorphism may be used as a selection criteria in pig breeding.

**C054**

**Polymorphism in the *FSHB* and *OPN* gene and their association with reproductive traits in synthetic pig line 990.**

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Two DNA markers, one at the *FSHB* locus and the second at the osteopontin (*OPN*) locus, were evaluated for their associations with reproductive traits in swine. Synthetic 990 line from Institute of Animal Production–Pawlowice was used as experimental material. A total 126 and 114 sows were genotyped for *FSHB* and *OPN* loci, respectively. The first litter of each sow was examined for a total number of born piglets, number born alive, litter weight on 21th day and at weaning. The polymorphism of the *FSHB* gene was determined by the PCR/RFLP method. The PCR product was digested by the restriction enzyme *HaeIII*. We revealed two alleles designated 1 and 2 ( Rohrer et al., 1993 Mamm.Genome 5, 315). The allele frequency observed was 0.892 for allel 2 and 0.118 for allel 1. The polymorphism in *OPN* gene was also determinated by PCR/RFLP method. The different length of both alleles (A, B) is due to presence or absence of 305 bp fragment in intron 6(sequence *PRE-1*) (Knoll et al., 1999 Anim. Genet. 30,1). The frequency of individual alleles amounted to 0.82 and 0.18 for B and A alleles, respectively. Relation between the *FSHB* and *OPN* genotype and reproduction traits were evaluated using the least squares method. Among the animals examined only 3 homozygous 1/1(*FSHB*) and 4 - A/A(*OPN*) was found, what means that only two genotypes for each marker could be compared: 1/2 with 2/2 and A/B with B/B. No significant difference between genotypes was observed but the research is still continued and more data will be collected soon.

**C055**

## Estimation of reproductive characters of Large White breed pigs with regard for genetic factors

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There has been studied the possibility to use the genetic affinity indices as to blood (r) of parental couple in breeding of pigs for reproductive characters. The analysis was carried out among the pigs of 'Omsky Bacon' hog corporation farm (direct descendants from 628 sows and 99 boars) as to characteristics of 1487 farrows. The value r was determined as to ten blood systems. The parental couples were divided into 6 groups as to value r. The higher characteristics were in group having r from 0.5 to 0.59: 11.9 piglets born, 11.2 piglets survived, litter weight 15.3 kgs. In group with r from 0.9 to 1.0 there were the low characteristics while weaning the pigs in the age of 2 months: litter number 7.5, litter weight 143.3 kgs, safe keeping of piglets 76%. One can see that the genetic affinity of parental couples having r from 0.5 to 0.59, with the definite level of heterozygosis, is optimum. In this case, the moderate genetic affinity is sufficient to sustain a genetic stability of their taxon (population, breed), and it does not bring to inbreeding depression. The moderate genetic remoteness does not provoke an immunological conflict 'mother - fetus', and at the same time, gives a necessary diversity for descendants to produce competitive combinations from heritable genes.

**C056**

## About a connection of genetic propinquity of parental couples with production characters of offspring

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In literature there is the information about the influence of genetic propinquity of parental couples on their reproductive and production characters. We have taken an interest in this connection with the economic characters of offspring. The test object was Siberian Black-and-White cattle of the farm 'Zavarzino', Tomsk Region (n- 198), and sows of Large White breed of experimental farm 'Borovskoe', Novosibirsk Region (n - 204). The characteristics of genetic propinquity of the animals were the genetic affinity indices with regard for erythrocyte antigens of nine blood systems. The results of studying the productivity of two cattle generations in the first lactation show a higher milk-yield of daughters of parents having 0.4 - 0.6 genetic affinity index. The reproductive characteristics of sows (fertility, milk-yield, litter weight, quantity of sucking-pigs up to weaning) were higher in the group of sows whose parents had a genetic affinity at the level of 0.5 ($p<0.05$ - 0.001). Such genetic remoteness of parental couples ensures the best production characters of descendants.

**C057**

**Pig anterior pituitary ESTs isolated by differential display analysis of gene expression in two lines selected for fertility.**

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Differential display PCR (ddPCR) was used to investigate differences in gene expression in anterior pituitary of sows from two different lines selected for fertility. Second parity sows from a line selected for 16 generations on an index of ovulation rate and embryo survival and a randomly mated control line were used. Anterior pituitary was collected immediately after slaughter in order to evaluate expression of genes that could be involved in the significant reproductive differences observed between these populations. Tissue was collected during follicular development, two (d2) and four (d4) days after injection of prostaglandin analog between days 12 and 14 of the estrous cycle. Total RNA was extracted followed by purification of mRNA using Oligotex columns (Qiagen). Four independent pools of mRNA were made from one d2 and one d4 sow each; two pools were from control line sows and two pools were from index line sows. cDNA from these pools were used as template for ddPCR using all 200 combinations of 10 anchor (3') and 20 arbitrary (5') primers. Bands were prioritized for evaluation (n=372) based on a combination of strongest differences between lines and highest consistency within line. Selected bands are being cloned (Topo TA, Invitrogen) and sequenced. Preliminary cloning and sequencing results indicate that 95/126 clones are unique EST species. These clones have homology to known genes (n=35; 36.8%), unknown genes (n=49; 50.5%), or are novel genes (n=12; 12.6%). The cloned EST's will be used as probes in gene arrays to further investigate differences in gene expression between the lines and confirm the ddPCR results.

**C058**

## Differential display PCR and microarray evaluation of ovarian follicle gene expression in pigs selected for reproduction

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Differential display PCR (ddPCR) and human microarray analysis were used to evaluate follicular gene expression differences between index (ovulation rate and embryo survival) selected (I) and randomly selected (C) lines of pigs. Follicles (4-7 mm) were dissected from ovaries collected immediately after slaughter of multiparous sows (n=27), two (d2) or four (d4) d following PGF2α analog injection on d 12 to 14 of the estrous cycle. Gene expression in follicles from I and C was compared with ddPCR. Differentially expressed bands (n=274: I vs. C; d2 vs. d4; follicle size ranges) were excised from gels and 107 were sequenced, yielding 85 porcine follicle ESTs. For microarray analysis, two mRNA pools, each with six follicles (d2; 4.50-4.75 mm) from two I or two C sows, were evaluated on an Incyte UniGEMV1.0 human chip (~7,000 gene probes). An additional analysis on the Incyte UniGEMV2.0 chip (~9,100 gene probes) is in progress. UniGEMV1.0 results indicated significant differences between I and C sows (minimum two-fold relative expression) for 33 genes. Northern blot confirmation is in progress for 12 genes of interest from ddPCR and microarray results, including *cytochrome P450 aromatase* (expression increases with increasing follicle size), *poly(A) binding protein* (I>C), *G-beta* (C>I) and *follistatin* (C>I). These results demonstrate changes in follicular gene expression as the result of long-term selection for reproduction. These changes may represent direct (i.e. QTL) or correlated responses due to selection.

How to Search

**D001**

## Polymorphous systems of blood of polecats, minks and sables

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Biochemical polymorphism of polecats of 3 color groups (n=366) was studied for the first time. Results were compared with minks (n=1212) and sables (n=806). IN polecats genetical polymorphism was discovered in systems of albumin, postalbumin, transferrin, posttransferrrin of serum and hemoglobin of red corpuscles. Methods of horizontal electrophoreses were used for identification of genotypes. We made a hybridological analysis and established codominanteous character of inheritance of all system alleles in polecats. Allele B predominated in albumin locus in golden and pastel polecats (0.4042 and 0.3980, respectively).

Albumin A predominated in mother-of-pearl polecats (0.4055). IN posttransferrin locus allele C (0.5052), allele C (0.5510) and allele A (0.4330) predominated in golden, pastel and mother-of-pearl polecats, respectively. High concentration of allele A was character for postalbumin locus and allele 4 (D) prevailed in hemoglobin locus of all types of polecats.

In minks there was the highest frequency of albumin B (0.6351), hemoglobin 3 (C) allele was absent. Sables were only notable for high frequency of posttransferrin B (0.6723). Average level of real homozygosity was within 39.1-43.2% in polecats. Hybridological analysis showed a high level of accordance of parent and progeny genotypes. Thus in polecats as well as in minks and sables there is a possibility to fulfill genetical control of animal's origin and directional selection of certain genotypes.

**D002**

## Biochemical polymorphism of caged marmots (Marmota bobac)

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Adaptation of marmots began in 1989 from snaring 1500 animals in Rostov region and their further reproduction in state pedigree farm "Pushkinskiy", situated in Moscow region. For the first time biochemical polymorphism was studied on the second year marmot (n=153) by horizontal electrophoresis methods. Blood was taken from heart during slaughter. Two allele genes were detected in albumin locus, three allele genes were found out in postalbumin, transferrin and posttransferrin loci, and four - locus of hemoglobin of red corpuscles. 15 allele variants were determined altogether. In all systems level of homozygosity was within 50, 4-54,5%, that corresponded to 44, 9-45,1 theoretically expected percentage (x2>0,05). Body length of the marmots wasn't connected with definite alleles and genotypes of studied polymorphous systems. However connection between posttransferrin A allele and live weight of females was established. Stabley for 3 year posttransferrin A allele had been defining in marmot females which had higher weight in comparison with those which had alternative allele. Degree realization of possible variability in five loci was within 49,9-64,7 that testified to considerable genetical reserves of marmots of this population.

**D003**

**Combined estimation of QTL effects using a mixed model in an inbred pig family*+**

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An inbred pig family was started from a father-daughter backcrossing; inbreeding was continued, chiefly by performing sib mating, for five successive generations. This pig family provided an informative reference for QTL analysis and mapping, as well as for investigation of inbreeding and inbreeding depression. The use of this pig family in QTL analysis is limited however, by the small number of backcrosses, which consists of 39 pigs, of which only 30 have complete records of performance. Combined estimation of QTL effects is therefore suggested for QTL analysis in this family, with a view to increasing the number of animals and consequently increasing the power of detecting QTLs. The basic idea is to combine estimates of QTL effects from the backcrosses and the $F_t$ generation using a mixed model by giving them different weights. The expectation of additive effect (a) in the combined estimation equals $a+w_1d$, which is biased by $w_1d$. The bias, however, is much smaller than that in backcross estimation if considering $w_1$ <1. The expectation of dominance effect in the combined estimation is unbiased. Single-locus analysis is utilized to illustrate the theory, using results of microsatellite genomic scanning and individual records of performance in the BC and $F_3$ generations of the CUP family. Analysis of variance has revealed the existence of a significant QTL on swine chromosome 17, which positively contributes to slaughter weight. Combined estimation shows that the additive effect of the QTL is 1.786 .733kg ($p<0.05$), and the dominance effect is 1.364 .194kg ($p>0.05$). Variance contribution of this QTL is estimated to be 9.60%. A comparison of different estimations has also been conducted. The estimate of additive effect in the backcrosses is the largest but not significant ($p>0.05$), and that of the F3 is the smallest ($p>0.05$). The combined estimates fall in between the BC estimate and F3 estimate, and are significantly greater than zero ($P<0.05$). The standard error for the combined estimation is the smallest, which is a sign of the improved power of detecting a QTL. Dominance effect of the QTL in the combined estimation is exactly the same as that in the F3 estimation.

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**D004**

**Association of crossbreeding parameters for growth and meat-production traits with average band-sharing of RAPD markers in commercial crosses of Duroc, Landrace and Large White Pigs*+**

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By using individual records of growth, meat production, and genotypes of RAPD analysis in commercial crosses of Duroc, Landrace and Large White pigs from 1996 through 1998, crossbreeding parameters have been estimated for: average daily gain (ADG); dressing percentage (DRP); backfat thickness (BFT); percent of hind legs (HLP); and average band-sharing of RAPD markers. The results indicate that individual and maternal genetic effects are significantly greater than zero for all four traits of growth and meat-production ($p<0.05$). Effects of individual heterosis and maternal heterosis have been found to be significant for ADG ($p<0.05$), but not significant for DRP, BFT, and HLP ($p>0.05$). Positive maternal genetic effect and negative effects of individual heterosis and maternal heterosis have been revealed for ABS of RAPD markers. Correlation analysis shows that the total correlation between RAPD-marker ABS and traits of economical importance, when including all parameters at a time, is small (0.2735~0.4414) and not significant ($p>0.05$). The partial correlation, however, is greatly improved (-0.8240~0.8919) when considering one parameter at a time, or two in the case of heterosis. Partial correlations for maternal genetic effect and combined effects of individual and maternal heterosis between ADG and RAPD-marker ABS are significant ($p<0.05$), and the correlation for individual genetic effects between them is very significant ($p<0.01$). It is therefore concluded that the efficiency of predicting heterosis is low by simply applying total correlation. Alternatively, the efficiency will be much improved by investigating correlation of individual parameters between markers and traits of economic importance, instead of using all parameters. Increasing QTL coverage (percent of markers linked to QTL) is also expected to significantly contribute to increased accuracy of predicting heterosis. Correlation of additive effect between ABS of RAPD markers with ADG, DRP, and HLP is positive, but that of individual and maternal heterosis between them is negative. This result suggests that, while genomic similarity of crosses increases, individual genetic effect will increase (or decrease in certain cases), but the effects of individual heterosis and maternal heterosis will decrease.

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**D005**

**Microsatellite and blood protein diversity in the sheep breeds of Finland and North-Western Russia**

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Genetic diversity at 15 microsatellites and 7 blood proteins were examined in 9 sheep breeds or local populations, e.g. different Finnsheep colourtypes, two distinct Romanov populations and autochthonous populations from Russian Karelia. In addition, a Finnish Oxford Down population was used as a reference group. Within-population expected mean heterozygosities varied from 0.64 to 0.74 for microsatellites, and from 0.15 to 0.35 for proteins, respectively, with the Oxford Down being the least variable. No statistically significant differences in heterozygosity between the breeds were found, but this could be due to the limited number of loci analysed. It was estimated that a minimum of 20 to 35 polymorphic loci were needed to detect a true difference of 0.10 in heterozygosity between two populations by a paired t-test (α=0.05). However, an allelic deficiency was observed, e.g. in Oxford Down and the brown colourtype of Finnsheep, suggesting a recent decrease in their effective population sizes. Finite population size may also explain linkage disequilibrium between physically unlinked loci in some of the studied breeds. Statistically significant population differentiation was observed. $\theta$ values based on microsatellite data and protein data were 0.080±0.007 and 0.149±0.057, respectively. Respective Nei's *DA* distances varied between 0.051–0.351 and 0.018–0.156. Distances were used to construct a neighbour-joining tree illustrating the grouping of these breeds.

**D006**

**Marker-assisted introgression experiment in mice**

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African trypanosomiasis is an endemic disease affecting sixty million cattle in 37 African countries, with annual direct and indirect losses estimated at more than US$5 billion. Most West African *Bos taurus* breeds are genetically resistant to trypanosomiasis and chromosomal localization of cattle trypanotolerance quantitative trait loci (QTL) is underway. In an earlier experiment, using $F_2$ and $F_6$ crosses of susceptible (A/J) and resistant (C57BL/6) mouse strains, 3 trypanotolerance QTL were mapped to chromosomes 1, 5 and 17. A marker-assisted introgression experiment involving the two inbred lines has been set up to study the effectiveness of an introgression program. The experiment started in 1997 with reciprocal crossing of the two parental lines producing an $F_1$, followed by four generations of backcrossing to the susceptible strain, an intercrossing phase and a multiplication phase. During backcross and intercross, animals were selected based on markers flanking the identified QTL (5, 18 and 7 cM on chromosomes 1, 5 and 17, respectively). Ten groups of animals carrying different combinations of marker-genotypes were produced. These groups and the two parental lines were challenged with *Trypanosoma congolense* and trypanotolerance was measured as the survival time following infection over a period of 22 weeks. Difference in survival time between the two parental lines was 58 days.

**D007**

## Towards a strategy for the conservation of the genetic diversity of European cattle

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There are growing concerns that the almost exclusive use of highly productive cattle breeds, which are the result of a systematic and intense selection, will lead to a reduction of genetic diversity and the disappearance of valuable phenotypes such as local adaptation and disease resistance. The project *'Towards a strategy for the conservation of the genetic diversity of European cattle'* from the European Community coordinates the efforts of several laboratories and has the following targets:

1. Microsatellite genotyping of 50 animals from 50 breeds with standardized set of 30 markers. This will combine and complement previous national and regional studies, which were done with different sets of markers.
2. AFLP genotyping of 20 animals from 50 breeds with 100 biallelic markers.
3. Analysis of the effect of selection by studying the diversity in loci coding for milk proteins, the myostatin locus and regions known to contain a QTL.
4. Formulation of recommendations for conservation It is expected that the RESGEN project will generate a unique and valuable set of data, which represents most of the genetic diversity of European cattle. This will lead to fundamental new insights in the relation between selective breeding and genetic diversity as revealed by different categories of molecular markers.

**D008**

**Polymorphism of *Calpain* locus and relationship with meat tenderness in Piedmont cattle breed**

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Calpain-mediated degradation of myofibrillar proteins is known to be responsible for post-mortem meat tenderization. Polymorphism at calpain II regulatory subunit gene (*CAPN*) has been detected in several cattle breeds. Therefore the present investigation was carried out to study the variability of *CAPN* locus in the Piedmont breed and to determine whether the polymorphism is related to beef tenderness. Seventy-five unrelated subjects were analyzed for *CAPN* genotype by PCR-RFLP of genomic DNA from blood, and for meat tenderness by Warner-Bratzler shear force (WBs) on LTL at 1, 3, 7 and 11d post-mortem. GLM procedure was used for statistical analysis. The digestion of PCR products with *HhaI* revealed two alleles: $CAPN^{A}$ (900, 620 and 280 bp) and $CAPN^{B}$ (1520 and 280 bp), with frequencies of 0.24 and 0.76 respectively. No significant associations were observed between *CAPN* genotype and Wbs:

| | WBs1 | WBs3 | WBs7 | WBs11 |
|---|---|---|---|---|
| *AA* | 12.20 | 7.99 | 6.82 | 6.27 |
| *AB* | 10.70 | 6.57 | 5.79 | 5.49 |
| *BB* | 9.54 | 7.28 | 6.62 | 5.50 |

The results indicate that this polymorphic site at *CAPN* locus cannot be used to predict beef tenderness.

**D009**

**Polymorphism in the Upstream Regulatory Regions of *BoLA-DRB* genes.**

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The *MHC-DRB* are highly polymorphic genes. These polymorphims were mainly located in the peptide-binding site (*PBS*). In human and mouse, variant sites were also reported in the upstream regulatory regions (*URR*). In bovine, the polymorphism of the second exon of the class II genes were extensively studied. However, to our knowledge, *URR* have not been studied yet. Consequently, the aim of this study was to analyze the *BoLA-DRB URR* polymorphism using single-strand conformation polymorphism (*PCR-SSCP*) technique. Genomic DNA from 37 Argentine Creole cattle of one paternal half-sib family was amplified for *URR* of the *DRB* genes by *PCR*. The oligonucleotide reported by Turco *et al.*, 1990 Immunog 32, 117-28 were used as forward primer, while the reverse primer were deduced from *BoLA-DRB3*, *HLA-DRB*, *SLA-DRB*, *H2-Mb2* and *H2-IAb* published sequences (from position -2 to 20). These oligonucleotides amplified a single product of the expected size (~ 220 bp) corresponding to previously reported data. Amplification products of the putative *URR* reveals four distinct patterns using *SSCP* method. In order to identify allelic variants, the PCR product from the sire was cloned. All bands detected in the sire could be explained by the observed patterns in the clones. These *SSCP* patterns appear to be inherited in a simple Mendelian fashion in the studied family. Additional studies, like DNA sequencing of the *PCR* product, are required to confirm the identity of the sequences and their implications with regulation of the *DRB* genes expression.

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**D010**

**Preliminary *PCR-RFLP* analysis of *DQB* exon 2 polymorphism in Argentine Creole horses.**

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The *DQB* gene of the Equine Leukocyte Antigen (*ELA*) seems to be encoded by a single locus. Up to date, the complete coding sequence of *ELA-DQB* gene is known and thirteen *DQB* exon 2 sequences were reported in domestic horses (*Equus caballus*). Genotyping systems have been defined for *MHC* class II *DQ* molecules for a number of species, including cattle and humans. However, there is no widely used system for typing *DQB* gene in horses. The aim of this study was to analyze the *DQB* exon 2 polymorphism using the *PCR-RFLP* technique. Genomic DNA from 22 Argentine Creole horses of two paternal half-sib families was amplified using the oligonucleotide primers *GH28* and *GH29* (Gyllensten *et al.*,1990 Proc. Natl. Acad. Sci. USA 87,1835-9). The identity of the PCR products was confirmed by digestion with *Taq* I restriction enzyme. PCR products were digested with either *Eco* RI, *Hae* III, *Rsa* I, *Hinf* I and *Msp* I. Restriction fragments were separated by electrophoresis in 8% (19:1) acrylamide-bis acrylamide gels. In the sample studied, *Eco* RI digestion profiles were monomorphic, while the remaining enzymes showed from two to three distinct restriction patterns. The restriction sites present in the thirteen reported *DQB* sequences could only explain five out of ten observed restriction patterns. The remaining patterns would represent additional alleles or the result of coamplification of another class II gene. Further studies have to be done in order to improve the specificity of *DQB* primers to use *PCR-RFLP* as a typing method for *ELA-DQB*.

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**D011**

## cDNA cloning of a swine major histocompatibility complex (SLA) class II DMA gene and genetic polymorphisms in the SLA-DMA, -DRB, and -DQB genes

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In order to isolate cDNA clones from the swine histocompatibility complex (SLA) class II DMA gene, a Göttingen miniature swine spleen cDNA library was screened using the PCR products from the third exon in the human HLA-DMA gene. Seven cDNA clones were isolated, and the longest of these clones encodes a primary translated product of 260 amino acids. Amino acid comparative analysis and construction of a phylogenetic tree revealed that this clone was more closely related to the human and bovine DM α genes than to the genes for the conventional swine class II α chains, DRA and DQA. These results suggest that the SLA-DMA gene is expressed and may function, like HLA-DM, as an important modulator in class II restricted antigen processing in the SLA. Furthermore, we have analyzed genetic polymorphisms and allelic variations of the SLA-DMA, -DRB, and -DQB genes in five different breeds of swine. Based on sequences and PCR-RFLP patterns in the SLA-DMA gene, no allelic variation was recognized in the second exon, and five allelic variations were recognized in the third exon. These DMA alleles are defined by variation at four nucleotide positions. Two alleles among them result in an amino acid substitution. These results suggest that SLA-DMA has little polymorphism as observed in HLA-DMA and mouse H2-DMA. Sequence analysis of the second exon of the SLA-DRB genes after PCR amplification has allowed the identification of three and two novel alleles in the SLA-DRB1 and -DRB2 genes, respectively. In addition, two novel alleles were also identified from the sequence analysis of the second exon of the SLA-DQB gene.

**D012**

## Development of a microsatellite parentage and identity verification test for dromedary racing camels

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Camel racing in the U.A.E. is a traditional sport comparable to horse racing in the Western world. With the increase in value of transactions for these camels, a need arises for a test to confirm the parentage or identity of a camel. Since no microsatellite markers were available for *Camelus dromedarius*, two approaches were used: firstly, the evaluation of published South American camelid microsatellite primer pairs, and secondly, the *de novo* development of microsatellite markers. In the first approach a total of 37 recently published polymorphic microsatellites were assessed. According to our results, 24 of the 37 primer pairs amplified in *C. dromedarius*. The second approach involved the development of a small insert genomic DNA library. The library was screened with a DIG$(GT)_{15}$-labelled oligonucleotide. Positive clones were sequenced and primers designed to amplify the microsatellites resulting in 8 markers. Polymorphism of all 32 markers was evaluated in a group of 52 unrelated racing dromedaries. A total of 7 markers proved to be monomorphic, while 6 were dimorphic and 19 polymorphic with allele numbers ranging from 3-19 alleles. Mendelian segregation of alleles was confirmed in 9 paternal half-sib families with a total of 24 offspring-dam pairs. One marker was found to be X-linked, the rest were unassigned autosomal. A total of 16 highly polymorphic markers with probability of exclusion values ranging from 0.2-0.7 resulted in a total $P_E$ of 0.99997. Current work focuses on the establishment of non-linkage and the multiplexing of the microsatellite markers. Suitable genetic markers were quickly developed with the two-tier system used.

**D013**

**Relationships among the Spanish Pure Breed and other horses bred in Spain**

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The Andalusian, or Spanish Pure Breed, horse is the most emblematic Spanish horse from economical, zootechnical and cultural points of view. This study uses DNA microsatellites to complete previous studies of its biodiversity with classical markers. The main objective is typing a random sample of 850 animals including 150 Spanish Pure Breed and other breeds from the Iberian Peninsula. The methodology consisted of a whole blood DNA extraction, PCR amplifications, and allelic characterisation with silver stain technique. For routine typing we have been using the following multiplex groups with great success: M1 = *ASB2* + *AHT4*, M2 = *HMS2* + *HMS7*, M3 = *HMS3* + *HTG7* + *HTG6*, M4 = *HMS6* + *HTG4* + *VHL20*. Four of these groups (M2, M3, M4 and M1) are suitable for multiloading into a single line, although M4 and M1 require waiting 7 min between loads. We found a clear differentiation of Spanish Pure Breed from other horses bred in Spain, confirming historical data and data found in studies with blood groups and biochemical polymorphisms.

**D014**

## Genetic analysis of the Chato Murciano pig and its relationships with the Iberian pig using microsatellites

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The Chato Murciano is a Spanish pig whose origin was in old Iberian pigs from the Mediterranean area. During the first quarter of the 1900s, it was crossbred with Berkshire, Tamworth and other European breeds to improve its production traits. In the last decade it was near extinction, but today there is great interest in conserving its genetic resources. The primary objective of this work is to elucidate and clarify the genetic relationships between this breed and the Iberian Pig using DNA microsatellites. This information could be interesting for the development of future conservation plans. Blood samples were taken from 80 animals. Microsatellites used in this research (25) are enclosed within those recommended by the ISAG-FAO advisory group for diversity studies in pig. Genotypes were compared with those obtained in previous studies of the Iberian Pig and the Duroc populations. Descriptive statistics, genetic diversity, and distances have been studied. Results have shown that the Chato Murciano pig has a low level of variability, probably because of the bottleneck effect. Also, this breed has shown allelic frequency differences and a great genetic distance from the Iberian pig at the microsatellite level. In spite of sharing the same origin, the influence of European pigs and the decreasing size of their populations could be the origin of divergence between these two breeds of pigs.

**D015**

**Relative quantitation of *CSN3* allele mRNAs in heterozygous Holstein cows**

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Many alleles have been identified at the *CSN3* locus (A, B, C, E, F, G, H, I), of which three (A, B, & E) have been shown to be segregating in the U.S. Holstein population. A difference in the relative protein expression of the A and B alleles (B > A) has been shown previously. However, little is known about the relative expression of the mRNA of any of the three alleles in Holsteins. The objective of this study was to determine the relative amounts of the *CSN3* allele mRNAs expressed in heterozygous Holstein dairy cattle. Total RNA was extracted from cells obtained from milk samples taken post-milking near peak lactation of each cow. *CSN3* mRNA was amplified by RT-PCR using 5' fluorescent labeled primers. RT-PCR products were digested with *Hinf*I and size-separated on an ABI 310. *Hinf*I digests the A allele product resulting in bands of 92 and 186 bp while the B allele does not digest yielding the full length product of 278 bp. Bands were quantified by sizing the detected bands using Genescan™. Peak areas and sizes were extracted from data files using Genotyper™. Relative amount of allele A was determined by calculating the relative proportion of the 92 and 186 bp peak areas divided by the total peak areas of the 92, 186, and 278 bp peaks. A standard curve, using cloned cDNAs of each allele in known proportions, was used to predict the relative amount of A vs. B mRNA ($R^2$ = 0.956). 27 animals were analyzed in this way and yielded an average relative quantity of 43.3% (SEM = 1.98) for the A allele, which was significantly different from the expectation of 50% (T-test $p < 0.001$).

**D016**

## Detection of polymorphism between full-sib calves by representational difference analysis

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Representational difference analysis (RDA) is a subtractive hybridization method by which the difference between two complex genomes can be isolated. We applied this technique to detect the polymorphism between two Japanese Black full-sib male calves. Genomic DNA was digested with a restriction enzyme recognizing six base pairs and an oligonucleotide adaptor was ligated to the digest. PCR was used to amplify the populations of the fragments of approximately less than two kilobases in length (called mplicons . Both tester and driver amplicons were re-digested with the same enzyme and only tester amplicon was ligated to another adaptor. Then tester amplicon was hybridized with an excessive amount of the driver amplicon so that the fragments that existed only in the tester could be effectively amplified by PCR. The amplified fragments were hybridized with the same driver amplicon to remove pseudo-positive fragments and PCR was performed again. This hybridization-amplification step was repeated once more. After these operations, five to ten clear bands were visualized in agarose gel electrophoresis. Southern blot analysis revealed that some of these bands existed only in the tester, although others existed in both tester and driver. The result demonstrated the usefulness of RDA to detect the polymorphism among full-sib calves.

**D017**

**Molecular weight determination of *L* system alloantigens by SDS-PAGE**

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The *L* system is one of twelve alloantigen systems in the chicken. White Leghorn populations generally are characterized by two alleles designated *L1* and *L2*. Genotypic effects have been demonstrated for both macrophage function and regression of Rous sarcoma-induced tumors. Total membrane protein was isolated from Leghorn erythrocytes of both homozygous and the heterozygous types. The isolates were run on eight and twelve percent SDS polyacrylamide gels under reducing and non-reducing conditions. After transfer, the membranes were probed with polyclonal alloantisera specific for either *L1* or *L2* antigens. Two pairs of bands were detected using horseradish peroxidase conjugated secondary antibodies and Amershams Enhanced Chemiluminescence kit. The *L1* and *L2* antisera detected identical bands for their respective homozygote genotypes and both detected the same bands for the heterozygote. The heavier pair had molecular weights of approximately 80 and 70 kD and the lighter pair 35 and 25 kD. Both reduced and non-reduced samples gave the same results. Therefore, the specific alloantisera made against *L* alloantigens collectively identifies four polypeptides of approximately 80, 70, 35 and 25 kD in White Leghorns.

**D018**

## Polymorphism of 10 tetranucleotide repeat microsatellite markers in Korean dogs

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Microsatellite markers have been used for various purposes, from individual identification to population genetics. To obtain genetic data for a population of Korean dog breeds, DNA samples from Jindo, German Shepherd, and miscellaneous other dog breeds were analyzed for 10 different tetranucleotide repeat microsatellite loci (Francisco *at al.*, 1996 Mamm. Genome 7, 359). The genotyping was carried out using techniques of PCR, polyacrylamide gel electrophoresis, silver staining and DNA band size determination compared to standard marker DNAs. The results showed that Jindo dogs have a higher degree of polymorphism than German Shepherd for all markers analyzed. In the Jindo dog, 9 out of 10 loci have a high PIC value (>0.7), and 4 loci are over 0.9. On the contrary, the German Shepherd dog had only 4 loci with a PIC value exceeding 0.7. Even though the observed hetorozygosities were slightly lower than expected in all loci examined, the differences were not statistically significant. The results indicate that the Jindo dog has a larger gene pool than the German Shepherd. The number of loci with exclusion powers exceeding 0.6 was 8 in the Jindo dog and 5 in the German Shepherd. The present data may serve as a basis for developing a parentage testing and individual identification system for dogs in Korea.

**D019**

## A *Cervus* genotyping kit based on automated fluorescent multiplex PCR for rapid characterization of genetic diversity in several deer populations

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Microsatellite loci are highly conserved among related species (Kühn *et al*, 1996) such as cattle and sheep (Moore *et al*, 1992). Transfer of a large number of polymorphic microsatellites between families of the *Artiodactyla* order has been demonstrated several times (Engel *et al*, 1996; Kuhn *et al*, 1996, Slate *et al*, 1998 and Roed, 1998). Therefore, a multi-allele system should be useful in studying the genetic diversities of populations. This study was developed using four different deer species (Rusa, Eld, Swamp and Vietnamese Sika deer). A set of 38 microsatellites derived from bovine and ovine origin were chosen based on two criteria: (i) known to amplify in other deer species (Red deer); and (ii) showing an interesting polymorphic level as described in previous studies (Slate *et al*, 1998 and Talbot *et al*, 1996). From these 38 screened markers, 30 gave an amplified product in the 4 deer species (78.9 %). Of those 30 markers, 14-20 (40%-60%) were polymorphic, depending on the species. Using 12 microsatellites polymorphic in the 4 species, we set up a unique multiplex PCR optimized for annealing temperature and reagent concentrations. The 12 primer sets were labelled with 3 different fluorochromes, depending on the allelic range for each species. The automatic analysis was performed using an ABI 377 sequencer and PE Genotyper software. This method for parentage testing or genotyping gave good and reproducible results for the 4 studied species as well as for several other tested deer species and subspecies. This tool could be considered a first generation “Cervus” genotyping kit useful for the rapid characterization of genetic diversity.

**D020**

**Linkage mapping of rabbit microsatellite markers generated from chromosome-specific libraries**

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The rabbit is frequently used as an animal model for human diseases like atherosclerosis and hypercholesterolemia and as a production animal for meat, wool and fur. In order to identify and localize the genetic factors which are involved in these diseases and traits, a dense genetic map of the rabbit is needed. In collaboration with Keygene N.V. (Wageningen) a genetic map containing about 280 AFLP markers in 23 linkage groups has been developed. Up until now we could only assign one AFLP linkage group to a specific chromosome (ocu 1). Extending the map with polymorphic microsatellite markers with known chromosomal location would increase the usability of such a map. The availability of flow-sorted chromosomes (Korstanje et al., 1999 Cytogenet. Cell Genet. 86(3-4):317-322) allows us to use a chromosome-specific approach. Flow-sorted chromosomes are randomly amplified (DOP-PCR) and enriched for CA-repeats. The DNA-fragments are ligated into vectors to facilitate sequencing. Primers spanning the CA-repeat are designed and the amplification products are tested for polymorphisms in a panel consisting of different rabbit breeds and strains. Polymorphic microsatellites are subsequently analyzed in an $F_2$-intercross and/or three backcrosses. With this method we have been able to incorporate microsatellite markers in the linkage maps of chromosomes 1, 3, 5, 6, 7, 12, 15, 18 and 19. Research is ongoing to complete the chromosomal assignment for all linkage groups.

**D021**

**Identification of *BoLA-DRB3* in Japanese black cattle by PCR sequence-based typing**

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The bovine leukocyte antigen (*BoLA*) class II genes code for highly polymorphic transmembrane glycoproteins that present antigenic peptides to helper T cells. The *BoLA-DRB3* is thought to be the most functionally significant, as it is actively transcribed and at least 97 alleles have been characterized for this locus. In this study, we developed the technique of polymerase chain reaction-sequence based typing (PCR-SBT) which can assign *DRB3* alleles in DNA sequence level and would allow fast screening of large numbers of animals. Since an alignment of the predicted amino acid sequences of the β1 domain encoded by previously characterized *DRB3* alleles appeared that 93 distinct alleles were divided into 8 allelic groups, we designed 8 kinds of sequence specific primers (SSP) for the specific amplification of exon 2 of all alleles in each group for sense primer. First, *DRB3* alleles were amplified from animals obtained from the 5th international BoLA workshop by the primer set of one of 8 SSP and a locus-specific primer for antisense and each PCR product was directly sequenced. The typing results of 53 samples obtained by SBT were coincident with results had been typed by PCR-restriction fragment length polymorphism (RFLP). Next, a sample of 200 Japanese black cattle had studies by this method. We identified 20 distinct published alleles and 4 new alleles. The three most frequently isolated alleles, *DRB3*1001*, **1601* and **1101* were accounted for 17.5, 12.8 and 11.0% of the alleles in population of this breed, respectively. Results of this study indicate that the *BoLA-DRB3* locus is highly polymorphic in Japanese black cattle.

**D022**

## Analysis and frequency of *OLA-DRB1* alleles in Suffolk, Cheviot, Corriedale sheep

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To investigate the genetic diversity of the sheep MHC (OLA) class II *DRB1* locus, we amplified exon 2 of *OLA-DRB1* alleles by polymerase chain reaction (PCR), and determined the nucleotide sequences from both strands after cloning. Studying 78 sheep of 3 breeds such as Suffolk, Cheviot and Corriedale, 25 *OLA-DRB1* alleles were identified with frequencies ranging from 0.7% to 17.5%. Nineteen alleles were similar to those reported previously; and 6 new alleles. At the protein level, the identity between these alleles is rather high, ranging from 80.5% to 100%. Moreover, by a two-step PCR followed by digestion with restriction endonucleases *Rsa*I, *Sac*I, *Sac*II and *Hae*III, the 19 different restriction fragment patterns were identified that exactly matched the patterns predicted from DNA sequences. Thus, only 19 of the 27 sequenced *DRB1* alleles may be distinguished by PCR-RFLP. The 18, 14 and 9 allele types were identified in Suffolk, Cheviot and Corriedale sheep: their allelic frequencies differed within 3 breed sheep. Most frequently isolated alleles *OLA-DRB1n*2*, *OLA-DRB1n*23* and *OLA-DRB1n*22* accounted for 28.8%, 25.0% and 25.0%, respectively of the alleles in the population of Suffolk, Cheviot and Corriedale. Results of this study demonstrated that the *OLA-DRB1* locus is highly polymorphic in Suffolk, Cheviot and Corriedale sheep.

**D023**

## A BAC library of elite genomic BOVINE DNA constructed in a modified BAC vector

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Our aim is to clone and analyse multigenic trait loci in cattle. Therefore, we constructed a bovine genomic DNA library in a BAC vector that would enable ready manipulation and ligation of inserts. We replaced the polylinker of the vector pBeloBACII with one containing multiple 8-base rare-cutting restriction sites to generate the modified vector, pRazorBAC. Chromosomal DNA from the leukocytes of a purebred Holstein bull was partially digested with *Hind III* and then resolved via pulsed-field agarose gel electrophoresis (PFGE). Optimal PFGE separation was achieved by first applying short pulse times in the "reverse" direction to run smaller fragments off the "short" end of the gel (i.e. behind the wells) and then reversing the current to return the DNA through the origin and into the gel. Size-selected DNA was then electroeluted from the gel and ligated to *Hind III* digested pRazorBAC. The most critical parameters that affected DNA insert size were DNA preparation, size selection and elecroporation conditions. DNA of superior integrity was obtained through electroelution into dialysis tubing rather than agarase digestion from the agarose gels. This BAC library consists of approximately 100,000 clones (average insert size 120 kb; approximately 3-fold genome coverage) and represents a valuable resource for our ongoing structural and function dairy genomics research.

**D024**

**Assessment of biodiversity in a wide range of chicken breeds by genotyping DNA pools for microsatellite loci**

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During domestication, a vast range of genetic diversity has been accumulated in the chicken. Each breed or strain might comprise a unique set of genes. A widely accepted assumption is that potentially important genetic resources are represented in those populations that are most divergent within the species. In a project on chicken biodiversity (AVIANDIV) funded by the European Commission, eight laboratories collaborated to assess the genetic variation between and within a wide range of chicken breeds. In the first phase of the project, genetic diversity of 43 chicken breeds has been evaluated by genotyping a set of 22 microsatellites. The genotyping was carried out on breed-specific DNA pools made from 50 individual samples per breed. The estimated heterozygosity (Gene Diversity) varied between 26% and 66%, with an average of 47%. The average number of alleles per locus across breeds was 10.1. Using allele frequencies and sizes, four kinds of genetic distance were estimated. Based on these estimates, the mean genetic distance from other breeds (MGD) was calculated. This measure may indicate the degree to which a particular population shares its genomic polymorphism with the other analyzed populations. It was concluded that Red Jungle Fowl, traditional unselected breeds and broilers are highly polymorphic, and all had relatively small MGD values, in contrast, to both highly selected breeds (layers and experimental lines) and some local fancy breeds. The latter populations were characterized by low polymorphism, relatively high MGD values and are outliers from the spectrum of the detected biodiversity.

**D025**

## Multi-primer target PCR for rapid identification of bovine *DRB3* alleles

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*BoLA* DRB3 is the most polymorphic MHC class II gene in cattle. Currently, more than 70 bovine *DRB3* alleles have been characterized. The traditional method of typing based on PCR-RFLP is expensive, laborious and unable to distinguish all the alleles. Consequently, in order to efficiently screen large numbers of animals for the presence or absence of alleles associated with increased risk (DRB3.3*23 (DRB3*1501 & 1502)) and decreased risk (DRB3.2*16 (DRB3*2701-2703, 2705-2706)) of mastitis in Canadian Holsteins, we have developed a multi-primer PCR-based method. Two outer primers are used to amplify exon 2. Simultaneously, two inner, allele-specific primers amplify the individual alleles. Initially, 36 animals previously typed by PCR-RFLP were tested using the multi-primer target approach. The alleles tested include 20 of the original 30 *DRB3.2* alleles and all those at a frequency of greater than 5% in the Canadian Holstein population. All animals carrying alleles DR3.2*16 or DRB3.2*23 were correctly identified and there were no false positives. We are currently confirming the validity of this technique by typing 50 daughter/dam pairs first by the multi-primer approach, then by PCR-RFLP. Results can be obtained in four hours for the cost of a simple PCR reaction. As a result, this method can be used for rapid screening of a population for a specific allele and has the potential to be modified for numerous *DRB3* alleles.

**D026**

**Variation at microsatellite loci in the Lantang, Dahuabai and Landanrace breeds of swine**

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Fifteen highly polymorphic microsatellites were utilized to study the relationship between the Lantang, Dahuabai and Landrace breeds of swine. Lantang and Dahuabai are Chinese native breeds that are highly reproductive and very good meat tasty, but low daily gain and very fat. Although Lantang and Dahuabai are produced in the same province of China, they have had limited contact until recent years because Lantang was only produced in a little county with high inbreeding. Because of this situation, it is questioned whether the two breeds represent the same gene pool in Guangdong province. In order to more clearly define the relationship between Lantang and Dahuabai, Landrace was selected to serve as a potentially unrelated reference breed. Heterozygosity levels, $F_{st}$ and genetic distance were calculated from the allele frequencies in each breed. Heterozygosity levels indicated that Lantang was highly inbreeded. $F_{st}$ values indicated that Lantang and Dahuabai are moderately different. Genetic distances indicated breeds were related in following order (from most to least): Lantang, dahuabai, and Landrace.

**D027**

**Use of microsatellites to analyse genetic variability in four populations of *Columba palumbus***

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*Columba palumbus* (woodpigeon) is a very important game bird in European countries such as Portugal, Spain and France, so, it is essencial to understand the amount of genetic variability remaining within the existing populations and also the extent of gene flow between them. Eight microsatellite *loci* were studied in different populations of *C. palumbus* from Azores Islands (n = 23), Portugal Continental (n=33), Spain (n = 30) and France (n = 29). Genomic DNA was extracted from feathers samples using phenol/chloroform and commercial kits and analysed in 6% polyacrylamide gels using a fluorescence 4200S Li-Cor automated sequencer. The primers used were CliμDo1, CliμD16, CliμD17, CliμD32,CliμD19,CliμT13 and CliμT17 from *C. livia* (domestic pigeon breeds) and PTC3 from *Ficedula hypoleuca* (pyed flycatcher). Results showed that PTC3 and CliμD32 were monomorphic . The other ones were polymorphic with allele variation ranging from 5 to 12. Preliminary analyses of CliμT13, a tetranucleotide, showed dinucleotide variations in 6 individuals from Azores, Portugal Continental and France populations. The PCR products will be sequencing to confirm if we can assume this mutation as a population and/or specie specific marker.

**D028**

## Genetic distance among bovine populations estimated with AFLP markers

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Relative genetic distances of cattle individuals within and among European breeds were estimated on the basis of biallelic AFLP markers. The accuracy of these estimates was evaluated by bootstrap analysis. In a panel of 47 Holstein-Friesian cattle, genetic distances correlated with the additive relationship derived from herdbook data. In a panel of 116 animals from three different Italian breeds the mean pair-wise genetic distance within breeds was 89 % of the value across the breeds. A Principal Co-ordinates analysis of AFLP markers clearly clustered the animals according to the breeds. In a panel of 45 animals of 14 European breeds, we found a significant clustering of black-pied dairy animals and British breeds, but not of French or beef cattle. Our findings indicate that (a) AFLP can provide quantitative estimates of genetic distances of individuals; (b) breed formation had only a small effect on the overall diversity; and (c) genetic distances of breeds depend partly on geographical origin. These findings may be relevant for conservation efforts and breeding strategies.

**D029**

**Detection of origin between two bloodlines of Romosinuano cattle using microsatellite screening.**

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Two primary bloodlines of Romosinuano cattle exist (i.e., a Costa Rican [COR] and a Colombian [COL] bloodline) and have been imported for evaluation at STARS near Brooksville, Florida. The two bloodlines are phenotypically indistinguishable except for occasional scurs or rare white spotting observed in the COR bloodline. The objective of this study was to determine whether or not the genetic variation between the two bloodlines was significant and how accurately an individual's bloodline of origin could be determined based on genetic markers. The COR bloodline originated through upgrading from Hereford dams at the University of North Carolina from 1948-50's and was imported in 1990-92 as frozen embryos from the Centro Agronomico Tropical de Investigation y Ensananza (CATIE), Turrialba, Costa Rica. The COL bloodline was imported in 1996 from Venezuela as frozen embryos with no evidence of outcrossing from the pedigrees tracing to Colombian origins. Forty-seven individuals from each bloodline were identified and screened across 46 microsatellite loci selected by proximity to published carcass merit QTL, usefulness in previously published genetic distance studies, or chromosomal location maximizing genomic coverage. Unique alleles (n=66, COR and n=47, COL) were detected in 41 of the microsatellite systems comprising up to an allele frequency of 0.511 for a single system. Polymorphism information content values (both maximum and average) and average heterozygosities for the 46 systems were (COR) 0.891, 0.752, 0.571 and (COL) 0.918, 0.705, 0.579, respectively, facilitating correct bloodline assignments.

**D030**

## The use of microsatellites for measuring genetic diversity of European local beef cattle breeds for conservation purposes

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This study was undertaken to determine the genetic structure, the genetic relationships, and the genetic diversity of a set of 18 local cattle breeds from Spain, Portugal and France using 16 microsatellites. Heterozygosites, estimates of Fst, genetic distances, dendrograms, multivariate, diversity analyses and assignment tests were performed. Heterozygosities ranged from 0.54 in the Pirenaica breed to 0.72 in the Alentejana breed. Seven per cent of the total genetic variability could be attributed to differences among breeds (mean $F_{st}$ -0.07; $P<0.01$). The six computed genetic distances have been compared and no correlation was found to be significantly different from 0 between distance based on population effective size and those which use the sizes of the alleles. Support for internal nodes in phenograms estimated by bootstrapping was, in general, low, except for the Alistana/Mirandesa and the Salers/Aubrac groups, which appeared with an occurrence of 94% and 96% respectively. Multivariate analysis distinguished 4 breed groups. The diversity of the breeds was measured by the Weitzman's recursion approach which suggests that the most important breeds to be preserved are those included into two clusters: the one formed by Mirandesa and Alistana breeds, and the other one composed of the Sayaguesa and Tudanca breeds. The hypothetical extinction of one of those clusters presents a 17% of loss of diversity. In addition, the variation between breeds was sufficiently high as to assign individuals to their breed of origin with a probability of 99% for simulated samples.

**D031**

**Homozygosity mapping approach for the Chondrodysplasia gene in Dexter Cattle**

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Dexter cattle are a dwarf breed of cattle originating in Ireland which have been bred in Australia for several decades. There have been reports of mutant, aborted foetuses in this breed of cattle, described as chondrodysplastic foetuses. The affected foetuses display disproportionate dwarfism, a short vertebral column, marked micromelia, a relatively large head with a retruded muzzle, cleft palate and protruding tongue and a large abdominal hernia. Dexter chondrodysplasia is considered to be inherited in an incompletely dominant manner. As part of an approach to controlling the disease in Australia, the Australian Dexter Association has chosen to support research to develop a DNA test to prevent carrier/carrier matings. A homozygosity mapping approach is being used to localise the disease gene. Firstly, 12 candidate genes were identified by searching for diseases with similar phenotype in other species. Using comparative mapping, nine regions on the cattle genome were selected. A total of 90 microsatellite markers were used to cover these regions. A selection of animals was genotyped and the results analysed by searching for regions of homozygosity in the affected samples. Of the 90 selected markers, one demonstrated a homozygous pattern amongst the affected samples, but not among the parents and unrelated animals. A gene in this region is currently being screened for mutations. If a mutation is found, a DNA based heterozygote test can be developed.

**D032**

**Genetic polymorphism of Goat k-casein**

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Investigation on milk protein polymorphism in goat mainly concerns *CSN1S1* fraction, which is characterized by a high qualitative and quantitative genetic variability. Isoelectic focusing (IEF) in ultrathin polyacrylamide gels with carrier ampholytes was used to demonstrate *CSN3* polymorphism in milk samples of Italian (Orobica n=36; Saanen n=60) and German goat breeds (Weisse Deutsche Edelziege n=85; Bunte Deutsche Edelziege n=25; Thüringer Waldziege n=57). A genetic polymorphism resulting in three phenotypes (A, AB, B) could be demonstrated at *CSN3* locus in addition to the already described polymorphism in *CSN1S1, CSN1S2* and *CSN2*. The further *CSN3* casein band exhibited a more cathodic migration than $CSN3^{A}$. $CSN3^{B}$ can only be resolved using an ultranarrow pH range pH 4-5.5. Otherwise there is an overlapping by *CSN1S2* C and other protein fractions. After chymosin action, the genetic polymorphism was also observed in the para-k-casein fraction. Thus, the further allele might correspond to the B variant described by Di Luccia *et al* (1990). The genetic basis of $CSN3^{B}$ was confirmed by genetic studies. The frequency of $CSN3^{B}$ ranged from 0.36 (Orobica) to 0.26 (Bunte Deutsche Edelziege). The populations were in Hardy-Weinberg equilibrium at *CSN3*. $CSN3^{B}$ occurred in low frequency (<0.03) in Thüringer Waldziege and in the related breeds Weisse Deutsche Edelziege and Saanen. The results indicate that goat *CSN3* locus is more polymorphic than described until now and studies are in progress to evaluate the influence of the different *CSN3* genotypes on cheesemaking properties as already demonstrated in bovine milk.

**D033**

## Genetic relationships among some Criollo populations bred in Caribbean area

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The polymorphisms of 19 genetic systems, 9 blood groups, 5 blood proteins and 5 milk proteins were investigated in a Carora population (Criollo x Brown Swiss) bred in Venezuela to characterize the genetic structure. Since 9 loci (ALB, TF, CA, HB, CSN1S1, CSN2, CSN3, LALBA, LGB) were in common with other researches carried out in three Criollo populations of Caribbean area, the data were pooled and submitted to statistical analysis in an attempt to define their genetic relationships. In addition, three breeds (Holstein Friesian, Brown Swiss and Zebu) were included in the study for their possible relations with the investigated breeds. The gene differentiation coefficient Gst was computed by DISPAN program and the genetic distances were calculated by PHYLIP program. Moreover, Principal Component Analysis was performed. The results show a clear and expected separation among Zebu, Holstein and Brown while the other breeds are rather closed to the last two ones. The more interesting loci for gene differentiation were ALB, CSN1S1, LALBA. These results confirm that the traditional blood and milk genetic polymorphisms provide an efficient marker system to discriminate *Bos taurus* from *Bos indicus* and to quantify the blood percentage among cross-bred cattle populations.

**D034**

## Cloning and characterisation of CYP51 transcripts in pig

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Lanosterol 14 α-demethylase (CYP51) is a microsomal cytochrome P450 enzyme involved in postsqualene cholesterol biosynthetic pathway. CYP51 removes 14 α- methyl group from lanosterol, forming FF-MAS (**f**olicular **f**luid **m**eiosis **a**ctivating **s**terol). The gene shows remarkable conservation between species and is the only member of the cytochrome P450 superfamily whose sequence is conserved across phyla. The quantitity of CYP51 mRNA varies between tissues. The highest expression is in mammalian (mouse, rat, human) testis. Besides somatic transcripts, shorter CYP51 transcripts are present in testis of these species. These shorter transcripts are germ cell specific, with the highest level of expression in round and elongated spermatids. The goal of our study is to determine the expression of CYP51 in pig and to evaluate if a shorter, testis specific CYP51 transcript is found also in testis of this mammal. RACE (**r**apid **a**mplification **c**DNA **e**nds) PCR techniques was used for characterisation of pig CYP51 transcripts. Combination of classical PCR and RACE-PCR on the pig testis cDNA library enabled isolation of three polyadenilated CYP51 cDNA clones with lenghts 1620 bp, 1623 bp and 1725 bp. A CYP51 cDNA from pig liver which has been reported in GenBank recently, has a longer 3' untranslated end compared to the testis clones. Northern analysis showed two CYP51 mRNAs (3.1 kb and 2.2 kb) highly expressed in pig testis. The 3.1 kb trancript is probably identical to the liver one. The partial pig testis polyadenylated clones which end at 1620 bp, 1623 bp and 1725 bp probably all belong to a broadened 2.2 kb long transcript. Immunohistochemical studies of pig testis show highest level of CYP51 protein expression in Leydig cells and in round and elongated spermatids. Colocalisation of the electron transferring enzyme, NADPH citochrom P450 reductase, indicates that the CYP51 protein is functional. Preliminary results of immunogold EM in mouse testis show CYP51 localised in the membrane of smooth endoplasmic reticulum in Leydig cells and round spermatids and on the acrosomal membrane in round and elongated spermatids. High levels of CYP51 mRNA and protein expression in germ cells sugessts that CYP51 may play a fundamental role in reproduction *via* formation of meiosis activating sterols MAS.

**D035**

## Lipogenesis genes and fatness in the chicken

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Molecular genetic mechanisms involved in the meat chicken fatness were investigated, based on the lipid metabolism pathway. Experiments were conducted in experimental lean and fat chicken lines obtained by divergent selection on abdominal fat weight. Several genes coding for enzymes of fatty acid synthesis (ATP-citrate lyase [ACL], acetyl-CoA carboxylase, fatty acid synthase, malic enzyme [ME], stearoyl-CoA desaturase), an apolipoprotein (apolipoprotein A1 [apoA1]) and a transcription factor (C/EBPα) were studied. A first set of experiments was done to compare the hepatic transcription rates (run on assays) and mRNA levels of the quoted genes between lean and fat chickens. These criteria were significantly higher in the fat line for the ACL, ME and apoA1 genes. This result suggested that these genes contribute to the fattening variability. Besides, a cosegregation analysis of ACL and apoA1 alleles and adipose tissue weight was achieved in full- and half-sib F2 families derived from a cross between the fat and lean lines. In the studied families, no relationship between the gene sire alleles and the trait values appeared, meaning that these genes could not be considered as responsible for the phenotypic variability. These results emphasized the need of complementary analyses (gene expression and genetic association) in the search for genes of quantitative trait.

**D036**

**Characterization of Epitheliogenesis Imperfecta in *Equus caballus***

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Epitheliogenesis Imperfecta (EI) is a severe, mutilating mechanobullous disease in horses. Affected foals have been observed in the American Saddlebred and Belgian Draft breeds. Inheritance patterns indicate that it is an autosomal recessive disease. Transmission electron microscopy (TEM) of skin from affected horses revealed a separation between the epidermis and dermis at the level of the Lamina Lucida. TEM also revealed reduced numbers of small abnormal hemidesmosomes. Based on these findings the three subunits of the Laminin 5 protein were identified as possible candidate genes. Linkage disequilibrium analysis was performed using markers chosen based on information from the horse/human comparitive map and the equine gene map. The analysis suggests an association between EI and Equine chromosome 5, a likely location of the Laminin α3 and Laminin γ2 subunits.

**D037**

## Isolation and characterization of equine ß-casein cDNA

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Lactoprotein genes belong to the best studied loci in most farm animal species. However, until recently the only equine lactoprotein cDNA sequence available in the GenBank was cDNA sequence encoding β lactoglobulin. We focused our research on milk protein genes in horse because of the possibility to use mare milk as replacement for cow's milk for children, sensitive to cattle milk proteins. From the lactating mare mammary gland mRNA was extracted and used for cDNA synthesis. The cDNA was cloned into the pBluescript vector and propageted in competent *Epicurian Coli Cells*. Screening of positive colonies was performed using DIG-labeled PCR product representing the 445 bp fragment of the equine *β-casein* (*β-CN*) cDNA, obtained with heterologous primers based on bovine *β-CN* sequence. Sequencing of the cDNA was performed on ABI Prism 310 Genetic Analyzer. The obtained cDNA sequence is full length at the 3'-end, but 41bp shorter than the bovine homologue at the 5'-end. However, the exact determination of the transcription start site has not been performed. The entire coding region for equine *β-CN* has 75.1%, 74.4 %, 73.8%, 72.3%, 69.9% sequence identity with camel, porcine, human, bovine and rabbit *β-CN* mRNA, respectively. The sequence identity within the signal peptide coding region is considerably higher than in the remaining parts of the cDNA sequence.

**D038**

**Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*).**

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The control region is the most rapidly evolving segment of the mitchondrial genome and its sequence should provide a sensitive assay of residual genetic variation in cheetahs, which are the most widely quoted example of a genetically depauperate species. 525 bp from this area were sequenced and analysed for 20 *Acinonyx jubatus* and one *Felis catus*. These sequences were analysed and compared with published sequences from another domestic cat, 20 ocelots (*Leopardus pardalus*) and 11 margays (*Leopardus weidii*). The intra-specific population divergence in cheetahs was found to be less than in the other cats. However variation was present and two main haplogroups which correspond to a north-south geographical division are discernible. The 80bp RS2 repetitive sequence motif previously described in other felids was found in four copies in cheetah. The two central repeat units in cheetah show homogenisation which may have arisen by convergent evolution.

**D039**

**Arthrogryposis in Piedmontese cattle breed: a genetic analysis**.

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Arthrogryposis is a congenital syndrome affecting a considerable number of new-borns in Piedmontese cattle. It is characterised clinically by limb juncture hyperflexion and muscle atrophy at birth. The high frequency within families, particularly in males, and similarity with other animal and human heritable syndromes (particularly Spinal Muscular Atrophy), justify a study on this disorder by genetic approach. The present work is part of a wider study on arthrogryposis in Piedmontese breed. The aim of this work was the study of this syndrome by disease frequency analysis at population level and by candidate gene approach. Starting from 1980 syndrome incidence in the breed has been analysed on a studbook database. The *Survival Motor Neuron* 1 (SMN1) gene has been chosen as candidate gene. By comparative alignment on DNA sequence databanks, several primer pairs have been drawn to amplify a great part of the gene. RT/PCR from spinal cord of 2 affected calves and one control, plasmid cloning and ABI Prism 377 P.Elmer sequencing have been performed. From population data the disease resulted inherited as a recessive trait, showed an incidence of 1.8% on the total new-born population and an incomplete penetrance in female. Complete sequences (from exon 1 to 7) and sequences with exon 2 or 5 deletions have been found by SMN1 cDNA clones sequencing, indicating an alternative splicing mechanism, not directly correlated to the presence or the absence of the syndrome in our samples. Our results could suggest a pathological effect conditioned by a modulation of the mRNA maturation or the presence of gene duplication.

**D040**

**Mapping of QTL involved in growth, backfat thickness and intramuscular fat content in pigs. Regional RH mapping in the QTL region identified on chromosome 7.**

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A QTL detection experiment has been performed at INRA since 1991 in F2 crosses between Meishan (MS) and Large White (LW) pig breeds. Six F1 males and 23 F1 females issued from 6 LW boars and 6 MS sows produced 530 F2 males and 573 F2 females. F2 animals were measured for a large number of traits related to growth, backfat thickness, carcass composition and meat quality. QTLs were evidenced on chromosomes 1, 4, 7, 8 and 11 for growth traits, on chromosomes 1, 6, 7, 8 and X for backfat thickness, on chromosomes 2, 4, 7, 17 and X for carcass composition traits and on chromosome 7 for intramuscular fat content. To refine position of QTL, production of backcross animals and haplotype analysis on various populations are underway. On chromosome 7, the Meishan allele has a positive effect on growth, backfat thickness and intramuscular fat content. The most likely position of the QTL is close to SLA region. Construction of a BAC contig and sequencing of the SLA Class I region is performed (see Renard et al, this congress). To improve knowledge of the region surrounding SLA, we simultaneously developed a high-density RH map of swine chr7p-q12 using IMpRH radiation hybrid (RH) panel. We added on the RH map 23 new loci (8 microsatellites, 14 genes and one EST). Our results show that gene order is conserved between chromosomal region HSA 6p and SSC 7p-q12. All these studies will help to identify gene(s) responsible(s) for QTL effects observed on chromosome 7.

**D041**

## The relationship of sequence homology between species to within species rate of polymorphism: evidence from 108 pig genes

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The genetic dissection of complex traits is one of the most challenging problems in livestock genome mapping. A dense set of nucleotide polymorphic markers, together with automated genotyping approaches brings the prospect of widespread use of linkage disequilibrium to map quantitative trait loci (QTL) in livestock. We report here the results of a pilot study on characterization of nucleotide polymorphisms in 108 porcine gene-fragments (one fragment per gene). We identified 134 nucleotide polymorphims in 37.5 kb of porcine genome, including 120 SNPs, 9 sequence repeat variations and 5 deletion/insertions. We observed differences between exon and intron regions in the rates and types of polymorphisms and in proportion of transition and transversion that occurred at CpG dinucleotide sites. In coding sequences, the rate of synonymous and non-synonymous SNP decreased significantly with increase of homology among mammalian species ($P < 0.0001$). In introns, those that exhibited variation in interspersed repeats, such as SINE, LINE, MIR, MER and STR, between species also exhibited twice the frequency of within breed nucleotide polymorphism than introns without interspersed repeats. This study suggests strategies that would maximise the efficiency of developing genetic markers within genes in order to facility mapping of mammalian genome.

**D042**

**Molecular and functional characterization of coat color mutations at the *I/KIT* locus in the pig**

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Together with *Extension/MC1R*, Dominant White (*I/KIT*) is the major coat color locus in pigs and has been extensively characterized at the molecular level. *KIT* encodes a tyrosine kinase (mast/stem cell growth factor receptor) with an important role in several tissues during embryonic development and in adult animals. In mice, most of the viable *KIT* cause a variety of pigmentation defects due to altered expression which are often associated to long-range genomic rearrangements. Four *KIT* alleles have been documented in the pig so far: Dominant white (*I*), Patch ($I^P$), Belted ($I^{Be}$) and the recessive wild type allele (*i*). *I* and $I^P$ are associated with a long-range duplication that contains the entire coding region of *KIT*, while the molecular basis of $I^{Be}$ has still to be unraveled. The further characterization of these mutations is of great interest, in that provides the opportunity to gain insight in the regulatory mechanisms of tissue-specific expression. We characterized the *KIT* expression profile in various adult tissues of *I/I*, $I^{Be}/I^{Be}$ and i/i animals by Northern Blot analysis and quantitative RT-PCR (TaqMan technology). We show that *KIT* expression is dramatically reduced in the lung of $I^{Be}/I^{Be}$ animals, stressing the similarity between $I^{Be}$ and some mice W mutants, while in *I/I* animals both copies of *KIT* are expressed in most tissues. We also carried out physical characterization of the same alleles by Pulse Field Gel Electrophoresis (PFGE), and constructed a BAC contig spanning *KIT* and its flanking region. Current work is aimed to refine the precise location of the duplication breakpoint proximal to *KIT*.

**D043**

**Genetic diversity analysis and parentage control in Korean native cattle using Microsatellite**

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Korean native cattle have been characterized by using 8 microsatellite markers (ETH3, ETH10, ETH225, TGLA122, TGLA126, TGLA227, BM1824 and BM2113) and parentage control have been investigated. Allele frequencies were calculated and used for the characterization of Korean native cattle. The diversity value, number of alleles per locus, heterozygosities and PIC values, is calculated for each marker on the basis of frequency data. And exclusion probabilities is calculated and determined on exclusion efficiencies for each marker. The number of alleles varied between 6 and 13 with an average number of alleles per locus of 8.75. ETH3, ETH10, ETH225, TGLA122, TGLA126, BM1824 and BM2113 display 7, 9, 7, 13, 7, 12, 6 and 9 alleles, respectively. Among them TGLA122 display most (13) and BM1824 display smaller numbers (6) of allele. The highest heterozygosity is observed at TGLA227 (0.880). In contrast, TGLA126 is the least heterozygosity (0.644). The mean expected heterozygosity is 0.789. Polymorphic information content is observed from 0.608(TGLA126) to 0.861(TGLA227) and mean polymorphic information content is 0.757. This result exhibit that Korean native cattle is higher polymorphic than other European improved cattle breeds. The exclusion probability is observed from 0.427(TGLA126) to 0.748(TGLA227). The highest exclusion probabilities are observed with TGLA227(0.748) and ETH10(0.740), whereas TGLA126 is found to be the least exclusion probability (0.427). The total exclusionary power of all markers is 0.999. This result exhibit to be suitable for parentage control in Korean native cattle using 8 microsatellite polymorphisms investigated in this study.

**D044**

**Simultaneous PCR of equine *SRY* and *AMEL*, and its application in infertile mares.**

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Chromosomal abnormalities are common in infertile mares with small gonads lacking follicular development or oestrus cycles. To help the diagnosis prior to karyotyping, a quick method for sex determination of horses was developed. Simultaneous amplification of equine *sex-determining region of Y chromosome gene* (*SRY*) and *amelogenin gene* (*AMEL*) accomplishes to determine the presence of both sex chromosomes and *SRY*. By the agarose gel electrophoresis, PCR products from normal stallion samples represent 1 *SRY* band and 3 *AMEL* bands (*AMELX*, *AMELY* & *AMELX* / *AMELY* heteroduplex), besides normal mares show single *AMELX* band. In 3 XY-mares tested, no *SRY* bands were detected while 3 *AMEL* bands were detected as well as normal males. This method enables a quick diagnosis for the infertile mares prior to karyotyping.

Genbank Accession: AB004572 (*SRY*)
: AB032193 (*AMELX*)
: AB032194 (*AMELY*)

**D045**

**Allelic variation at the porcine α-inhibin(*INHA*), $\beta_A$-inhibin/activin (*INHBA*) and $\beta_B$-inhibin/activin (*INHBB*) loci**

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Inhibins and activins, members of the *TGF-β* superfamily, are involved in the male and female reproductive axis and in growth and differentiation. *INHA* maps to SSC15 where evidence of a QTL for ovulation rate has been reported. We have investigated the porcine *INHA*, *INHBA* and *INHBB* genes of up to 33 boars of the Pietrain (14), Deutsche Landrasse Sauenlinie (11), Deutsche Landrasse Universal (3) Deutsches Edelschwein (1), Large White (1), Schwäbisch Hällisches Schwein (1) and Meishan (2) breeds for allelic variation by Southern blot RFLP analysis with 10 restriction enzymes using full length porcine and rat cDNA probes. *INHA* was highly polymorphic, where *Bam*HI, *Bgl*II, *Hind*III, *Pvu*II, *Sac*I and *Taq*I detected a large number of polymorphisms that describe at least 7 different alleles. *Msp*I was also polymorphic with the *INHA* probe, but could not be consistently typed. *Hind*III, *Msp*I, *Sac*I and *Xba*I detected RFLPs at the *INHBA* locus which identify up to 6 alleles. *INHBB* was less variable with at least 3 alleles detected by *Bgl*II and *Taq*I. Published porcine cDNA sequences were used to design primers for PCR amplification of 730 bp (*INHA*), 843 bp (*INHBA*) and 667 bp (*INHBB*) fragments that contain the mature subunits and a portion of the pro region of these genes. PCR products derived from Wild Boar, Meishan, Pietrain and Edelschwein were cloned and are currently being sequenced to evaluate the extent of coding region sequence polymorphism.

**D046**

**Diversity in the ovine MHC *DQA* region**

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Approximately 2000 sheep from five different breeds have been genotyped at their MHC *DQA1* and *DQA2* loci using Southern hybridisation. This revealed 8 alleles at the *DQA1* locus and 16 alleles at the *DQA2* locus. A rapid PCR-SSCP typing system was developed for each of the loci and this, along with DNA sequencing, revealed a further 6 alleles at *DQA2*, some of which appear to be breed-specific. It is expected that further polymorphism will be found as more breeds and sheep are screened. Sequence analysis revealed that some alleles had potentially ineffective splice sites at the end of the hypervariable second exon. This suggests these alleles may be inactive, or possibly pseudogenes. Sequence alignment showed that some *DQA2* alleles that had greater sequence homology to bovine *DQA3* sequences, than to other ovine *DQA2* sequences.

**D047**

## Association of single nucleotide polymorphisms in the growth hormone receptor gene with blood serum IGF-I concentration and growth traits in Angus beef cattle

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To evaluate the association of four single nucleotide polymorphisms (SNPs) in the bovine growth hormone receptor (GHR) gene with blood serum IGF-I concentration and growth traits, 470 Angus calves divergently selected for blood serum IGF-I concentration at the Eastern Ohio Resource Development Center were examined for SNP genotypes as described earlier (Ge *et al*., 2000 J. Anim. Sci., in print). Blood serum IGF-I concentrations on d 28, 42, and 56 after the beginning of postweaning test, mean IGF-I concentration, postweaning gain, and weights at birth, weaning, and beginning and end of the 140-d postweaning test were analyzed using animal models and the MTDFREML computer program. The full animal model included year and season of birth, sex, selection line, age of dam, age of calf and four SNPs as fixed effects, and direct genetic, maternal genetic, and permanent environmental effects as random effects. Maternal genetic and/or permanent environmental effects were deleted from the full animal model if the effect accounted for less than 20% of the total phenotypic variance. One SNP located in the promoter region of the bovine GHR gene was significantly associated with all IGF-I concentrations. Significant association with IGF-I concentration on d 28 and 42 of the postweaning test was also found for another SNP located in the $10^{th}$ exon. A third SNP showed significant association with mean IGF-I concentration and tended to be associated with other IGF-I concentration measurements (P = .10). More tests are needed to fully evaluate the association of these SNPs with blood serum IGF-I concentrations and growth traits.

**D048**

## Null-alleles in the standard set of loci for cattle parentage control

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In cattle parentage control, DNA typing of microsatellite markers is becoming an increasingly useful tool and many cattle laboratories are now processing towards a complete shift from the conventional blood typing to DNA typing. Different panels of microsatellites have been evaluated in the last 3 international cattle comparison tests, and a set of guidelines concerning marker set, exclusion parameters, reference sample and nomenclature has been proposed and will be further discussed at the present ISAG Conference. Another important topic for discussion is how and where to report instant problems, like discovery of null-alleles, to ensure that all laboratories get the information in order to keep parentage control a dynamically evolving procedure with a high degree of confidence. We report here the identification of three null-alleles that were discovered during routine parentage control using the Cattle StockMark Kit II from PE Biosystems. Two null-alleles were discovered in *TGLA122* in two different breeds, and one null-allele was discovered in *INRA023*. New primers were designed outside of each of the original primer sites to amplify the silent alleles, and the amplified fragments were cloned into pCR2.1 (Original TA Cloning Kit from Invitrogen). Sequencing of the clones revealed point mutations in the original primer sites. Lowering of the annealing temperature from 61 to 56 degrees Celsius during PCR with the Cattle StockMark Kit II resulted in amplification of all three “null”-alleles.

**D049**

**Calcium chloride induction of an enhanced green fluorescent protein gene driven by the metallothionein promoter**

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Many of the initial experiments with transgenic animals used a combination of a metallothionein promoter and a growth hormone gene. This promoter was chosen for its inducibility by the presence of heavy metals (i.e. zinc, cadmium, lead, and copper). However, there were concerns that the promoter was active, even in the absence of such heavy metals. The sockeye salmon metallothionein promoter was used to drive the enhanced green fluorescence protein (*eGFP*) gene in transfected Chinese Hamster Ovarian (CHO) cells. Aberrant expression of the transgene shortly after calcium-phosphate transfection, and in the absence of heavy metals, was noted. After establishment of stable transfected cell lines, we found that concentrations as low as 12.5 mM of calcium chloride were sufficient to induce *eGFP* expression under control of this promoter. Thus, it is possible that leaky expression of metallothionein driven transgenes is the result of calcium-based induction.

**D050**

## Microsatellite DNA testing for parentage verification of thoroughbreds

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For individual identification and parentage verification of horses, especially thoroughbreds in Japan, we developed a microsatellite DNA typing with the routine 12 markers, AHT4, AHT5, ASB2, ASB17, HMS3, HMS6, HMS7, HTG4, HTG10, TKY19, TKY28 and VHL20, and the backup panel of four markers, ASB15, LEX33, TKY3 and UCDEQ425. This method consisted of multiplexing PCR procedures, and it showed reasonable amplification of all PCR products. Sizing precision with standard deviations of PCR fragment size at all loci was confirmed to be less than ± 0.4bp. Using the routine panel, 2,124 thoroughbreds in Japan were analyzed. Observed allelic frequencies provided a discrimination power of $6.74 \times 10^{-12}$, and it was considered that there were no null alleles at 12 loci in this population based on agreement between observed and expected heterozygosities with no significant deviation from H-W equilibrium. The exclusion probability (PE) on the routine panel was 0.9997 and a combined PE of the routine and backup panels was exceeding 0.9999. These results demonstrate that the present DNA typing is so useful for individual identification and parentage verification of thoroughbreds.

**D051**

## Establishment and characterization of 600 canine (*Canis familiaris*) cDNA clones

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The dog shows a variety of disorders well suited as a model for understanding the molecular mechanisms underlying corresponding human diseases. Nevertheless, canine molecular genetics is still far behind that of human and mouse. A limiting step in using the dog as an animal model is the still small number of cloned genes. We established a cDNA library from canine testis tissue and picked random clones for analysis. By sequencing and characterizing 500 cDNA clones we showed that based on the gene level the homology between man and dog is much higher than estimated so far. The characterized cDNA clones showed a homology of 85 % - 100% to known human genes or ESTs, as e.g. the fragile X mental retardation syndrome related protein 1 (*FXR1*), interacting with *FMR1* (fragile-X-mental retardation), the Huntingtin interacting protein or the ubiquitin specific protease 4. In addition, we compared the sequence homology in the open reading frame and the 3'UTR separately. As the dog is well accepted now as an animal model for analyzing human diseases, it is very important to characterize and provide sequence data for canine genes in the near future. Combining these data and mapping genes will be a powerful tool for analyzing many human diseases as well.

**D052**

**Canine Mitochondrial DNA, a Novel Criminal Case Study**

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Evidence from an attempted capital murder was submitted for analysis using canine STRs. The evidence was in the form of canine hair that was retrieved from the crime scene. An attempt at STR analysis failed due to the fact that the hair was shed hair with no follicles. The hairs were then cleaned, ground and prepared for extraction. Mitochondrial DNA was extracted and amplified using two primers designed to amplify hypervariable region one in the dog. The product obtained produced template DNA to be sequenced using two more primers. One primer was 5' and the other 3'. The resulting products were quantified on an agarose gel prior to sequencing. Sequencing was performed with an ABI 377 Sequencer using the Big Dye Terminator sequencing kit by Perkin Elmer. Sequences were consistent with published sequences for canine HV1. Upon analysis of the sequences, there was heteroplasmy observed at a site known to be polymorphic in the evidentiary samples. The same heteroplasmy was seen in the known samples submitted with the case. Therefore, the suspect was linked to the crime scene on the strength of this evidence. This case establishes that the use of mitochondrial DNA from animals can be a valuable tool to the forensic community.

**D053**

## Identification and characterization of a second interferon-regulated bovine Mx gene

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Mx proteins belong to the GTPase family, and are induced by type I interferons (IFNs), including IFN-tau, and which express antiviral activity. We have isolated a new bovine Mx gene (Mx2) which is distinct from bovine Mx1 from an endometrial (caruncular) cDNA library in pregnant Japanese Black Cattle (Day30 of gestation). We analyzed the structure of the isolated gene and performed genomic PCR using genomic DNAs from eight bovine breeds to investigate whether or not all breeds possess both Mx genes. Further, semi-quantitative RT-PCR analysis was performed to evaluate levels of mRNAs coding for bovine Mx1 and Mx2 in the endometrium (caruncle and intercaruncle) during the non-pregnant to pregnant period. Mx2 has a single functional open reading frame as same as Mx1, although the 5' untranslated region and the 5' coding region of Mx2 cDNA were rather different from the regions of Mx1. As a result of genomic PCR, it was indicated that eight bovine breeds all had both Mx genes, so the new bovine Mx was strongly suggested to be bovine Mx2. Further, both Mx genes were expressed from the non-pregnant to pregnant period, especially Day 17 and 20 of gestation when trophoblast secretes IFN-tau. In conclusion, cattle have at least two functional Mx proteins which might provide antiviral activity and/or perform a fundamental function in the endometrium, especially in early pregnancy.

**D054**

## Sequencing and mapping of PERVs (Porcine Endogenous Retroviruses) in Westran pigs

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Pigs are regarded as a potentially good source of organs and tissues for transplantation into humans. However, porcine endogenous retroviruses have emerged as a possible problem as they can infect cultured human cells. Two main types of pig retrovirus, determined by envelope protein, PERV-A and PERV-B are widely distributed in different pig breeds and a third less common type, PERV-C has been recognised. Endogenous retroviruses have been analyzed from the Westran (Westmead transplantation) inbred line of pig, specially bred for biomedical research. Thirty one 1.8 kb *env* PCR product clones were sequenced after preliminary screening with the restriction enzymes *Kpn*I and *Mbo*I. Five recombinant clones between A and B were identified. 55% of clones (17/31) sequenced have stop codons within the envelope protein-encoding region, which would prevent the retrovirus from making full length envelope protein recognizable by cell surface receptor for the virus. The endogenous viruses have been physically mapped by FISH (Fluorescence *In Situ* Hybridisation) using PERV-A and PERV-B envelope clones as probes. Preliminary FISH data suggest that there are 15 plus PERVs (6 PERV-A and 9 PERV-B) and the chromosomal location of these are quite different from European Large White pigs. The sequences and mapping results of inbred Westran pig suggest that there are relatively few PERV integration sites compared with commercial pigs and further a large proportion of clones are defective due to premature stop codons in the envelope gene.

**D055**

## Predicting Heterosis Using Biochemical and RAPD Markers in Animal Species

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The animal industry has a history of using breed crosses and/or strain crosses in commercial production, mainly to take advantage of heterosis. Association of heterosis of economic traits with biochemical and RAPD markers were therefore investigated, which involved 23 alleles at 8 polymorphic biochemical loci in poultry populations and 5 RAPD markers amplified by 2 random primers in swine stocks. Genetic distance between parental populations based on markers of polymorphic blood groups and isozymes were favorably associated with percent heterosis in poultry. However, neither RAPD-based parental difference nor average band sharing was predictive of the level of heterosis in swine. The result was not an evidence that RAPD markers were not promising for predicting heterosis, but rather that choosing proper markers and populations were more important than choosing types of markers. Sequent random sampling experiments on choosing biochemical markers applied in predicting heterosis supported the hypothesis that association of heterosis with markers might varied greatly with markers employed. Therefore, improper use of markers in predicting heterosis was likely to result in deviation.

**D056**

## Mitochondrial DNA diversity and possible dual origins for BOS INDICUS

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The origins and demographic history of domesticated cattle are controversial. The two major domestic cattle forms, taurine (*Bos taurus*) and zebu (*Bos indicus*), are differentiated primarily by the absence or presence of a hump, and are regarded as separate subspecies. Prior analysis of mitochondrial DNA (mtDNA) control region sequences has suggested that zebu cattle were domesticated independently from taurine cattle, presumably from a different aurochsen subspecies from the Indian subcontinent. In an attempt to address the origins of indicine cattle we have examined a 240 bp segment of the mtDNA control region from seven Indian breeds. Phylogenetic analyses revealed that haplotypes generally fall into two distinct groups corresponding to a geographical north-south division. These observations suggest more than one wild ancestral strain of indicine cattle.

**D057**

**Genetic relationships between nine French Horse breeds.**

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The aim of this study is to investigate the genetic variability of a semiferal french breed namely *Pottok* (PO; n = 461) and its genetic relationships with two breeds from the same area *Landais* (LA; 78) and *Merens* (ME; 21), a draught breed *Breton* (BR; 88) and five French race and riding breeds, namely *Trotteur Français* (TF; 1836), *Selle Français* (SF; 1452), Thoroughbred (TB; 4065), Arabian (AR; 1080), Anglo-Arab (AA; 214). The analyses were performed using a set of 11 microsatellites (AHT4, AHT5, ASB2, HMS1, HMS3, HMS6, HMS7, HTG4, HTG6, HTG10 and VHL20) including the international parentage panel. Phenetic trees were generated by UPGMA with Nei's standard genetic distances and Neighbor-Joining with Cavalli-Sforza's chord distances. Multidimensional analysis was processed by correspondence analysis, which allows horse breeds and alleles to be ploted simultaneously. All phenograms showed a similar topology, with two major clusters. One group involved the three Pyrenean-"*Aquitaine*" breeds, *i.e.* PO, ME and LA, and the draught breed BR. A second cluster grouped TF, SF, AA and TB. Within this group, TF, originated from "old" French populations, was well differentiated from the others. SF and AA were still close to the Thoroughbred, resulting from its great genetic contribution. Finally, Arabian was in an intermediate position. Multidimensional analysis showed a similar pattern. These results are discussed on the light of historical and genealogical data, considering especially the possible ancient contribution of Arabian to Pyrenean-"*Aquitaine*" breeds.

**D058**

## Analysis of hereditary diseases genes in Lithuanian and Finnish cattle populations

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BLAD (Bovine Leukocyte Adhesion Deficiency), Citrullinaemia and DUMPS (Uridine Monophosphatase Synthesis Deficiency) genes were examined in Lithuanian and Finnish Holstein-Friesian cattle populations. Testing of mutations was performed by PCR-RFLP method with genomic DNA isolated from blood, semen and hair roots. Rapid method for extraction DNA from hair roots was optimised. No DUMPS or Citrullinaemia genes were found in Lithuanian and Finnish cattle populations. BLAD gene with the frequency of 3,7% within bulls, 6,7% within cows and 27% within daughters of the BLAD gene carriers bulls was found in Lithuanian HF cattle population. Pedigree analysis of bulls BLAD gene carriers showed that the bulls were paternal or maternal linked to the bull Osborndale Ivanhoe - ancestor of the mutation.No statistically significant differences in morfphological and biochemical blood testing between heterozygous for BLAD gene and having no mutation cows were found. No homozygote for BLAD was identified in tested population. No BLAD gene was found in Finnish Holstein-Friesian cattle population.

**D059**

## The *RN* gene involved in meat quality in pigs encodes a muscle specific isoform of a regulatory subunit of AMP-activated protein kinase

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The *RN* gene has an unfavorable effect on meat quality in pigs. The dominant *RN*$^-$ allele affects not only yield of cured cooked ham but also tenderness and mellowness of meat. In order to identify the *RN* gene, we developed a high resolution linkage map using more than 1000 meiosis, a local Radiation Hybrid map in Pig and in Human, a BAC contig of 2.5 Mb, and a linkage disequilibrium map using Hampshire boars. We finally sequenced a BAC containing markers in total linkage disequilibrium with the *RN*$^-$ allele. Analysis of BAC sequence allowed us to identify a gene showing 63 % identity with a regulatory subunit of AMP-activated protein kinase (AMPK). The whole coding sequence of this new gene has been determined using RT-PCR and 5' RACE. Northern blot analysis revealed that this new isoform is specifically expressed in muscle. A non-synonymous mutation in this gene has been found in complete concordance with the presence of *RN*$^-$ allele. The mutation affects a protein domain well conserved from human to yeast. The mutation can be easily detected by PCR-RFLP and thus eradicated from populations in which it segregates. The discovery of this new muscle specific isoform of an AMPK subunit, and the identification of the *RN*$^-$ mutation, may also provide insights into muscle physiology in general as well as in the pathogenesis of disorders in energy metabolism, such as diabetes mellitus.

**D060**

## DNA-based methods for *Mhc B* and *Rfp-Y* haplotyping in chickens

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Genetic variability in chickens at the major histocompatibility complex is extensive. Accurate *Mhc* typing in chickens is made particularly complicated by the arrangement of structurally related *Mhc* genes into two clusters (*B* and *Rfp-Y*) that transmit haplotypes independently of one another. Genetic recombination occurring apparently within both *B* and *Rfp-Y* further increases the complexity of the *Mhc* variability that may be present within breeding populations. Methods are needed that will allow polymorphism at *B* and *Rfp-Y* loci to be detected separately and yet not require the determination of allelic sequences or the development of cluster-specific alloantisera. Such methods would be especially advantageous for efficiently working out *Mhc* variability in previously uncharacterized genetic stock. To develop such methods available, we have designed a series of DNA probes for Southern hybridization and a series of primer sets for SSCP assays that are specific for loci within the *B* and *Rfp-Y* gene clusters. We have tested these and found that they reveal heritable differences at *B* and *Rfp-Y* among individuals in fully pedigreed families. We further demonstrate the utility of these methods in revealing *Mhc* variability in a commonly available line of SPF chickens and anticipate their use in the characterization of *Mhc* variability in additional genetic stocks.

**D061**

## SNP screening at the myostatin gene level in European cattle breeds

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The Muscular Hypertrophy syndrome (MH), which appears in many cattle breeds, is produced by mutations in the GDF8 gene encoding the myostatin protein involved in muscle growth regulation. The higher lean value this phenotype shows in some cattle breeds allows overcoming the often-coupled dystocia problems. As a result, certain breeds show a high occurrence of the phenotype while others do not. Although several mutations have been described, there are still some MH individuals belonging to different breeds which show a wild type myostatin sequence.

EuroMH (BIO4-CT98-0421) is a UE project grouping eleven breeds from France, Spain and Belgium. Its aim is to track all possible mutations accompanying different phenotypes, and address one of the main concerns of breeders, which is to know the status of their breed in order to allow a better management of the trait. For this purpose and as a first part of the project, the use of the SSCP (Single Strand Conformation Polymorphism) technique has allowed to detect all possible mutations, and to find new mutations in some breeds. These new mutations affect the first two exons corresponding to the latency-associated peptide and are silent (D108D, T129T) or missense (S105C, D182N). The OLA (Oligonucleotide Ligation Assay) technique has later been used to set up a one-tube assay to genotype all mutations in every individual of any breed, facilitating the mutation screen for management purposes.

**D062**

## Mitochondrial D-loop nucleotide sequence variation in central and south american creole cattle breeds

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Creole cattle are the descendant from those bovines brought by spaniards during the first 100 years of colonisation. The cattle mated and reproduced freely suffering bottlenecks and expansion periods. Cross-breedings with introduced breeds could also have occurred in the history in some country. The aim of this works is to analyse the relationships among Creole Cattle breeds as determined by mtDNA D-loop sequence. It has been obtained the nucleotide sequence of aDNA fragment (850bp) comprising the $^{Thr}$tRNA gene and most of D-loop region of 28 animals representing 6 Creole Cattle breeds from Argentina (Argentinean Creole, AC), Guadalupe (Guadalupe Creole, CG) and Brazil (Mocho Nacional, MN, Caracu, CC, Curraleiro, CU, Pantaneiro, P). These sequences were aligned using CLUSTALX, then phylogenetic trees were constructed by neighbor-joining procedure. Considering all breeds, 8 haplotypes were found. Two haplotypes (# 3, # 4 and #5, #7) were exclusive of AC and GC respectively. None of the 4 haplotypes found in these breeds was dominant. In contrast 2 haplotypes (#1 and #6 were predominant in the brazilian breeds). As expected, an increased transition/transversion bias was noted. All the sequences obtained were included within the taurine group of the dendrogram, indicating the absence of any Asian zebu introgression. Even though, this do not discard the possibility of contamination with other zebu strains. mtDNA d-loop sequence of other native breeds is under investigation.

**D063**

## Cloning and characterization of microsatellites from enrichment library of equine genome

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Microsatellite analysis is useful for the construction of a linkage map and the parentage testing of equine. We constructed the equine genomic library enriched for DNA fragments containing (CA)n and (CAG)n repeats. The method includes the hybridization capture of repeat regions using biotin-conjugated oligonucleotides, nucleotide substrate-biased polymerase reaction with the oligonucleotides and PCR amplification. About 95% of the sequenced clones contained for the (CA)n repeats. In addition, about 90% of these clones had over 12-repeats, thus are more likely to be polymorphic. Microsatellites containing (CAG)n repeats were obtained at the ratio of one per 3~4 clones. Those results indicate that the enrichment value is about ~104 -fold, resulting in a significant reduction in the time and cost of microsatellite cloning. In this study, 55 clones containing (CA)n repeats and 66 clones containing (CAG)n repeats were identified. The number of the CA and CAG repeats ranged from 10 to 28 and from 4 to 33, with an average repeat length of 18.2 and 8.5 units, respectively. The flanking sequences of (CA)n and (CAG)n repeats of some microsatellites were identical with a few interruptions, suggesting that the identical sequences may be novel equine repetitive elements. Thus the primers to amplify the microsatellites must be carefully designed when multiplex PCR is performed.

**D064**

## DNA microsatellite analysis in Austrian pigs for parentage control

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We have compiled a DNA microsatellite profiling system for individual identification and parentage control in pigs. The system was designed to be suitable for use in both German and Austrian herd-book breeding. We have combined 10 porcine DNA microsatellite markers for fluorescent labelled multiplex amplification and developed a standby battery of 5 markers. The microsatellites in our batteries are selected from the recommended panel of markers for diversity studies. The PCR fragments are analysed on an ABI PRISM™310 Genetic Analyzer using Genescan 2.1 and Genotyper 2.1 software. The French PiGMaP reference DNAs were used to calibrate the fragment sizes. The multiplex system of 10 markers contains the microsatellites *S0090, S0101, S0155, S0355 S0386, S0005, SW24, SW240, SW857* and *SW951*. In Austria we have investigated the two most important Austrian breeding populations, Large White and Piétrain. In our material the highest polymorphism information contents (*PIC* > 0.70) were present at the following loci and breeds: *SW24, SW240* and *S0005* in Piétrain, *S0005, SW857* and *S0355* in Large White. The combined exclusion probability of the 10plex battery exceeded the desired value of 99.5% in both investigated breeds: *CEP* 99.76% in Austrian Large White, *CEP* 99.74% in Austrian Piétrain.

**D065**

## Assessment of the genetic diversity of sub-Saharan African sheep populations using microsatellite markers

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Indigenous African sheep have evolved in diverse environments and represent unique combination of genes that underline productive and adaptive traits. These populations are today often at risk of extinction through crossbreeding, replacement with exotic breeds, unstable political climate and environmental disasters. Our purpose is to understand the genetic diversity among the indigenous sub-Saharan African sheep populations in order to develop a rational approach to their conservation, utilization and improvement. Fifteen indigenous African and six reference breeds (North Ronaldsay, Swaledale, Dorset (UK), Lanzhou (China), Karakul (Central Asia) and Awasi (Syria)) were characterized with 23 autosomal microsatellite loci using fluorescent genotyping (ABI 377 DNA sequencer). Observed heterozygosities range from $0.623 \pm 0.038$, Blackhead Persian (South Africa), to $0.776 \pm 0.0243$, Ossimi (Egypt). Neighbor-joining tree (Da), principal component and multidimensional scaling analysis reveal two main clusters of sheep. The first cluster includes the East and South African fat-tailed sheep; the second cluster includes the West African thin-tailed sheep, the North African fat-tailed sheep (Ossimi), the fat- and thin-tailed reference breeds from outside Africa. The results suggest that there are at least two major genetically distinct groups of sheep in Africa, possibly corresponding to two different phases of sheep introduction within the African continent.

**D066**

**Parentage testing with microsatellites in cattle: pitfall twins**

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Over 90% of multiple pregnancies result in anastomoses. In microsatellite typing one could assume that there would be enough DNA to display all alleles of co-twins. In routine case work we also test for freemartinism. Criteria that a female calf is considered sterile: more than two alleles, microsatellite profile typical for chimaerism, discrepancies blood-hairbu-lb analysis, male specific amplification product. Among 85 tests 2 cases (one described) had similar results: In the blood DNA of the twin female calf (male co-twin slaughtered) male-specific Bov157 was detectable but no chimaerism in any 11 microsatellites (standard set). Blood-hairbu-lb comparison showed that in 3 microsatellites only the co-twin's genotype was displayed in the blood; not the slightest peaks for the genuine alleles were perceptible. Since hairbu-lb testing is not always possible in parentage verification the phenomenon of hidden genotypes can be a cause of false exclusions in microsatellite typing too.

**D067**

**Characterization of the caprine T cell receptor alpha chain**

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The examination of T cell receptor (TCR) repertoires plays an important role in the study of immune mediated diseases involving expansion of particular T cells. Expanding T cells can be traced by following the expression of the different TCR variable (V) chains in these cells using specific tools. In order to complete our study on the caprine TCR repertoire, which we have started by characterizing the different V regions of the TCR β chain, we have generated a cDNA library of TCR α chains. Total RNA, isolated from caprine peripheral blood mononuclear (PBMCs) was retro-transcribed and the cDNA used for AnPCR as described elsewhere. The amplified fragments were cloned and sequenced. From a total of 67 clones, 56 were functionally rearranged and classified according to their human counterpart. Sixteen caprine-specific Vα and 28 Jα regions could be identified. Based on this sequence information, it will be possible to develop Vα and Jα specific oligonucleotide probes and PCR primers. These tools, together with the previously developed Vβ specific reagents, will permit an identification of expanding T cell clones in several immunopathological processes such as caprine arthritis encephalitis (CAE) virus-induced arthritis, a unique natural model to study human rheumatoid arthritis, or Cowdriosis, induced by the hemoparasite *cowdria ruminantium*, which affects goats and other ruminants. Both are infectious diseases of goats where the clinical course appears to be controlled by immunogenetic factors that may also influence the T cell response to the infecting agents. Vα and Jα TCR specific markers will help to analyze and compare the T cell response in disease susceptible versus disease resistant animals.

**D068**

**Diversity of the prion-protein gene *PRNP,* in Norwegian sheep**

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The prion-protein in its normal form ($PrP^{C}$) exists as a cell surface protein in tissues of mammals. Prion diseases like scrapie, BSE and CJD, are associated with conformational change of $PrP^{C}$ followed by intracellular accumulation of the abnormal form of the protein ($PrP^{Sc}$) in the central nervous system. This leads to vacuolization and malfunction of the brain and finally to the death of the diseased individual. The conformational change is catalyzed by exposure to the abnormal protein. Several allelic variants of the prion-protein gene (*PRNP*) have been observed in sheep and some variants are associated with increased susceptibility to scrapie. On this background we have analyzed a total of 250 animals from 11 Norwegian sheep breed groups. The samples were typed for variants in the coding region of *PRNP* by sequencing a PCR product covering bp 340 to bp 740. The reported polymorphisms at codons 136, 154 and 171 are covered by this approach. The animals were also typed for a microsatellite in the 5' region of the gene. For codons 136, 154 and 171 five haplotypes were found as reported in the literature. In addition we found polymorphisms at codons 137, 141 and 151. The microsatellite displayed five alleles. We observed large *PRNP* variation within and between the Norwegian sheep breeds, but all groups including the original Old Norwegian sheep showed a certain frequency of the *PRNP* haplotype associated with the majority of scrapie cases in Norway.

**D069**

## Molecular basis of esterase D polymorphism in pig

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Human esterase D (EC 3.1.1.1) is a member of the carboxylic ester hydrolase family ubiquitously distributed in the cells of the most tissue. The genetic polymorphism of the esterase D (EsD) isozyme is well known one of most useful genetic markers of population studies in human or animals. Two human EsD cDNAs, EsD1 and EsD2, are differed by one base nucleotide exchange G to A resulting in the amino acid substitution Gly191Glu (Tsuchida et. al., 1994 Hum. Genet. 93, 255). We demonstrated the isolation of the pig EsDA cDNA from a Ohmini pig, and showed the pig EsDA cDNA had an open reading frame composed of 849 bp nucleotides, which encoded a predicted protein of 282 amino acids. The pig EsDA cDNA exhibited 86% identity and 87% similarity with the human EsD1 cDNA. Furthermore, we found that the two alleles, EsDA and EsDB, were characterized by a missense mutation in the pig EsD polypeptide. These results will provide useful information for future research into the molecular evolution of the ESD gene in mammals.

**D070**

## Genetic structure of several populations of Pottoka-Basque Poney

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The Pottoka-Basque Poney is a local horse breed, traditionally used in countryside and mine work and presently bred at large. Its main interest is its potential for meat production in mountain areas and for sportive and cultural uses. To characterize this breed four serum protein loci (*AL, A1B, TF* and *GC* ), eight enzyme loci (*PGD, GPI, PGM, MDH, SOD, LDHA, LDHB* and *ME1* ) and three microsatellite loci (*VHL20, HMS7* and *AHT5* ) were studied. Four populations from different geographic locations (72 individuals) were analysed. Allele frequencies, observed and expected heterozygosity values, percentage of polymorphism and mean number of alleles per locus were estimated for each population. Mean values of $F_{is}$, $F_{it}$ and $F_{st}$ are significant ($F_{is}$=0.06772 ± 0.08594, $p<0.05$; $F_{it}$ =0,16105 ± 0.11791, $p<0.01$; $F_s$=0.10011 ± 0.05757). Only Population 1 shows a significant global $F_{is}$ value ($F_{is}$=0.17647 ± 0.15475, $p<0.01$). Global $F_{st}$ values for pairs of populations are significant, except for Populations 1 and 2. Gene flow between these two populations is possible, in the light of their geographic proximity. Significant levels of inbreeding in Population 1 can be caused by a small efective size or a particular reproductive scheme.

**D071**

**Differential display RT-PCR revealed expression difference caused by culture condition in bovine embryos**

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The synthetic oviduct fluid (SOF) system is widely used in the bovine embryonic culture in vitro, similar to the situation in bovine uterus 5% oxygen stress in applied in SOF system. But some other culture systems, for instance COC and TCM199 systems, still employ 20% oxygen tension in the culture atmosphere. Excess oxygen could form the reactive oxygen species (ROS), which has been reported relating to the induction of programmed cell death. Therefore, we applied 2%, 5% and 20% oxygen stress to our SOF system respectively, and used the sensitive differential display RT-PCR to study the gene expression in embryos which derived from the SOF system with different oxygen stress. To investigate the differential gene expression, one 17-base- pair anchor primer and four 10-base-pair random primers were applied in the RT-PCR. The most prominent different bands were excised, cloned and sequenced to identify the corresponding genes.

**D072**

## A Parentage Test For Rhesus Macaques Using Human Primers for Microsatellite Markers

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Rhesus macaques (*Macaca mulatta*) are the primary source of non-human primates for biomedical research. Experimental animals are derived from captive breeding colonies maintained by primate centers. Pedigree assignments have been mostly based on behavioral observations and the use of methods such as DNA typing for parentage determination has been limited. We identified 13 microsatellites in Rhesus that are amplified by heterologous human primers using a single PCR protocol and two sets of multiplexes. One set contains primers for D3S1768, D7S513, D7S794, D11S925, D13S765, D16S403 and D17S804. The other set contains primers for D6S276, D6S291, D6S1691, D8S1106, D10S1412 and D18S72. The HSA 6 markers are linked to the major histocompatibility complex (MHC) in humans. In a sample of 107 animals, the number of alleles ranged from 6 to 21 and heterozygosities ranged from 0.309 to 0.905. The expected probabilities of exclusion (PE) are 0.999 for one-parent ($PE_2$) and 0.998 for no-parent ($PE_1$) comparisons. The 13 markers provide a highly effective parentage test for macaques. The HSA 6 markers are useful as a screening tool to evaluate association between MHC markers and disease traits.

**D073**

## A new protocol for genotyping microsatellites in Goats

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Because of the small economic value of single animals in this species the parentage test must be very low money and time consuming. For this reason a protocol was developed with a number of microsatellite loci large enough to give a good percentage of success in parents identification with the smaller economic and time effort possible. Five economically important italian goat breeds (Camosciata, Derivata di Siria, Ionica, Maltese Saanen), have been genotyped for 15 microsatellites loci with a protocol of multiplex PCR. It consists of two multiplex amplifications one of 9 (INRA005, MAF65, SR-CRSP24, ILST S19, SR-CRSP5, INRA063, SR-CRSP8, PZ963 and HSC) and one of 6 microsatellites (SR-CRSP23, FCB20, ILST S87, McM527, INRA023 and CSRD247) loci and their allele size scoring by automated fluorescent DNA sequencing. Parentage exclusion probability has been calculated for each locus and for all 15 loci together and is equal to 0.999. The first set of 9 microsatellites was enough in most cases to give a satisfactory paternity identification. Not very much is known about the genetic diversity of goats in Italy so allelic frequencies have been also used to evaluate the genetic variability (He ranged between 0,4 and 0.9, number of alleles from 3 to 16) and to calculate genetic distances. The population structure for the 5 breeds studied so far, gives a picture of inbreeding in each single breed with an over all heterozygote deficiency accompanied by an heterogeneity in the total goat population.

**D074**

**Study of two candidate genes for production traits in pig: the lysosomal proteinase Cathepsin B (*CTSB*) and the proteinase inhibitor Cystatin B (*CSTB*).**

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Excessive softness is a defect of dry cured hams which seems related to a higher *postmortem* activity of lysosomal cysteine proteinases, such as cathepsin B, in pig muscles. Using a candidate gene approach we studied two genes: Cathepsin B (*CTSB*) and Cystatin B (*CSTB*) which codes for an inhibitor of the cathepsins. A fragment of the porcine *CTSB* gene encompassing part of exon 6, the intron and part of exon 7 was amplified by PCR and sequenced. SSCP analysis revealed 4 alleles that have been characterized by sequencing. *CTSB* was assigned to porcine chromosome 14 by linkage and physical mapping. A fragment of the *CSTB* gene, encompassing part of exon 2, the intron and part of exon 3, was amplified and sequenced. The two alleles identified by PCR-RFLP have been sequenced and a non-synonimous mutation in exon 3 was identified. *CSTB* was localized to porcine chromosome 13 by linkage and physical mapping. Allele frequencies at the *CTSB* and *CSTB* loci have been studied in a sample of 390 pigs (Large White, 118; Landrace 53; Duroc, 57; Belgian Landrace, 29; Hampshire, 22; Piétrain, 32; Meishan, 14; Calabrese 9; Cinta Senese 14; Casertana, 14; Nera Siciliana, 28). Association analysis of the two loci with several meat quality and production traits (cathepsin B activity, ultimate pH, back-fat thickness, lean content, ham weight and average daily gain) was performed in a sample of 220 pigs (Large White, 128; Landrace, 38; Duroc 54) coming from central Sib-Test stations. The results indicate a significant association of *CSTB* ($P \leq 0.01$) with average daily gain.

**D075**

## Use of Single Nucleotide Polymorphisms for low cost parentage testing in cattle & sheep

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Increasingly efficient methods for the discovery and typing of single nucleotide polymorphisms (SNPs) has sparked a new revolution in DNA-based genetic studies. Single nucleotide substitutions can now be detected by methodologies which can be miniaturized and automated, and consequently, can be exploited for low cost parentage and identity testing in livestock. Published cattle and sheep sequences were used to amplify DNA segments from sets of unrelated animals and SNPs were identified by detecting differences in dideoxyfingerprinting (ddF) patterns. The presence of a SNP was confirmed by bi-directionally sequencing PCR products. Approximately 200 SNPs have been discovered and the allele frequency of the SNPs will be determined by sequencing pooled DNA samples. Addition SNPs will be generated by sequence information obtained by the end-sequencing of FISH-mapped bacterial artificial chromosome clones.

**D076**

**Differential expression of mRNA in ileal Peyer's patches from sheep inoculated with scrapie agent and in matched controls.**

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Prion diseases are a group of neurodegenerative diseases that also can be infectious. The infectious agent is thought to be the prion protein (PrP) which has been conformationally tranformed ($PrP^{SC}$). The *PRNP* gene is highly expressed in brain, however, expression is also detected in several other tissues. It has been shown that *PRNP* expression is essential for development of the disease. Whether other proteins are involved in this process is not well understood. In brain tissues of mice and hamster with an experimental prion disease, up-regulation of several genes has been reported. In an effort to unravel factors involved in the initial steps of the disease development, we are studying differences in mRNA expression in ileal Peyer's patches from lambs orally inoculated with scrapie agent and control lambs matched with respect to age and *PRNP* genotype. Increased levels of PrP have been observed 1 week after inoculation in Peyer's patches and also preliminary results indicates increased levels of PrP mRNA in the same tissues. We are in the process of identifying differences in expression using representational difference analysis (RDA) of cDNA on Peyer's patches from both control and scrapie infected animals. This technique, using cDNA instead of genomic DNA, is very sensitive. Subtracting cDNA from normal tissue with cDNA from infected tissue and vice versa will identify both down-regulated and up-regulated genes. Results from this study will be presented.

**D077**

## Polymorphism in the tyrosinase (TYR) gene in the PIG and its influence on coat color and quantitative traits

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Tyrosinase (*TYR*) is known to be a decisive factor in the metabolic pathway leading to melanine, the pigment giving color to the mammalian coat. Mutations in the *TYR*-gene have been shown to be responsible for phenotypic changes as impressive as the albino phenotype which is known to influence not only coat color but also quantitative traits in mice. Though widely distributed among mammalian species, no such phenotype has ever been observed in the pig. Based on the partial cDNA-sequence of the mouse (D00440) and man (M27160) we amplified a 820 bp fragment of exon 1 of the porcine *TYR*-gene on Chr. 9 by heterologous PCR. The sequence is homologous to the mouse sequence by 84,4 % and to the human sequence by 87,9 %. We detected a single-base-polymorphism (C / T) that created a restriction site for MluN I. Based on a PCR-RFLP (forward: AATGCTCCTGGCTGTTTTGTA; reverse: CTGCCAGGAGGAGAAGAAGGATGCT) we genotyped 212 $F_2$ animals of a Duroc and Miniature Pig intercross resource population and additionally purebred animals (Saddleback, Large White, Piétrain, Hampshire, Duroc, Miniature Pig). The mutation tested was polymorphic in all breeds except the Miniature Pig. No correlation could be detected between the polymorphism and the coat color phenotype in the resource population. An association analysis between the tyrosinase genotype and quantitative traits (growth, carcass and meat quality) revealed a suggestive influence of the tyrosinase genotype on growth traits in the time period 35$^{th}$ to 100$^{th}$ day of age.

**D078**

**Phylogenetic analysis of some Caucasian cattle breeds**

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The results in the field of blood groups of 9-11 EA-systems – 22045 animals of 6 breeds, four of which are reared in the Caucasus, have been summarised. Immunogenetic distances (d) between the breeds have been calculated and a phylogenetic dendrogramme has been constructed. The smallest distance (d=0,1090) is observed between the Caucasian Brown and Swiss Brown breeds, the largest distances (d=0,249) and (d=0,281) between the Red Megrel and Red Steppe and Small Caucasian, correspondingly. On the dendrogramme all the 6 breeds are distributed into three clusters. One of them includes the Caucasian Brown and Swiss Brown breeds, the second includes the Khevsurskaya and Red Megrel breeds, and the third – the Small Caucasian and Red Steppe Breeds. Such a distribution of the breeds on the dendrogramme is in good agreement with the real interrelation of the breeds with known history and genealogy of their formation.

**D079**

## Neuronal ceroid-lipofuscinosis in Australian Merino sheep

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The neuronal ceroid-lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative diseases characterized by the accumulation of autofluorescent lipopigment in a variety of tissues. Clinical features of NCL are dementia, loss of vision, motor disturbances, and premature death. At least eight different forms of NCL occur in humans and the disease has been identified in various animal species, including sheep. Recently NCL was reported for the first time in Merino sheep in Australia. The aim of this project is to characterise the genetic defect in Merino sheep and to compare it to a clinically and genetically well defined form of NCL in South Hampshire sheep. Due to the limited number of animals and the lack of pedigree structure a homozygosity mapping approach was used successfully to localise the disease gene in Merino sheep to the same region on chromosome 7 in which the NCL gene was recently mapped in South Hampshire sheep. This region shows conserved synteny to a region on human chromosome 15 in which the human NCL variant CLN6 was mapped. NCL in Merino and South Hampshire sheep are therefore regarded as animal models for the human variant CLN6 and further investigations are undertaken to identify the disease gene.

**D080**

## Effects of leptin polymorphisms on bovine carcass traits

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Effects of leptin polymorphisms on bovine carcass traits were examined in 179 Angus bulls divergently selected for blood serum IGF-I concentration at the Eastern Ohio Resource Development Center. The bulls were born in spring and fall of 1995 and 1996, and in spring of 1997 and 1998. Microsatellites BM 1500 and WDMS (Wilkins & Davey, 1997 Anim. Genet. 28, 376) were genotyped by denaturing PAGE following PCR amplification. SNPs LSNP (Lien *et al*., 1997 Anim. Genet. 28, 245) and FSNP (Fitzsimmons & Schmutz, 1998 PAGVII, P493) were genotyped by agarose gel electrophoresis following digestion by *Bsa*AI and *Aci*I restriction enzymes, respectively. Carcass traits included hot carcass weight, ribeye area, KPH, backfat thickness, marbling score, quality grade, and yield grade. Ultrasound measurements of ribeye area and backfat thickness were made at days 56 and 140 of the postweaning period. Data were analyzed using SAS general linear models procedures. Fixed effects included in the model were year and season of birth, IGF-I selection line, age of dam, leptin genotypes, and a covariate for age of calf. Interactions among the BM 1500, WDMS, LSNP, and FSNP genotypes were tested and were generally found to be unimportant (P>.10). Significant effects were found for FSNP upon ribeye area (P=.03), FSNP upon hot carcass weight (P=.03), and WDMS upon ultrasound ribeye area at day 56 (P=.01). Noteworthy relationships (P<.15) were observed between BM1500 and ultrasound backfat thickness at day 140, WDMS and ultrasound back fat thickness at days 56 and 140, and WDMS and hot carcass weight.

**D081**

**Polymorphism of the *MC1R* gene and feather color in the Chicken : between breeds diversity and within family analysis.**

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The *Melanocortin 1 Receptor* is a candidate gene for the *Extension* locus in the chicken, which controls the distribution of black pigment over the body (Takeuchi et al., 1996, B.B.A., 1306, 122-126). In the present study, the relationship between a missense mutation of *MC1R* (Glu92Lys due to substitution G →A274 ) and allelism at the *E* locus was investigated with two approaches : (i) a family of 132 chicks segregating for the (G →A274) mutation was produced by mating an heterozygous sire with black phenotype to 8 non-carrier dams (ii) 25 chicken breeds were sampled within the European project AvianDiv, showing various phenotypes corresponding to 5 different alleles known at the *E* locus. The (G →A274) mutation was detected with a PCR-RFLP test. The results from the within-family study showed a tight linkage between *MC1R* and *E* loci because all chicks (n=65) showing the extended black phenotype at hatch (*E*E* allele) were heterozygous carriers of the mutation, but 4 chicks classified as brown (*E*EB*) at hatch were also carriers and showed red plumage at 8 weeks. The between-breeds analysis showed that 4 out of 5 black breeds were homozygous carriers and the 5$^{th}$ one was heterozygous, but the mutation was also found in 3 out of 4 breeds carrying the *E*ER* allele and in 2 brown breeds. Most of the wild-type and the wheaten breeds did not carry the mutation, but 2 over 10 appeared to be segregating. It was concluded that the *MC1R* (G →A274) mutation was necessary but not sufficient to determine the extended black phenotype in the chicken.

**D082**

**Comparison of rapid methods for the detection of mutations in the myostatin locus in cattle breeds of Central Italy.**

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In several European beef breeds double muscling can be attributed to mutations at the myostatin locus. While in some populations the alleles are fixed, in others they are still in segregation and there is an objective interest for a genotyping-assisted selection in order to build up homozygous double muscled breeds. We have recently described a new mutation causing double muscling in some individuals of Marchigiana breed. A genotyping of all performance tested bulls of this and of other related breeds of Central Italy (Chianina and Romagnola) is in progress using different techniques, as sequencing, RFLP-PCR, allele specific PCR and BESS (Base Excision Sequence Scanning). Also cows surveyed on farms and showing a relevant muscle development are genotyped. On the 60 Marchigiana individuals analyzed so far we found only the wild type and the described mutation, therefore it seems safe to proceed with rapid and cost effective methods like allele specific PCR, which proved to be quicker and more repeatable than RFLP-PCR. Interestingly, none of the 120 individuals of Chianina and Romagnola analyzed showed such a mutation, although it is reported that in 18$^{th}$ century Marchigiana was crossed with these breeds. For Chianina and Romagnola, the BESS technology allows a quicker screening relatively to sequencing, while revealing at least half of the present mutations and partially identifying also the heterozygotes. The BESS protocol standardized for the specific locus and the first results are reported.

**D083**

**Primer extension preamplification: a tool in embryo diagnostics.**

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With the advent of techniques like IVF, IVP and multiple ovulation and embryo transfer, molecular DNA tools to determine genotypes of bovine preimplantation embryos have become of great importance for animal breeding strategies. Biopsied embryos remain capable of normal development after deep-freezing and transfer to recipients. However, only a limited number of cells can be removed from the embryos without markedly reducing their viability. Since the amount of DNA that can be isolated from this few cells is limited, it has been tried to adjust and improve the primer extension preamplification (PEP) for whole genome amplification of embryonic bovine DNA. The PEP method is a PCR-based in vitro method for amplification of a large fraction of the DNA sequences present in a single haploid cell by repeated primer extension using a mixture of 15-base random oligonucleotides.

The PEP amplification efficiency fluctuated much between experiments and between samples amplified during the same experiment. Stringent and sterile setting up conditions are very imported to avoid contamination. Other problems of the PEP method are preferential amplification of one allele at heterozygous loci and non-reproducible results between different primer batches. The just discussed problems rise serious doubts about the potential of a successful and reproducible PEP procedure for the production of good quality bovine embryonic DNA that can be used as template for subsequent PCRs. Other methods are currently under development to achieve the same goal.

**D084**

**Functional characterization of the Atlantic salmon MHC class II promoters.**

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The Major Histocompatibility Complex (MHC) molecules are necessary components of the primary immune response. The MHC class II are dimeric cell surface glycoproteins expressed primarily on antigen presenting cells and B-cells. They present short polypeptides to the T-cell receptors which thereby induces the immune response. We have isolated the MHC class IIβ and MHC class IIα promoters from Atlantic salmon (Salmo salar). In order to investigate the activity of the promoters they were cloned into LacZ and Green Fluorescent Protein (GFP) reportergene plasmids and used for transient transfection in an Atlantic salmon head kidney cell line called SHK-1. Preliminary positive results were achieved. In addition, the function of the MHC class II promoter/LacZ reporter constructs is investigated by injection into somatic tissue of the Atlantic salmon. Immune response was measured after two weeks of injection for both the constructs.

**D085**

**Generation of transgenic pigs by sperm-mediated gene transfer using a linker protein (mAb C)**

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Sperm-mediated gene transfer has been recognized as a potentially powerful method to make transgenic animals for many years. It might overcome the difficulty of microinjection reported to have only limit success in higher or larger animals. A monoclonal antibody C (mAb C) has been identified after screening many hybridomas immunized with mouse sperm cells. mAb C is reactive to a surface antigen on mouse sperm cell and is also cross-reactive with sperm cells from many different species such as pig, cow, sheep, goat and chicken. mAb C has been characterized as a basic protein, and is shown to bind DNA through ionic interaction. Therefore exogenous DNA can be specifically bound to the sperm surface via mAb C, and successfully integrated into the chromosome of offsprings in mouse and pig. Furthermore expressed foreign proteins can be detected in the serum of transgenic pigs. Diluted pig sperm cells were mixed with mAb C to form a sperm-mAb complex and pSEAP-2 reporter gene (Clontech) was added to react with the sperm-mAb complex. Then half-million treated sperm cells were injected into each side of the oviduct from ovulating pigs by surgical oviduct fertilization. Forty-three pig offsprings have been analyzed, 30% of piglets' tails were shown to contain exogenous DNA integrated into host genome by Southern blot analysis and PCR. Furthermore 65% of these offsprings were found to express human placenta alkaline phosphatase secreted into the serum. All these data suggest that transgenic pigs can be generated with greatly improved efficiency by sperm-mediated gene transfer using the linker protein, mAb C.

**D086**

## Glycogen storage disease type IV: an inherited deficiency in glycogen branching enzyme in the Quarter Horse

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Glycogen storage disease type IV (GSD IV) is an hereditary disease characterized by abnormal polysaccharide inclusions and a decreased glycogen branching enzyme (GBE) activity. Clinically, GSD IV can range from liver failure to fatal cardiomyopathy to mild skeletal muscle myopathies. We now have documented the occurrence of fatal form of GSD IV in seven Quarter Horse foals (1), cardiac arrest (3) and stillborn (1). Increased serum activities of liver and muscle enzymes and leukopenia were present (5). 3 foals died suddenly by four weeks of age and 3 foals were euthanized by 7 weeks of age. Muscle, heart and liver samples from the foals were devoid of normal PAS staining for glycogen and contained abnormal PAS positive inclusions. Accumulation of unbranched polysaccharide was suggested by an alteration in iodine absorption spectra in isolated polysaccharide from tissues of affected foals. Assays for GBE function in multiple tissues showed virtually no activity, whereas activities of control enzymes compared to a normal control. Western immunoblot assays detected no GBE protein in the liver of an affected foal and diminished levels in one suspected carrier as compared to a control horse. Pedigree analysis further supports and automosal recessive trait. Clinical, histological, biochemical, and genetic finding in these horses is analogous to GSD IV as exhibited in humans and cats and may represent an important cause of neonatal mortality in Quarter Horses.

**D087**

**Study of genetic diversity of sub-Saharan African goat populations using microsatellite loci**

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There are at least 90 breeds of African goats. They represent a unique genetic resource due to their adaptation to the local environmental conditions of the tropics. Phenotypic and genetic characterisation of this unique livestock genetic resource is urgently needed for their efficient conservation and management. In this study we report the genetic diversity and relationship of 18 indigenous African goat breeds using 18 microsatellite loci. Within-breed genetic diversity was estimated by the number of alleles per locus and the average observed heterozygosity. Genetic differences between breeds were estimated by Nei's standard genetic (Ds) distance. Breed relationships were assessed by Neighbour-Joining trees, principal component analysis and multidimensional scaling. Average number of alleles per locus ranged from 4.61 to 6.44. Average observed heterozygosity was high (0.661 ± 0.036 (Afar) to 0.552 ± 0.038 (Newala). Ds ranged between 0.033 ± 0.013 (Ugogo and Masai) to 0.477 ± 0.145 (Tswana and Tanzanian Coast). The 18 African breeds cluster in four different groups, the Southern African breeds (Tswana, Ndebele, Venda, Landim), the Ethiopian-Kenyan breeds (Boran, Galla, North East Highland, Afar), the Nigerian breeds (Red Sokoto, West African Dwarf) and the Tanzanian breeds (Ujiji, Sukuma, Masai, Ugogo, Mbeya, Newala, Tanzanian Coast). In general, the relationships among breeds reflect their geographic origins more than the morphological classification based on sizes and the shape of the ears.

**D088**

**Identification and confirmation of differentially expressed genes in developing pig fetuses**

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Growth and development of pig fetuses is controlled by the coordinated expression patterns of multiple genes. The objective of this experiment was to identify differentially expressed genes in developing pig fetuses using the differential display reverse transcription PCR (DDRT-PCR) technique. Total RNA was extracted from 3 whole 21 d fetuses and from the anterior, medial, and posterior sections of three 35 d and three 45 d fetuses. For DDRT-PCR assays, RNA samples were pooled from anterior, medial, and posterior sections for each 35 and 45 d fetus. Sixteen differentially displayed fragments were excised, PCR re-amplified, and cloned. DNA sequence analysis resulted in identification of eleven unique gene products. Pig cDNAs were identified with homologies to *collagen type XIV (COL14A1), complement component C6 (C6), vitamin D binding protein (DBP), titin (TNT), craniofacial developmental protein (CFDP1), DNA binding protein B (DBPB), KIAA0456 protein*, and *epsilon globin*. Three novel pig ESTs were also identified. Differential expression of *COL14A1, C6*, and *DBP* was confirmed by northern blot analysis. For those three genes, relative abundance of mRNA increased in fetuses from 21 to 45 d of gestation. The spatio-temporal differential expression of *DBP* was discovered by northern blot analysis with the anterior, medial, and posterior samples. Relative mRNA abundance for *DBP* was high in posterior samples and undetectable in anterior samples. These results provide new information concerning gene expression in developing pig fetuses and indicate that DDRT-PCR is a powerful technique for identifying differentially expressed genes.

**D089**

## Polymorphisms of the porcine *C3* - a candidate gene for generalized defence power

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The complement system is an important defence mechanism of innate immunity. It mediates phagocytosis, controls inflammation and interacts with antibodies in immune response. The complement factor C3 is the central component of the complement system. The classical and alternative pathway of complement activation lead to the formation of a convertase that cleaves C3 to C3a and C3b, the central event of the complement activation. C3b in turn activates the terminal lytic complement sequence, which is identical in both pathways. Because of its central function within the complement cascade C3 is a potential candidate for complement activity. High complement activity can be expected to contribute to increased generalised defence power. We determined the cDNA-sequence of the porcine *C3-gene* (GeneBank accession number AF154933). The porcine cDNA (5131 bp) shows 82% identity to the human homologue. The sequence codes for 1661 amino acids that show 86% identity to the human C3 protein. Primer pairs where derived in order to amplify overlapping fragments of about 500 bp suitable to be screened for polymorphism by DGGE and comparative sequencing. PCR-fragments were produced from liver cDNAs of eight animals of the breeds Duroc, Hampshire, Pietrain, German Landrace, Duroc x Berlin Miniature Pig (F1 and F2) and were screened for polymorphism. Three polymorphic sites were found. PCR-RFLPs were derived suitable to be applied on genomic DNA. The polymorphic sites were found to segregate in our porcine F2-resource families (Berlin Miniature Pig x Duroc) in Mendelian manner.

**D090**

## Genetic polymorphism of 12 microsatellites in the Polish native horse Konik Polski

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Konik Polski is the native horse breed kept in Poland as genes reservation. This study is concerned with the conservation of its biodiversity and autochthonous genetic resources. Genetic polymorphism of 12 microsatellite loci (*AHT4, AHT5, ASB2, HTG4, HTG6, HTG7, HTG10, HMS2, HMS3, HMS6, HMS7, VHL20*) was studied in 215 individuals. Genotyping was performed on an ABI 377 DNA Sequencer. The results showed high polymorphism of 10 tested markers (6-9 alleles). Locus *HTG6* was monomorphic and in locus *HTG7* 3 alleles were identified. The highest heterozygosity was observed in loci *HMS6* (0.87), *ASB2* (0.82) and *HTG5* (0.79). The values for heterozygosity of 8 microsatellite markers ranged from 0.65 to 0.75.

**D091**

## Genetic polymorphism at *Mhc B* and *Rfp-Y* in the Camperos broiler chickens

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Camperos broiler chickens are produced at INTA, Argentina (see L. Soria et al., this volume). They are the F1 generation of two cross-breeds that have been maintained essentially without selection for 7 generations. With the aim of determining the degree of *Mh*c variability in these broilers, 51 individuals (15 males from the male parental breed, 18 females from the female parental breed and 18 Camperos) were analyzed with restriction fragment pattern typing methods utilizing probes specific for *B-G*, *B-F* and *Y-F* genes. The two *B-G* probes used in this analysis were: *G412*, a 752 bp probe generated by PCR from exon 1 (signal peptide), intron 1, and exon 2(IgV-like domain) of a Camperos *B-G* gene; and *bg28*, a 525 bp cDNA probe corresponding to a portion of exon 2 (IgV-like domain), exon 3 (transmembrane domain) and exons 4-11 (heptad domains) of a Leghorn *B-G* gene. We were able to identify a total of at least 45 *B-G* genotypes among the 51 samples. Some genotypes were distinguishable only with *G412* and others only with *bg28* suggesting that restriction fragment polymorphisms in these chickens are likely present in exons corresponding to both extracellular and intracellular domains. A *B-F*-specific probe revealed far fewer restriction fragment patterns at *B-F* loci in these chickens even though there is other evidence for *B-F* variability. The *Y-F* specific probe, *163/164f*, revealed the presence of at least 44 different *Y-F* genotypes assorting independently from the *B-G* genotypes. Thus, there is very high genetic variability at the *Mhc B* and *Rfp-Y* gene clusters in this broiler breed, considerably greater than that present in the Leghorn control population used in this study.

**D092**

**Genetic biochemical polymorphism of Yaroslav cattle and its intercommunication with other representatives of Bovine**

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There were investigated 62 antigenes of 11 EA-systems by 2845 animals of Yaroslav breed. We did not discover the Z' and M antigenes (in EAA and EAM systems accordingly). It was calculated the indices of similarity between Yaroslav cattle and with 86 other representatives of Bovinae (n=110000). The least similarity (r=0,5108-0,6821) this breed showed with buffaloes, yaks, aurochs and zebu and with 3 beef breeds: Chianina, Limousine, Svetlaja Akvitanskaja (r=0,7152-0,7343) and the largest similarity with the 4 craniological groups (r=0,8566-0,8730): B.t. brachyceros (Alatoo, Aberdin-Angus, Pinzgau), B.t. primigenius (Black and White ukrainian, Angeln) and B.t. brachycephalus (Red humped). This is demonstration that the Yaroslav cattle was created on the basis of Middle Russian cattle use of the method of nationality selection. Were calculated frequencies of alleles some blood proteins: TFA=0,481, TFD=0,467,TFE=0,052; HBA=0,899, HBB=0,101; CPA=0,192, CPB=0,808; CAS=0,848, CAF=0,152; fixed at 3 alleles of esterasa : ESS, ESP and ESO, and Alkaline Phosphatase: PPS, PPO and PPF. The frequencies alleles of milk proteins was following: CASA1B=0,807, CASA1C=0,193; CASBA=0,759, CASBB=0,238, CASBC=0,003; CASKA=0,383, CASKB=0,617; LGBA=0,350, LGBB=0,650. The Yroslav breed very discriminated from Europe breeds more frequencies of CASA1C allel.

**E001**

## Allelic variation of the D4 dopamine receptor polymorphic region in two dog breeds, Golden Retriever and Shiba

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The dog (*Canis familiaris*) has the longest history among domestic animals, and more than 400 breeds have so far been established 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 D4 dopamine receptor (D4DR) polymorphic region, which is possibly related to the personality trait known as novelty seeking in humans, was examined in 52 dogs from two breeds (Golden Retriever and the Japanese indigenous breed Shiba) by PCR and DNA sequencing of each allele. Golden Retrievers and Shibas are relevant breeds in Japan, and their behavioral traits such as excitability, aggression, and playfulness are quite different. The polymorphic region of the dog D4DR gene was composed of 39- and 12-base pair (bp) units, and six alleles were identified based on the difference of number and/or order of these units. Intra- and inter-breed allelic variations were observed. Two alleles (435 and 447 bp in length) were observed in Golden Retrievers and the frequency of the short 435 bp allele was dominant. On the other hand, the long 447 bp and 549 bp alleles were common among five alleles observed in Shibas. These findings suggest that allele frequency varies significantly between different breeds, and hence analysis of the polymorphism in D4DR might be of use for understanding the behavioral traits of dogs.

**E002**

## Parentage and identity testing by means twelve microsatellites in Dogs

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The Laboratorio Gruppi Sanguigni, which has been carrying on for years a support activity for the genetic improvement of domestic animals in Italy, has set up a protocol for parentage and individual identification in dogs. This protocol, based on STRs (Short Tandem Repeats) analysis through PCR, involves the use of twelve highly polymorphic microsatellites co-amplified in a single multiple PCR. The observed microsatellites were: (AHT140, AHT121; AHT137; 2001, 2137; CPH3; ZUBECA26; DGN3; 2161; DGN14; DGN13; ZUBECA25).

The procedure is almost fully automated: the amplification reaction is accomplished in a thermal cycler and samples electrophoresis and sizing analyses are carried out by using the GeneScan and Genotyper software of the automatic sequencer ABI Prism 377.

To demonstrate the validity of this protocol we analysed 120 non-sib dogs of four different breeds (30 German Shepherds, 30 Setter, 30 Labrador and 30 Yorkshire). Parentage exclusion probability has been calculated for each locus and for the all 12 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.

Besides, individual identification, as the probability of finding two identical genotypes for 12 loci, has been calculated in each of the four breeds.

**E003**

**A comparative radiation hybrid map of the X-chromosome of the Dog**

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The dog serves as an animal model for several human diseases including X-chromosomal diseases. To facilitate comparative genetic analysis, a linkage map and a radiation hybrid map of the dog genome have recently been established. Whereas the canine X-chromosome is the largest chromosome in the dog, only a few markers have been mapped to it. Using a commercially available whole genome radiation hybrid (RH) panel we were able to map 15 microsatellite markers, 25 genes and 19 STSs to the X-chromosome, extending the total number of mapped markers to 59, covering an estimated 830 cR. Nine distinct groups of markers could be established with an average spacing of 18,8 $cR_{3000}$, 13 markers remained unlinked.Using FISH analysis, 5 markers could be mapped physically to the p- or q-arm of the X-chromosome resulting in the addition of 2 new groups to the comparative map. One group includes FH2985, which is in former maps linked between AR and CHM/ PGK1 in a region known to be on dog Xq13. In our map we have clear linkage of this marker to the p-arm of the X-chromosome. Nineteen other markers mapped to 5 other RH groups consistent with their syntenic map position. Comparison with the human X-chromosome map revealed synteny up to 234 cR for the genes *TIMP-ALAS2-AR-IL2Rγ-XIST.* The extended and revised map presented in this poster will serve as preliminary map for the X-chromosome and makes comparative mapping with other species possible. More markers are needed to create a complete map of the dog X-chromosome.

**E004**

**Comparison of *GALK1* exon sequences in four breeds of dogs affected by juvenile cataracts.**

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Juvenile cataract is a heritable disorder in a number of dog breeds. The cause of juvenile cataracts has not been identified in dogs and no genetic tests exist to identify affected or carrier animals. Cataracts occur in many breeds, and vary in age of onset and severity. Therefore, it is likely that more than one gene is responsible for the numerous forms of cataract in purebred dogs.

We have chosen to look at galactokinase (*GALK1*) as a candidate gene for canine juvenile cataracts. The pattern of cataract occurrence in certain breeds parallels that of humans with galactokinase deficiency. That is, puppies develop cataracts as early as 8-9 weeks and these rapidly progress, producing blindness by 1 year of age. Cataracts are the only apparent effect of the mutation. The mode of inheritance is autosomal recessive.

Using PCR primers based on published human *GALK1* sequence, (Stambolian, et. al. 1995) we amplified and sequenced product from genomic dog DNA. BLAST results indicated an average of 89% homology between the dog sequence and the human *GALK1* exon sequence. Exon-specific primers were made based on the canine sequence.

These primers were used to amplify *GALK1* exon sequence in four breeds of dogs in which juvenile cataracts occur. Exon sequences of affected dogs, carriers and normal animals were compared and analyzed for potential mutation sites.

**E005**

**Identification of a X-autosome translocation in an intersex dog by chromosome painting**

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A 3-year-old female registered Yorkshire terrier had female external genitalia with an enlarged clitoris, uterus and bilateral ovotestis. Cytogenetic studies revealed a 78,XY chromosome complement and a mosaic condition for the X chromosome. In a subpopulation of cells the banding of the distal part of Xp was not in accordance with the one of the partial standardized canine karyotype. A whole X chromosome painting probe derived from this intersex dog was used for fluorescence *in situ* hybridization (FISH) on chromosome metaphase spreads of a normal male dog. The painting was seen on Xq and proximal on Xp and moreover on a small autosome indicating an X-autosome translocation. In order to identify the autosome the same canine probe was hybridized to human metaphase chromosome spreads. This revealed painting on the long arm of the human X chromosome and on human 20p. Using data from comparative chromosome studies the autosome of the sex-reversed dog involved in the translocation is CFA 24.

**E006**

**Microsatellite polymorphism at nine loci in the dog, silver fox and blue fox**

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Microsatellite polymorphism was analyzed at nine, previously described, canine loci: CPH1, CPH3, CPH6, CPH11, 2004, 2010, 2140, 2168 and 2319. Altogether, 151 dogs, 91 silver faxes and 53 blue faxes were included in this study. For all loci PCR amplification was successful and in general the length of the product was comparable with date published for the dog. The only exception concerned locus 2140 at which significantly shorter PCR products for silver fox (100 and 108 bp) and blue fox (92-124 bp) were observed, when compared with that of the dog (139-166 bp). Sequencing of this microsatellite revealed in the dog the following repetitive motif $(GAAA)_{10}(A)_2(GAA)_2GAAA$ which showed a quite high similarity to the originally published one - $(GAAA)_n$,. On the contrary, in the silver fox (genotype 108/108) no such motif was found. Interestingly, the two identified alleles: 100bp (p=0.04) and 108bp (p=0.96) segregated regularly in silver fox families. At other loci a variable number of alleles in these species was noticed, i.e. at locus 2004-15 alleles in dogs, 6 alleles in silver foxes and 4 alleles in blue foxes. Heterozygosity (Het) at these loci varied from 0.32 (locus 2140 in blue fox) to 0.93 (locus 2319 in dog). Probability exclusion (PE) for the studied loci was estimated: 0.9996 for dog, 0.989 for silver fox and 0.993 for blue fox.

**E007**

## Mapping gene(s) for inherited skin disease and underlying muscular atrophy: sebaceous adenitis in Standard Poodles and dermatomyositis in Shetland Sheepdogs

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Mapping the causal genetic defect(s) in sebaceous adenitis (SA) in Standard Poodles and dermatomyositis (DM) in Shelties are both problematic since the suspected autosomal recessive inheritance patterns have not been confirmed. Nor have successful treatments for affected dogs been developed for either disease. Age of onset seems more consistent in SA (around two years) with eventual loss of sebaceous glands that lubricate the skin and fur, accompanied by scaling and fur loss in affected areas. DM may strike at nearly any age. Severely affected puppies with atrophied head muscles have been observed. More commonly, the hallmark of DM is barely discernable scabs on ear tips and on extremity friction points, fur loss around the eyes and tail tip occurring in early adulthood or rarely as late as 6-8 years of age) and may progress to muscle degeneration in the same areas. While rare, muscle atrophy prior to skin involvement has been observed in Shelties but is common in related breeds. Environmental "triggers" are suspected in DM, and microbial involvement is under study by other groups. While SA has been observed in about 30 breeds, DM is documented in humans (juvenile) and only a few other breeds. Extensive linebreeding practiced in all of the affected kindreds examined so far, makes it quite possible that multiple gene loci are involved in producing both diseases and also that several defects in one gene might be responsible for the variation in symptoms within the breed or even particular lines. Testing multiple disease models for linkage to a map of informative microsatellite markers is being explored as a strategy with analyses of both nuclear and extended kindreds.

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**E008**

**Microsatellite analysis in South African wild dogs**

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The African wild dog (*Lycaon pictus*) is the second most endangered carnivore in Africa. The de Wildt Cheetah and Wildlife trust is the largest and most successful captive breeding centre for wild dogs. In the case of the wild dog the main goal of de Wildt is to breed animals for release in to the wild in order to contribute to other populations. To maintain a genetically diverse population inbreeding must be kept minimal. Analysis of microsatellite loci has been shown to be a valuable tool for such purposes. The aim of the study was to select a set of polymorphic microsatellites to be used routinely in South African wild dogs. Ten microsatellites were selected and typed in 50 dogs of the breeding stock at de Wildt showing up to 24 different alleles. In addition we evaluated a commercially available kit for paternity testing developed for the domestic dog and found that 9 out of 10 systems were also polymorphic (3-10 alleles) in the South African wild dog.

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