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 64th 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.

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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.