B001

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

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

B002

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

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

B003

Epistatic interactions with myostatin for carcass composition in beef cattle

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

B004

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

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

B005

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

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

B006

A genome scan for milk production QTL in Finnish Ayrshire cattle

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

B007

Detection of CA repeats insertion in IDVGA-48 interrupted microsatellite

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

B008

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

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

B009

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

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

B010

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

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

B011

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

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

B012

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

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

B013

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

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

B014

Towards a whole genome radiation hybrid panel in chicken

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

B015

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

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

B016

Candidate gene markers connected to litter size in German Pigs

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

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

B017

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

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

B018

Least squares interval mapping of QTL based on selective DNA pooling

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

B019

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

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

B020

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

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

B021

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

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

B022

Mapping quantitative trait loci for twinning rate in Norwegian cattle

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

B023

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

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

B024

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

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

B025

Full sequence of the SLA region containing all class I genes

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

B026

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

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

B027

A genome scan for umbilical hernia gene in cattle

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

B028

AFLP mapping of a resource family for muscular dystrophy in chicken

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

B029

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

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

B030

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

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

B031

Mapping quantitative trait loci in Danish Holstein Cattle

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

B032

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

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

B033

Physical mapping of the chicken genome

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

B034

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

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

B035

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

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

B036

Marker assisted selection of composite beef cattle for meat quality traits

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

B037

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

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

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B038

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

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

B039

Targeted development of microsatellite markers from bovine BACs.

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

B040

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

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

B041

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

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

B042

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

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

B043

Linkage mapping of *TYRP2* on cattle chromosome 12.

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

B044

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

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

B045

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

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

B046

A depository for standardized digital DNA signatures in animals

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

B047

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

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

B048

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

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

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

B049

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

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

B050

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

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

B051

Genetic analysis of seven Italian horse breeds using microsatellites.

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

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

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

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

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

B052

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

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

B053

An SNP based digital DNA signature for individual identification in cattle

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

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

B054

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

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

B055

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

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

B056

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

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

B057

Mapping feed intake genes in mice

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

B058

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

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

B059

Physical mapping of a putative recombination hotspot on BTA23

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

B060

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

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

B061

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

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

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

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

B062

An enriched version of the Dog RH-map.

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

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

B063

High Resolution Mapping of 148 Type I Markers in Pigs.

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

B064

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

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

B065

Chromosome assignment of differentially expressed bovine sequences by RH mapping

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

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

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

B066

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

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

B067

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

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

B068

An initial EST Radiation Hybrid Map of Porcine Chromosome 13

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

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

B069

Genetic mapping in salmonids.

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

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

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

B070

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

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

B071

Identifying *Trypanosoma congolense* resistance genes by positional cloning.

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

B072

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

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

B073

Identification of genomic regions for muscle pH in pigs

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

B074

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

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

B075

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

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

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

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

B076

Development of genetic markers using end sequences of swine BAC clones

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

B077

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

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

B078

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

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

B079

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

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

B080

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

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

B081

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

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

B082

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

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

B083

Genomic organization of *ELA* class II region

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

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

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

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

B084

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

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

B085

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

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

B086

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

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

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

B087

Genetic mapping of the BoLA complex using BAC contigs.

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

B088

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

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

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

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

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

B089

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

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

B090

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

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

B091

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

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

B092

Mapping quantitative trait loci in commercial pig populations

The PigQTech consortium*

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

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

B093

Detection of porcine ESTs that are preferentially expressed in liver

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

B094

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

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

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

B095

Development of genetic markers from a BTA29-specific library

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

B096

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

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

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

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

B097

An initial EST Radiation Hybrid Map of Porcine Chromosome 7

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

B098

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

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

B099

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

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

B100

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

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

B101

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

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

B102

H-FABP gene association study for body composition in pigs

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

B103

QTL for marbling maps to cattle chromosome 2.

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

B104

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

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

B105

A QTL for behavior maps to cattle chromosome 9.

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

B106

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

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

B107

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

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

B108

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

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

B109

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

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

B110

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

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

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

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

B111

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

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

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B112

Validation of QTL segregation in Angus half-sib families

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

B113

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

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

B114

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

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

B115

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

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

B116

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

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

B117

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

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

B118

The order of genes on porcine chromosome 12.

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

B119

Cytogenetic mapping in the domestic rabbit (ORYCTOLAGUS CUNICULUS)

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

B120

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

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

B121

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

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

B122

Two QTLs for growth map to bovine chromosome 14.

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

B123

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

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

B124

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

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

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

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

B125

Genetic variation of Cheju native horses in Korea

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

B126

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

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

B127

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

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

B128

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

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

B129

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

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

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

B130

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

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

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Identification of QTL for production traits in poultry

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

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

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