DNA microsatellites - the lab perspective

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The abundant source of highly polymorphic, well-dispersed, and conveniently typed microsatellites has aided to the generation of primary maps in livestock species. Parallel efforts of different labs have contributed to the generation of linkage maps for different species. Within species, common markers in different labs serve as ties to determine the orientation and distances among markers. Cattle and pig have thousands of markers as compared to hundreds for poultry and sheep. The mapping data of animals is organized in species-specific databases which can be accessed via the internet thus providing the infrastructure needed for mapping studies. Sequence conservation between cattle, sheep and goats allow about half of the primers developed from the bovine genome to be utilized in the ovine or caprine genomes although little correlation exists for polymorphism of these markers between the species. Comparative mapping between mammalian species has shown much higher syntenic conservation between human and cattle than with human and mice. The densed maps of cattle and pig cover the genome at ~5 cM level. However, once QTL has been located to a certain chromosomal segment, additional markers are needed for fine mapping. Several methods are successfully used for the targeted isolation of region-specific markers: Flow sorted chromosomes, Somatic cells, Radiation hybrids and Yeast Artificial Chromosomes (YAC) clones. As part of a collaborative effort with ABS Global (Deforest, WI) to fine map the gene controlling horn development we isolated two highly polymorphic markers from a single YAC clone and localized them to the centromeric region of bovine chromosome 1 within a few centimorgans of the gene.

In order to scan the genome at the 20cM spacing about 150 genetic markers are required. Application of either daughter or granddaughter design for detection of QTL requires determination of allele origin for the genetic marker in the progeny. If only the sires and their progeny are genotyped, the paternal allele origin of progeny having the same genotype as the sire cannot be determined. The expected frequency of informative sons can be predicted for each sire and genetic marker from the allele frequencies in the population. We estimated allele frequency in the population by genotyping seven sires. The regression of the frequency of informative sons on the predicted frequency was nearly unity. Thus, considering the large number of genetic markers available for analysis, predicted informative frequency is a useful criteria for selection of genetic markers.

Calculations of power to detect segregating loci in commercial populations of dairy cattle indicate the need for half-sib families with a total of thousands of animals. Thus, high-throughput genotyping is essential for any reasonable mapping approach. Methods developed by Sequana Therapeutics (La Jolla, CA) are best suited for this concept. Robotics systems using 96-well sample format enable uniform DNA extraction and direct quantification of DNA. Sample tracking is based on bar-code labels. Vacutainer bar codes are scanned into the LIMS system (Laboratory Information Management System) which records the sample ID and retains its history. Multiplex polymerase chain reaction (PCR) allow several microsatellite systems to be amplified in concert. The "plus A" feature (the addition of an extra A, at the 3' end of the amplified product) can cause splitting of the florescent peak thus complicating the allele calling step. It is most efficient to optimize the PCR conditions for each marker to push for either complete addition or lack of addition of the extra base, such as the recently described "FIG" tailing approach, in which a specific seven-base tail added in the reverse primer resulted in nearly 100% adenylation of the PCR products. Different multiplex PCR are pooled and loaded on a single lane gel using the ABI 377 DNA sequencer. Thus, 10-20 genetic markers can be analyzed simultaneously based on differences in either size or fluorescent dye. With 48 lanes per gel up to 1000 genotypes can be generated from a single gel and stored in LIMS. Data automation and analysis tools interact directly with the LIMS data base. The use of negative and positive

controls in each gel is essential to monitor errors in sample preparation and loading into the gel. PCR contamination must be dealt with in the high-throughput environment. Pipettes with special barrier tips are devoted for DNA handling while other pipettes are used for pre- and post- PCR analysis. We investigated the quality of our genotyping by comparing to another lab that had the same source of semen for sires. Each lab extracted DNA and genotyped 120 sires for the same markers. Discrepancies in genotype determination for bulls genotyped by both groups was less than 4%. Duplicates of 268 samples were run in Sequana, resulting in 0.5% discrepancies. High-throughput methods allow an individual worker to produce 5000 genotypes per week so that a 150,000 genotype genome scan can be completed in 10 weeks by a team of three people as was demonstrated by Sequana Therapeutics.

To efficiently utilize genetic markers that are linked to QTL in a Marker Assisted Selection (MAS) program, it is desirable to genotype preimplantation bovine embryos for multiple markers. This reduces the generation interval and enhances genetic improvement. In addition, direct selection at the embryo-level can also be applied for the desired sex, milk protein loci such as k-casein, and against disease encoding genes such as for CD-18 for bovine leukocyte adhesion deficiency. Individual cells can be removed from the embryo for DNA analysis without a significant reduction in viability. However, these cells contain only a minute amount of DNA. Thus, the embryo-derived DNA template can be used for the amplification of only a limited number of loci using multiplex PCR, due to reaction interference between the different systems. Furthermore, several replicates are needed to monitor random errors that occur in PCR. To increase the possible number of genetic typings, the primer elongation preamplification (PEP) procedure has been suggested for whole genome amplification from a single cell and subsequent multiple genetic analysis. Twenty-one in vitro-fertilized bovine blastocysts were quartered, lysed and subjected to PEP procedure allowing for the analysis of up to 40 genotypes per quarter embryo. We demonstrated, using replication, that multiple genotype analysis for marker loci and sexing can be accurately performed.

TOWARDS MARKER ASSISTED BREEDING IN POPLAR

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Due to the long generation times of trees, tree breeding is a long term process. For poplar, one of the most planted trees in Europe, only three generations of controlled crosses are available. Breeding of trees though is important given the high market position of wood world wide. At the Institute of Forestry and Game Management (IBW-Geraardsbergen, Belgium), poplar breeding has started in 1948 to generate new hybrids that produce suitable wood for the production of matches. Since then, numerous hybrids have been generated, several of which have superb growth characteristics and are planted throughout Europe. In poplar breeding programs, the main aim is to obtain clones resistant to diseases such as leaf rust, caused by the fungus Melampsora larici*f*populina and bacterial cancer, caused by the bacterium Xanthomonas populi. Other important selection criterare straightness of the stem which is a necessity for the production of veneer, height and diameter growth and frost resistance. Diagnostic methods that allow to predict the

characteristics of new hybrids at the seedling stage are very promising tools for breeding of poplar.

In our group, we have identified AFLPJ markers cosegregating with monogenic resistance against three different races of M. larici*f*populina (Cervera et al., 1996). To dissect quantitative traits, genome maps have been generated for tree clones of the mostly used species in poplar breeding, i.e. for Populus nigra, P. trichocarpa and P. deltoides. This was done using the pseudo-testcross strategy. (Grattapaglia et al., 1994). We are currently identifying the homologous linkage groups of the tree genome maps. These genome maps have been used to dissect quantitative resistance to Xanthomonas populi, height growth, and wood specific gravity with the mapmaker/qtl program. Now these QTLs need to be investigated whether they are stable across different environments and across different pedigrees. The ultimate aim is to develop strategies that allow a more efficient breeding program.

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MOVING FROM QTL EXPERIMENTAL RESULTS TO THE UTILISATION OF QTL IN CURRENT BREEDING PROGRAMMES.

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Results from quantitative trait loci experiments can not be readily implemented into breeding schemes through marker assisted selection. This is due to uncertainty about whether the quantitative trait loci identified are real. In addition, the results from QTL experiments, in most cases, are not directly applicable to the current breeding schemes.. For example, in dairy cattle, QTL results from granddaughter experimental designs are at least two generations away from the current breeding stock. Also, in the experimental designs commonly used in poultry and pigs, such as divergent crosses or inbred crosses, the reported QTL results are mainly from lines or breeds other than those in the current breeding population. One strategy to reduce uncertainty in the quantitative trait loci results is combining p-values from many quantitative trait loci experiments. This requires that p-values and QTL effects are published in literature and all of the chromosomes that have been evaluated should be presented to ensure an unbiased sample of experimental results are in scientific literature Another strategy is the establishment of a confirmation study. Power of a confirmation study must be high to ensure that the postulated quantitative trait loci can be verified. In the calculation of the power of the experiment there are many issues that have to be addressed; i) size of the quantitative trait loci to be detected - the estimated QTL size from an initial genome scan is usually biased upwards, ii) significance level required - can possibly have a combined type I error from the initial study and the confirmation study, iii) experimental design - confirmation study should be independent of the first QTL experiment, and iv) expected heterozygosity for the design - may be heterozygous at the QTL but the two allelic effects may be the same. The confirmation

experiment can have two objectives i) confirm that the QTL is a real effect in the family or line it was initially identified in, and/or ii) confirm that the QTL is present in the current breeding population.

A model for population growth of laboratory animals subjected to marker assisted introgression: how many animals do we need?

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Identification of trypanotolerance genes in N'dama cattle gives opportunities to use marker assisted introgression (MAI) to develop trypanotolerant cattle types. Trypanoresistance is well known in some laboratory mouse strains (Morrison et al., 1978), and three chromosomal regions have been identified (Kemp et al., 1997). Consequently mice are being used as an animal model in a pilot study to assess the effectiveness of MAI of trypanotolerance genes for cattle. The present paper intends to provide a mathematical model for estimating the mean and variance of the number of animals needed at each backcross generation and susceptible to end up to a targeted number of favorable animals at the end of the backcross process. The ultimate goal is to intercross those animals to produce homozygous individuals for the desired loci genotype. In developing the model we used inbred line of mice which can be adjusted for any biological material (animals or plants) as soon as the reproductive physiological background is taken into account. This study provides formulae for calculating the mean and variance of the number of animals with the desired genotype in each backcross generation for a marker assisted introgression experiment using inbred lines. The model assumes a Poisson litter size, and is similar to that used in stochastic versions of population dynamics models. Certain biological parameters must be specified (mean litter size, sex ratio, probability of inheriting the desired genotype), as well as parameters under the control of the breeder (initial number of founder animals, number of times each backcross male is used, number of backcross generations). These methods can be utilized in designing an experiment to determine the number of founder animals required, given the number of animals carrying the desired chromosomal region(s) and required at the completion of the backcross process and vice versa. Consideration is given to minimizing the total amount of genotyping over the entire experiment, by varying the number of times each backcrossed male is used. In addition, an outline is given for an adaptive design that allows for changes in male usage to be made during the experiment. The theory is illustrated by several numerical applications, and some general conclusions drawn. It is evident from these studies that large difference in the amount of genotyping required result from different design choices; consequently these techniques are seen as a useful tool in the design stage of an experimental breeding program.

EXPLOITATION OF HALOTHANE GENE IN SELECTION FOR PORK QUANTITY AND QUALITY

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Ways of exploiting the halothane gene in breeding for pork quantity and quality are discussed and demonstrated examples are given. The halothane gene is inherited as a single recessive gene with a penetrance for the recessive hommozygoty (nn) ranging from 50 to 100 % over a variety of breeds. Its frequency in the world breeds ranges from 0 to 89 %. A comparison of phenotypes has revealed that the halothane gene affects a wide range of important economic traits. Halothane positive reactors have shown consistent superiority in carcass lean content, ham proportions, loin eye area, backfat depth, growth rate and feed efficiency. However, these benefits are accompanied by deterioration in meat quality traits such as, intramuscular fat, water holding capacity, PH value, an increase in pure soft exudative (PSE) meat and post weaning mortality. This trade off between quantitative and qualitative traits calls for ways to exploit this gene to bring aa balance between the two types of traits. Performance of heterozygous is intermediate, an indication that while the gene is recessive for halothane sensitivity, it could be additive for some aspects of performance. Most selection strategies have focused on selection for growth rate, lean content, reproductive traits and against backfat. However, consumers demand for meat quality, growing interest in integrated production systems and increased possibility to select for meat quality are challenging individuals and pig breeding companies to shift emphasis to meat quality. Although statistically significant, the association between the halothane gene and PSE meat is far from unit an indication that PSE may be caused in part by environmental or other genetic factors. On average the disadvantages from halothane positive reactors from high mortality rate out weigh their advantage in carcass yield. The cost effectiveness of exploiting this gene will depend on factors such as, economic balance between harmful and beneficial effects on performance, the system of breeding strategy and its effects on expected response to selection for quantitative and qualitative traits. The major challenge for the future will be how to disentangle the harmful effects of the gene from the beneficial effects.

TOWARDS THE IDENTIFICATION OF TRYPANOTOLERANCE QTLS IN CATTLE

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Trypanosomosis in livestock is a blood parasitic disease which affects one third of the African continent and threatens 60 million cattle. Chemoprophylaxis is possible but resistance of the parasite to the drugs is increasing. Control of the vector, the tsetse fly, is often damaging for the environment. Some African indigenous cattle breeds are genetically tolerant to the disease but their productivities are lower compared to improved exotic breeds farmed in developed countries. The chromosomal localization of trypanotolerant QTLs should allow their introgression into susceptible breeds while conserving the productivity traits of the latter.

In an attempt to map the trypanotolerant QTLs, a two generation cross between N'Dama (trypanotolerant breed) and Kenyan Boran (trypanosusceptible breed) was established. The grand-parents included four N'Dama sires and four Kenyan Boran dams. The F2 generation comprises 211 offspring distributed mainly in seven major families including five sets of identical twins. They were challenged with Trypanosoma congolense (IL1180) at one year old and monitored for 150 days. By end of June 1997, 186 F2 had ended their challenge. The following phenotypic traits were recorded: Packed Cell Volume (PCV, a direct measure of anemia), body weight and parasitaemia. Phenotypic analysis, performed at the University of Jerusalem, shows a positive correlation between body weight and PCV but no correlation between parasitaemia and PCV, indicating that anaemia and parasitaemia, as we measure them, are under two different genetic control. Around 100 markers have now been genotyped in the 7 major families. A preliminary analysis

assuming that the N'Dama grandparents were homozygous for the major trypanotolerant QTLs was performed using Map Manager QTb10. Several markers linked to PCV or parasitaemia, with Lodscore values above 2, were identified in the major families.

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