INTERBULL MEETING, AARHUS, DENMARK

AUGUST 19-20, 1993

Objectives and present activities in the BovMap - The Bovine Gene Mapping project

A. Georgoudis

Aristotle University, Faculty of Agriculture, Dept. of Animal Production
540 06 Thessaloniki - HELLAS

SUMMARY

The paper describes the common European efforts for the development of a low resolution marker map of the bovine genome. The objective is to identify marker loci distributed across the bovine genome and to determine their physical and genetic relationship to one another. Markers which are highly polymorphic in cattle will be identified and placed on a linkage map by the analysis of their segregation in families of cattle. Selected markers will be assigned to particular chromosomes or syntenic groups based on patterns of hybridization with a panel of somatic cell hybrids. The precise chromosomal location of landmark markers will be determined by in-situ hybridization, yielding a physical map of the bovine genome.

The availability of such a map will permit the future mapping of Quantitative Trait Loci (QTL), which are of economic importance. The project will have three phases as outlined below:

(1) The development of a public domain marker map for cattle.
(2) Once the map is available, the goal will be to develop improved technologies and resources for the identification of loci controlling economically important traits.
(3) The practical implementation of the identified loci in breeding strategies. This will be the responsibility of smaller collaborative groups and commercial organisations.

I. INTRODUCTION

The map of the bovine genome is further advanced than that of other livestock species, ranking third behind man and mouse. For the map to be of value in identifying QTLs, it is important that the markers show high levels of heterozygosity, but in general the markers that have been mapped in cattle are monomorphic.

Current technologies will, however, enable rapid progress to be made in the identification and mapping of polymorphic markers.

The discovery of microsatellite sequences, which show high levels of polymorphism at high frequency and are distributed across the whole genome of several mammalian species, has made the identification of the 200+ polymorphic markers required feasible. The identification of such markers within cosmid clones will also help in the physical mapping of such markers. A considerable amount of between species synteny and the conservation of DNA coding sequences have been described, which will allow corresponding areas of the human and bovine genomes to be identified and, hence, the rapid identification of human cDNA markers for specific regions of the genome. The use of microsatellite markers has the advantage in that typing is achieved by the Polymerase Chain Reaction (PCR) and in most cases PCR technology can also be used for typing RFLPs.

The extensive use of artificial insemination results in very large half-sib families both within national herds and, for the more productive bulls, internationally. Such families can readily be identified and could be used for the mapping project. However, the use of full-sib families will increase the power of statistical analysis, in that phase of markers can be inferred even in the absence of grandparents. In practical terms full sib-families also reduce the number of animals that need to be genotyped over half-sib families, in that there is only one mother per family. When using half-sib populations the information from the maternal haplotype is discarded.

The cattle breeding industry across Europe is evaluating novel technology for enhancing breeding strategies, which has resulted in the extensive use of Multiple Ovulation Embryo Transfer (MOET) and hence herds comprising full-sib families can be used to form the reference population.

In order to keep track of progress of the map, a central data base will be required, this data base will hold information on reference animals, probes, and raw data on linkage and physical mapping. It will be compatible with the human and mouse data bases to enable rapid comparative mapping, but will contain more detailed information relative to polymorphism in the various cattle breeds and will go further in the analysis of data to form the linkage map.

II. GENERAL OBJECTIVES

The general objective of the programme is to place existing markers on the bovine gene map and produce additional markers to construct a low resolution map. Therefore, the following objectives are targeted:

(1) To constitute a panel of reference families.
(2) To generate a large number of markers which are highly polymorphic in cattle.
(3) To assemble these markers into a linkage map in which the markers are evenly spaced at about 20 cM intervals across 90% of the bovine genome.
(4) To assemble a panel of somatic cell hybrids in which all bovine chromosomes are represented.
(5) To use the somatic cell hybrids to assign markers to syntenic groups.
(6) To develop improved techniques for physical mapping and form a physical map of landmark loci with a minimum of 2 markers mapped to each chromosome.

(7) It will be essential to establish a single data base with statistical and computational methodology for the development of the map.
(8) Over the period of the project it is also intended to refine PCR based methodology to enable the rapid genotyping of animals for polymorphic markers, which will be essential for the application of the map. It will be necessary to develop automated genotyping technologies as the number of mapped marker increases and develop quantitative genetics theory to prepare for the application of the map in subsequent projects.

III. PROJECT METHODOLOGY

The work programme is divided into three interacting areas, genetic mapping, physical mapping and analysis.

The first priority is the identification of markers. Initially labs will work together in the identification of 200 microsatellite markers. In addition, homologous and heterologous cDNA clones will be screened for cross reactivity and polymorphism with bovine DNA.

As markers are being produced, the physical mapping methods will be refined and selected markers will be placed on the physical map.

To identify linkage, segregation of markers in families will be analyzed. For this, a common panel of reference families will be required.

Once data are generated on the reference panel, a central data base will be required to collect and collate information and identify linkage. As the linkage map develops, other methods will be developed to identify markers at specific locations, either by comparative mapping or by the construction of chromosome specific libraries.

In order to synteny map markers, somatic cell hybrids already available will be collected into a common panel and further somatic cell hybrids will be made to identify those representing missing chromosomes or regions of the genome.

It is important that duplication is kept to a minimum, therefore labs working in particular areas will interact closely to define their roles. Available expertise will be used to the full by sharing materials and exchange of personnel for training. DNA from reference animals will be produced by a single or restricted number of labs and distributed to the others.

(1) Reference families

The reference panel is a fundamental requirement of the collaborative work, allowing the construction of a single genetic map by linkage analysis, using first the available markers and later on, newly produced markers.

Twelve families of full-sibs, with an optimum size of 8 to 10 progeny will be collected, giving a total of 120 to 150 animals including parents. The use of full-sib families will increase the power of statistical analysis, as compared to half-sibs families, in that the phase of markers can be inferred even in the absence of grandparents. In

practical terms it also reduces the number of animals that need to be genotyped, in that there is only one mother per family.

(2) Marker production

The production of markers will be organized using several approaches. In the initial stages a random production of highly polymorphic markers (microsatellites) will be productive. However, as the map fills up, different areas will be either over- or under-represented. Consequently, later on, markers will be identified using a more directed approach to ensure an uniform coverage of the genome.

The strategy will first allow the mapping of all known markers and will progressively lead to an increasing number of STS (Sequence Tagged Sites) well dispersed in the bovine genome. The identification of cosmid clones containing either microsatellite or coding sequences, or both, will help in the physical mapping and will enrich the comparative mapping. The characterization of 300 to 500 microsatellites genetically mapped by linkage analysis should be achieved over the three year period, with up to 100 physically mapped using cosmid clones. This will allow the selection of the most suitable set of markers to constitute the low resolution map.

To produce microsatellite markers, agreement on usage of core sequences, screening of common libraries and sharing of clones for sequencing, testing and verification of putative primers, will be made to minimize duplication of effort and maximize productivity. To use and test cDNA heterologous and homologous probes, a central register of probes tested and results will be organized in order to minimize duplication.

(3) Physical mapping

Two approaches will be considered:

1. The use of somatic cell hybrids. The aim is to assemble a panel of somatic cell hybrids in which all bovine chromosomes are represented. As far as possible, a complete common panel will be defined and DNA distributed in order to assign any known or new marker to one of the 30 syntenic groups.

2. In situ hybridization. To improve in situ hybridization techniques in order to achieve the precise location of at least two markers per chromosome. The use of cosmid derived marker sequences in conjunction with fluorescent tagging for physical mapping makes the interpretation of results easier and potentially reduces the amount of work required in order to localize markers on the physical map. A restricted number of genetically mapped microsatellites markers will be physically mapped and chromosome assignment will be assisted by the identification of chromosome specific markers, as the bovine karyotype is very difficult to interpret without considerable experience.

Assembly of a complete panel of somatic cell hybrid lines will be achieved by pooling those produced by the laboratories involved. This will maximize the number of chromosomes covered, ensure universal availability of hybrid lines and reveal gaps in coverage. DNA will be prepared from the hybrid panel and distributed to laboratories identifying markers so that new markers can be rapidly assigned to syntenic groups.

Several laboratories will have the expertise of in situ hybridization technologies and will serve, on a coordinated basis, to precisely map the various genes or markers, characterized or produced by the participant laboratories.

(4) New Technologies

New technologies are fundamental requirement of the project because they will be needed to complete the map, for mapping closely linked genes and for the implementation of the map. Efforts put this area could lead to drastic gains in efficiency to achieve the different goals. It is difficult to predict advances in technology. The following tasks will be addressed.

(a) Production of chromosome specific markers using chromosome sorting and chromosome dissection. Sorted chromosome or chromosome specific material for example, could be used and distributed to establish restricted genomic libraries allowing the production of new markers.

(b) Other aspects to take into consideration are PFGE, cloning of high molecular weight fragments and sperm typing which would offer new possibilities in terms of physical or genetic mapping to study closely linked genes.

(5) Statistics and computing

To record, analyze and organize data, a single data base will be required. This part of the project will be implemented in strong collaboration between AFRC acting as Coordinator and INRA. Linkage and physical mapping information from all participants will be collected into a common data base which will enable monitoring of progress and reveal the degree of coverage of the genome. This will be a continuous process which will identify the point, at which it will be necessary for a change in the approach, from the random generation of markers, to the targeting of specific regions of the genome.

IV. BENEFITS

In the short term, benefits will include improved technological expertise acquired and developed within the project, which can be applied to other mapping projects in animals and man and to research in general.

Application of the bovine marker map to the study of disease could provide a model of human disease that is amenable to experimental manipulation. Such diseases might include neurodegenerative diseases, trypanosomiasis, ankylosing spondylitis, leukemia, achondroplasia etc. Alignment of human and bovine maps will help in the identification of homologous genes in man.

The ultimate objective of the marker map is the identification of loci controlling physiological traits in cattle, in particular loci controlling quantitative traits. The use of markers in breeding programmes could increase efficiency, resulting in social and economic benefits in reduced feed and energy consumption, reduced pollution and release of land for other uses. It should also be possible to breed animals that are healthier and less susceptible to infection, resulting in the reduced use of antibiotics and lowering of the risk of residual concentrations in food products. Efficient breeding

should also reduce the use of other artificial additives such as hormones and will allow the industry to respond more rapidly to changes in consumer demand with respect to carcass composition.

V. REFERENCES

CEC-DG,S-R&D,XII-F2, 1992. Development of Genetic and Physical Marker Maps of the Bovine Genome - Bovine Gene Mapping Project (BovMap)

FRIES R., 1991. The Bovine Gene Map. Swiss Federal Institute of Technology.

INRA-CEC, 1991. European Bovine Gene Mapping Meeting. Jouy-en-Josas, France, 3-4 October 1991.

INRA-CEC, 1993. European Bovine Gene Mapping Meeting. Freising - Weihenstephan, Germany, 23-25 March 1993 (In print).