# Animal genome mapping projects

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## 1. Objectives of genome mapping

Recombinant DNA methodology opens up new frontiers in the detection of genes and understanding of genome organization. During the latest years research within this field has shed light upon numerous structural and functional aspects of eucaryotic genome organization and knowledge has been obtained about evolutionary genome changes. Comparison of DNA structure within and between different species has revealed a common structure underlying all genomes. Surprisingly only approximately 10% of the roughly $3 \times 10^9$ bp which constitute the haploid genome in mammals can be categorized as protein coding sequences (Nowak, 1994). A prerequisite for the identification and analyses of important genes among the vast amount of non-coding DNA is the development of gene maps. During the latest years many examples have been provided illustrating that gene maps can be utilized for research aiming at (i) isolating genes causing or having an influence on certain diseases, (ii) studying the genomic organization and evolutionary relationship of mammalian species, and (iii) developing animal models of human disease. Furthermore, prospects of understanding the organization and action of genes controlling valuable quantitative traits and of possibilities for improving breeding stocks using marker assisted selection have been provided.

## 2. Construction of genome maps

A gene map is in part made up of linked polymorphic marker loci evenly distributed throughout the genome, and in part of cytogenetically located genes/cloned DNA sequences. The genetic map shows the distance between markers estimated from the amount of recombination that occurs between them in experimental crosses while the cytogenetic map provides the physical

localization of the markers on individual chromosomes. Thus, the physically mapped markers provide landmarks that locate and orient the linkage groups. The ultimate map of any given species is the complete DNA sequence of the haploid genome. It will not, however, be possible to interpret such a map without methods which can relate specific sequences with particular functions and without actually identifying the coding sequences. Two basically different approaches can be taken in order to clone genes of interest, i.e. functional cloning (the candidate gene approach) and positional cloning. While functional cloning requires prior characterization of the aberrant gene product, positional cloning relies on the genetic linkage map for the identification of the chromosomal location of the gene/genes in question. The different approaches used for the development of "meaningful" maps, i.e. maps that offer prospects for ultimately identifying the function of all genes in the organism, are briefly described in the next sections.

## 2.1. Genetic mapping

The informativeness of genetic markers are directly related to their polymorphism and frequency of heterozygosity. DNA technology has facilitated an actual exploitation of the DNA sequence variation for mapping purposes. This was initially demonstrated by Botstein and co-workers (1980) by the construction of a genetic map or linkage map in man using restriction fragment length polymorphism (RFLPs). Later studies have proved the simple sequence repeats (microsatellites) initially described by Litt and Luty (1989), and Weber and May (1989) to be the markers of choice for the construction of linkage maps. Thus, extremely comprehensive genetic maps have been constructed for the human and mouse genomes based on genotyping of several thousand microsatellites (Gyapay et al., 1994; Dietrich et al., 1994). Genetic linkage is defined in relation to meiotic recombination and relies on the fact that non-homologous chromosomes assort independently during meiosis. Thus, pairs of genes or markers in close proximity to each other on a chromosome will be transmitted together more often than pairs of genes with increased physical separation. The generally accepted method for detection of linkage is based on maximum likelihood estimation (Mather, 1960). The common test statistic for significant linkage is the lod score value, i.e. the $\log_{10}$ of the ratio between the likelihood that the observed data correspond to linked loci and the likelihood that the data would have arisen given that the two loci are unlinked (Morton, 1955). The distance between markers is measured in centiMorgans (cM). Computer programs, that can be used to perform multi-point analysis, have been developed. The most widely used are LINKAGE (Lathrop and Lalouel, 1988) and CRI-MAP (Lander and Green, 1987). It is assumed that on average, the physical distance represented by 1 cM (equivalent to .01 recombination fraction) is 1,000 kilobases.

As linkage relationships over short distances are often conserved across species, linkage information can be transferred from "map-rich" to "map-poor" species (O'Brien et al., 1993). In order to exploit this fact and in order to facilitate the identification and cloning of genes on the basis of the candidate gene approach, it is necessary to include coding sequences in the linkage map. Once the map has been constructed in a given species the candidate gene approach and/or positional cloning can be employed in order to clone genes of interest. In humans approximately 5000 genes have been mapped (Cuticchia et al., 1993), while the corresponding number in mice is approximately 3000 (Copland et al., 1993). Thus, both the human and the murine map provide valuable recourses from which information can be transferred to maps of other mammalian species.

### 2.2. Cytogenetic mapping

In cytogenetic maps, loci are ordered with respect to banding patterns or relative position along the chromosomes. These maps are generated by means of data from somatic cell hybrids and/or *in situ* hybridization. Fluorescent *in situ* hybridization (FISH) has revolutionized cytogenetic mapping enabling rapid and precise localization of DNA probes and facilitating the simultaneous sublocalization and ordering of several probes (e.g. Chowdary, 1991). The cytogenetic map plays an important role with regard to the anchoring of linkage groups to chromosomes. A recent advance in cytogenetic mapping, i.e. heterologous chromosomal painting (eg. Rettenberger et al., 1995), has demonstrated the feasibility of hybridizing heterologous chromosome specific libraries to metaphase spreads of chromosomes, thus visualizing chromosome segments that show conserved synteny. This procedure greatly accelerates the mapping of "map-poor" species.

## 3. Animal genome mapping

Presently projects have been initiated within Europe and elsewhere aiming at producing maps of the genomes of the pig, cow, sheep, chicken and dog. While the main incentives for the construction of linkage maps for the economically important domestic animals is the potential ability to genetically dissect phenotypic traits of economical or biological significance the main incentive for the construction of a canine genome map is the hunt for disease genes. In the following the projects concerning porcine gene mapping (PiGMaP), bovine gene mapping (BovMap), and canine gene mapping (DogMap) will be presented and the present status of the maps will be reviewed. In common for all genome mapping projects is the need for specific databases in which all relevant information for the specific species can be stored. Such databases have been established for all three species discussed here.

### 3.1. The pig gene mapping project (PiGMaP)

With support from the Commission of the European Communities a collaboration between 17 European laboratories was initiated in 1991. The aim of the collaboration was to produce an evenly spaced porcine marker map with linkage groups representing each of the 18 pairs of autosomes plus the X and Y chromosomes constituting the porcine genome. An important prerequisite for the collaboration was the establishment of a reference population amenable for mapping purposes. A highly informative three generation $F_2$ population was established from Meishan by Large White crosses and by crossing Wild Boar and commercial pigs (in total 153 animals).

The first linkage map developed by the PiGMaP consortium have been published recently (Archibald et al., 1995). This map is based on segregation analysis of 239 genetic markers in the reference population. Eighty of these markers correspond to known genes while the majority of the remaining are microsatellite markers. As cytogenetic mapping information is available for 59 genes the linkage map also contributes to comparative mapping. Linkage groups have been assigned to all chromosomes except the X and Y chromosomes. Rohrer and co-workers (1994) have reported on a map based on linkage analysis of 376 microsatellites and 7 RFLPs genotyped in 104 animals from two generations. In this study 23 autosomal linkage groups were identified of which 13 were assigned to autosomes (no assignments to chromosome 10,11,16,17 and 18) and one linkage group was assigned to the X chromosome. The two linkage maps are presently being merged providing a better coverage of the whole genome.

Presently one investigation in which the porcine linkage map has been applied for correlation studies between phenotypic traits and genetic markers has been made (Andersson et al., 1994). This investigation gives strong evidence for QTLs on chromosome 4 with large effects on growth, length of small intestine, and fat deposition. These results demonstrate that it is indeed possible to dissect polygenic inheritance into specific molecular components or genes. Also one very important example, in the pig, of identification of the molecular basis of a genetic disease deserves mentioning, although not identified in the context of the European collaboration. That is the identification of the mutation in the calsium release channel gene (CRC) associated with malignant hyperthermia (Fujii et al., 1991). The success in detecting the molecular background for malignant hyperthermia can be ascribed to exploitation of the candidate gene approach.

### 3.2. The bovine gene mapping project (BovMap)

Also the BovMap project has received funding from the European Communities. This collaboration have comprised a total of 30 laboratories throughout Europe. Much in line with the PiGMaP collaboration this project has also been centered around (i) mapping of reference animals, (ii) marker production,

and (iii) cytogenetic mapping. The collaborative efforts have resulted in the characterization of approximately 250 microsatellites, about 100 loci have been localized by fluorescent *in situ* hybridization, and somatic hybrid panels have been evaluated using more than 300 markers or genes (Leveziel, 1994). The final report on the results of the collaboration have not jet been published. However, a very comprehensive map of the bovine genome has been published recently (Eggen and Fries, 1995). This map comprise linkage and/or *in situ* mapping data on 877 bovine loci. Of these 314 are genes or pseudogenes and 563 are anonymous loci.

### 3.3. The canine gene mapping project (DogMap)

Of the three mapping projects discussed here the dog gene mapping project is by far in the most disadvantageous position both for technical and economical reasons. The collaboration aiming at establishing a canine gene map has been initiated without funding and thus is based on the ability of the individual groups to obtaining national funding. In total 31 laboratories have signed up for the collaboration. One very difficult drawback for the success of the project is that the canine karyotype (the prerequisite for performing *in situ* localization of genes) is very difficult to establish. However, initiatives have been taken both to establish the karyotype, to establish a reference population, and to identify good markers. A large number of markers have for instance been characterized by Ostrander and co-workers (1995). Thus, provided the collaborative efforts are aimed at genotyping a common reference population the prospects for the canine map is good.

## 4. Future prospects for mapping projects

A very important tool in the contexts of fine mapping and isolation of specific genes is the establishment of physical maps or contig maps of the animal genomes. Initiatives both in regard to establishing porcine and bovine contigs have been taken. In line with contig mapping, also radiation hybrid cell lines will be of importance for the future genom analyses projects. Furthermore, the assignment of more coding sequences to the maps is a very important aspect of the future work. This will greatly enhance the possibilities for using the candidate gene approach for the identification of loci of interest. The first example of a systematic approach to the identification of a large number of new genes in pigs has recently been provided (Winterø et al., submitted). Thus, based on the work already performed in the area of animal genome mapping and the work underway the future will no doubt bring a wealth of new insight into the structural and functional organization of animal genomes.

## Literature


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Summary

During the latest years many examples have been provided illustrating that gene maps can be utilized for research aiming at (i) isolating genes causing or having an influence on certain diseases, (ii) studying the genomic organization and evolutionary relationship of mammalian species, and (iii) developing animal models of human disease.

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