Application of molecular genetic techniques in animal breeding

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1. Introduction

Historically, one can recognize different phases of economic advancements, which have been influenced by scientific and technological ventures. Animal production is certainly included in such developments. Persons like Darwin and Mendel, as well as Watson and Crick, Arber or Mullis, — just to mention a few — contributed to the knowledge of todays' modern molecular genetic engineering. In addition to these newly developed genetic techniques, the reproduction technologies, computer sciences and management strategies also helped to intensify the production level worldwide. It should be clear for everyone, that without these developments, our world would look quite different, but not neccessarily better. Considering all possible alternatives, there is no other chance as to develop our knowledge on all levels of science and economic potentials, to use them in the best way for developing a better world.

1.1. Animal Science is a basic Science

Many people have the feeling that animal production can be done by everyone and only needs some experience to be successful. But in our time, for all biological systems, we need a basic knowledge of the internal and external functions of organisms in genetic, physiological, anatomical and behaviour aspects. The life functions of every living organism up to the very complex mammalian system must be understood, in order to treat and handle them accordingly and without drawbacks. This integral understanding of complex biological systems in all their interactions with the environment, including the human being, can only be scientifically handled by an animal science discipline.

No other discipline in an academic society will be able to combine so many basic functions as animal and plant sciences. From the beginning of domestication, the breeders took care of their plants and animals, and none

of these handled species are extinct or not functioning anymore. This progress was supported by scientists from many fields, but certainly also from animal- and plant geneticists.

1.2. Individual significant inventions

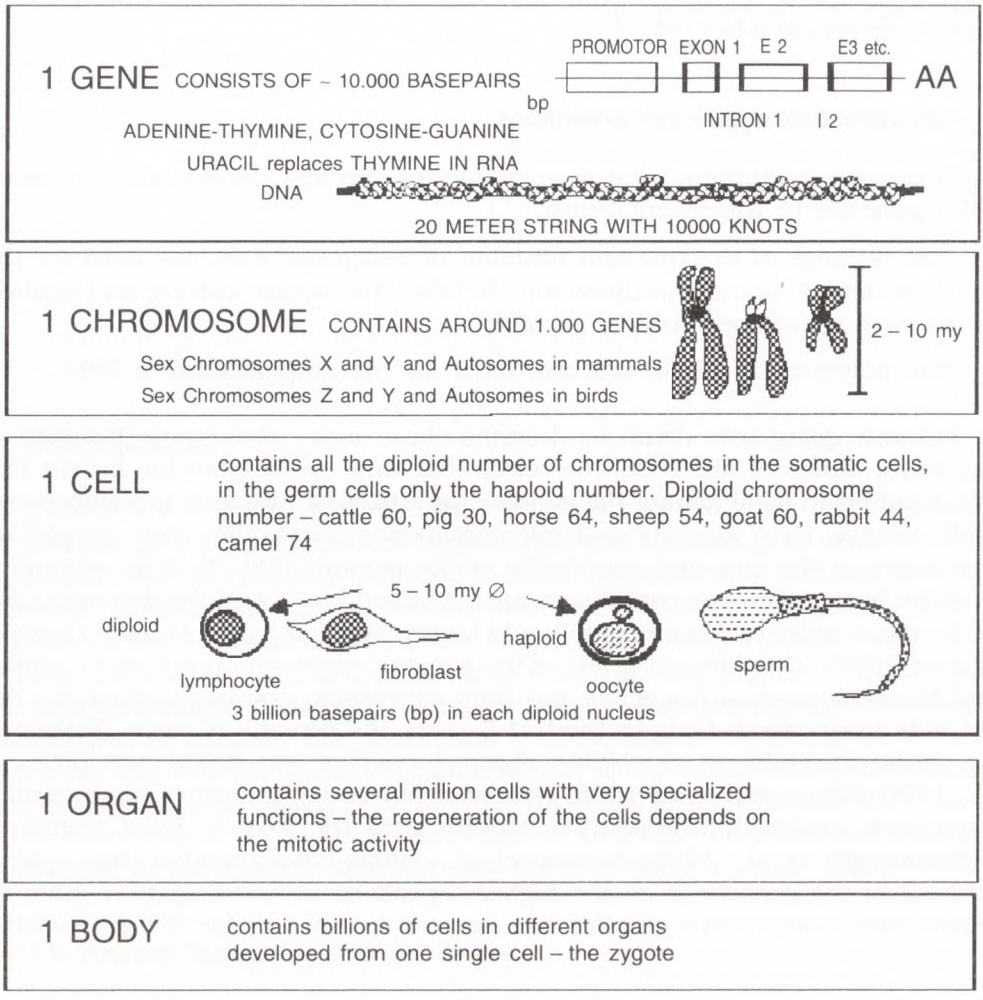
Two main inventions were responsible for the fast development in molecular genetics in the recent years:

- The findings of the role and function of restriction enzymes used for the detection of point mutations and RFLPs. The precise cutting and ligation of base sequences became possible.
- The polymerase chain reaction used for the amplification of DNA.

Without going into detail to describe these new inventions (Botstein et al., 1980; Mullis et al., 1986) it is clear that they are responsible for the fast development in establishing the genome data base of humans, laboratory animals, plants, farm animals and microorganisms. Generally, most people are not aware of the size and complexity of the genome (Fig. 1). It is estimated, that the human genome contains around 3 billion bases and the determination of the exact order of those bases should be investigated by the Human Genome Project (HGP). The different levels of the genome organisation are very complex and the structures of the genes and their expression and interactions are not yet fully understood. Only in the last 5 years the genome of cattle (Bishop et al., 1994; Barendse et al., 1994), sheep, (Ansari et al., 1993), pigs (Fries et al., 1990; Eggen and Fries 1995; Rohrer et al., 1994) and other farm animals have been analyzed. Comparative studies with the Zoo — FISH technique (Rettenberger et al., 1995; Solinas et al., 1995) have revealed that a large amount of the genome of all the higher organisms is homologous to such an extent that comparative genetics has become a new tool for the geneticist.

1.3. Research and Application

For any safe application of new techniques and methods, they have first to be investigated and published in a scientific way, to make sure that many people around the world can control and criticize the published datas. This is the quality and ethic control mechanism in scientific research, necessary to create safe methods for the practice. For the application of such methods other criteria are also important, such as scientifically created and controlled fieldtrials and a broad experience of the scientists, to set up a decision making procedure, which is also influenced by political and social impacts. The consideration of animal welfare guidelines will become more and more important, since the effects of the applied techniques can be harmful to the animals (Stranzinger, 1994 a). As outlined in Fig. 2, there are two large



The genetic diversity in higher organisms is created by the recombination event, by the random segregation of the homologue chromosomes, by mutations in the meiotic and mitotic cells, by the fertilization and by gene-environmetal interactions. Genetic engieering increases the diversity.

The function of the genes is regulated by activation/inactivation, transcription, splicing, accumulation, degradation, translation, splitting, phosporylation, glucosylation, aggregation.

The function of life is regulated by the ability for the body formation, metabolism and stimulation sensitiveness.

Fig. 1. The size of genetic informations in the different levels of genetic containments.

groups to be mentioned for the application of molecular genetic techniques in animal breeding:

First, one has to deal with the genetics of the single animal and within populations, to improve their genetic make up within breeding goals (Stranzinger, 1994 b).

Secondly, molecular genetic techniques also offer a broad range of methods for the diagnostics.

2. Genetic monitoring of animals and diagnostic applications

Many informations can be constructively used for selection and breeding strategies. Most important is the first step of basic and applied research, to make sure that all the applied techniques are correct, safe and efficient.

2.1. Marker for colour inheritance

More and more, the oldfashioned colour inheritance becomes important again, to be used as easily detectable markers in animal breeding. Especially colour marking in crossbreeding is a useful integration of genetic information of dominant black colour in the recessive red spotted populations, having a PCR marker for the recessive red to be used to increase the genetic variation. Recently, patenting and licencing have been done in this respect.

2.2. Markers for parentage control and pedigree studies

In breeding strategies, the correct parentage is very important, especially if expensive testing of animals is required for later use in artificial insemination. Programs to improve certain traits with genetic methods in populations include the use of genetic informations gained with many animals participating in the testing phase. The correct statistical evaluation of the testing results depends on the correct pedigree and parentage control (Glowatzki-Mullis et al., 1995).

2.3. Testing allelic variation for important trait loci

Every variation within genloci is caused by mutations producing different alleles which alter the productivity of the animal or the usability of the gene-product. As an example one can use the kappa casein variation in the milk of cows and goats, required for cheese making, since the coagulation and rennability is changed by the kappa kasein alleles. Every polymorphism in a given trait has its own genetic background (QTL, quantitative trait loci) and the optimal combination of different positively influencing alleles located

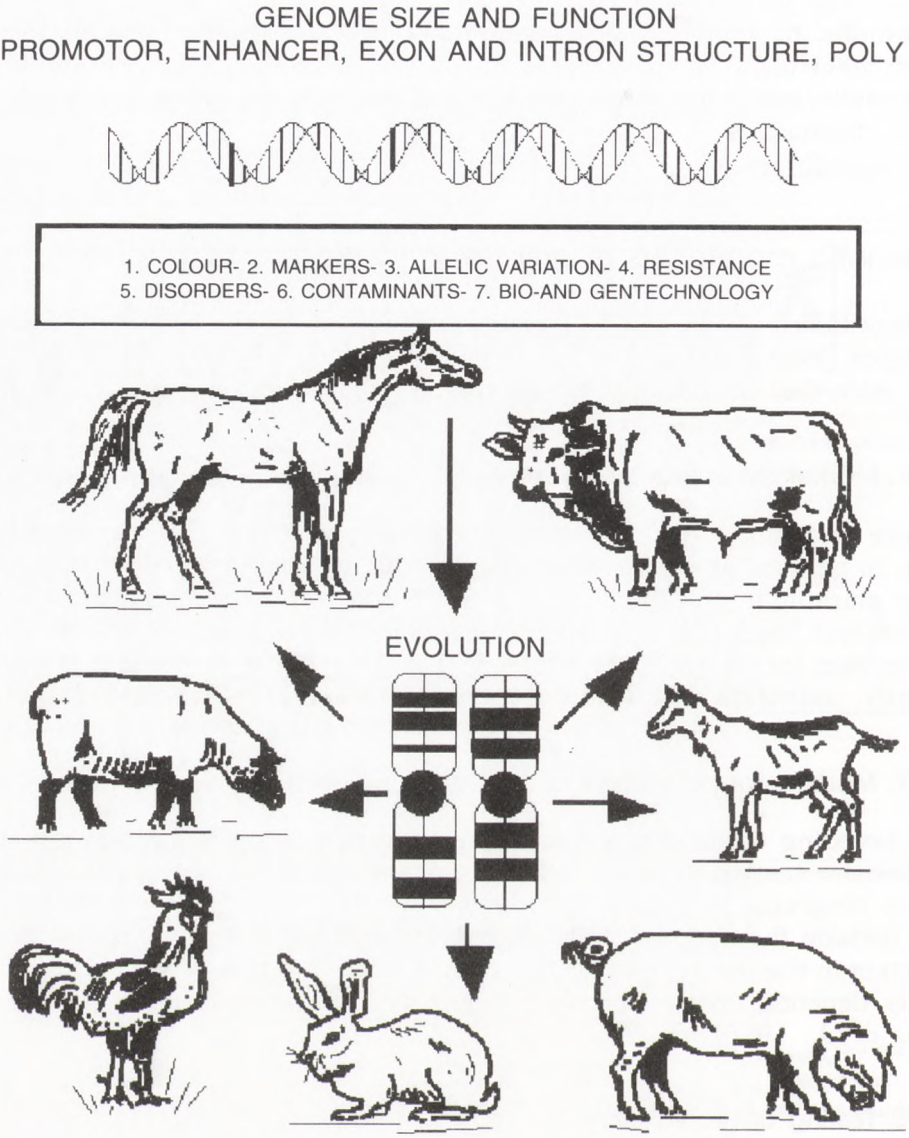


Fig. 2. The evolutionary development of species indicating several molecular genetic homologies and diversity.

on different chromosomes or recombined within a chromosome, can contribute to superior animals used for breeding purposes (ETL, economic trait loci). Identifying those combinations with molecular genetic techniques will enhance the usability and success of those animals (MAS, marker assisted selection).

2.4. The evaluation of resistance of animals to certain environmental stressful interactions

This can be done by challenge tests with contaminants like viruses, bacteria or fungi as well as with harmful climatic or feeding conditions. Stress susceptibility selection is best known for the malignant hyperthermia syndrome (MHS) in pigs, using the PCR based test for the C-T mutation in the calcium releasing channel gene sequence. In chicken there are other examples known for the selection to increase heat and disease resistance. As soon as particularly linked markers or the mutation within the responsible gene are known, a selection strategy can be made.

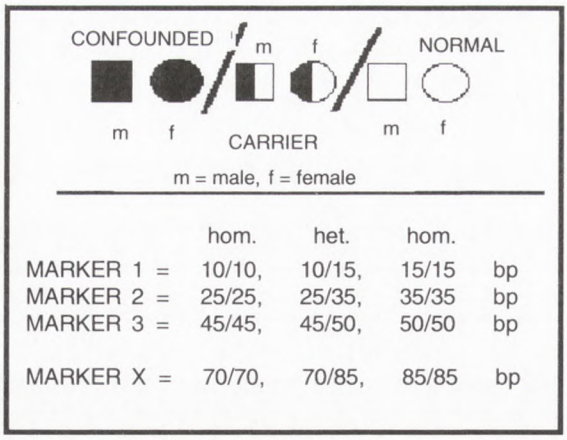
2.5. Markers used in combination with genetic engineering

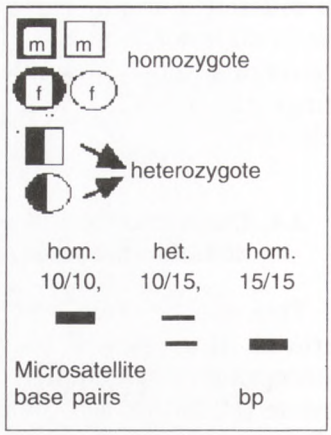
Many different techniques such as molecular genetic diagnostic tools, biotechnology such as embryotransfer and artificial insemination, *in vitro* maturation and fertilization of oocytes (Süss et al., 1988), embryo culture including embryonic stem cell culture (ESC) and homologue recombination techniques are useful tools for improving the quality of an animal. Unfortunately the ESC technique in farm animals has not yet been developed to a practical application, but convincing datas have been shown by Saito et al., (1992) in cattle, and in sheep by Campbell et al., 1995. How the ESC technique could be incorporated into a breeding plan, will be shown in Fig. 3. Since somatic cells from adult individuals can not yet be used for cloning, one has to use ESC cells from blastocysts and incorporate a testing phase for such ESC developed animals for further cloning of advantageous cases.

2.6. The diagnostic field in animal production

There are two main applications to differentiate:

First the diagnostic of markers in the animals and traits themselves. Genetic disorders, inherited diseases, genes with allelic variation, markers for parentage control and for the identification and tracking of animals (Teale et al., 1995) and plants for ecologists and wildlife managers will be analyzed. Secondly one has to consider the use of molecular genetic tests for the sale of diagnostic tools and kits for products as well as for testing diseases, poison and toxins expressed in the environment and causing death casualties or other losses with animals.





TRAIT CAN BE EXPRESSED IN RECESSIVE OR DOMINANT WAY, THIS CAN BE KNOWN BY A PEDIGREE STUDY

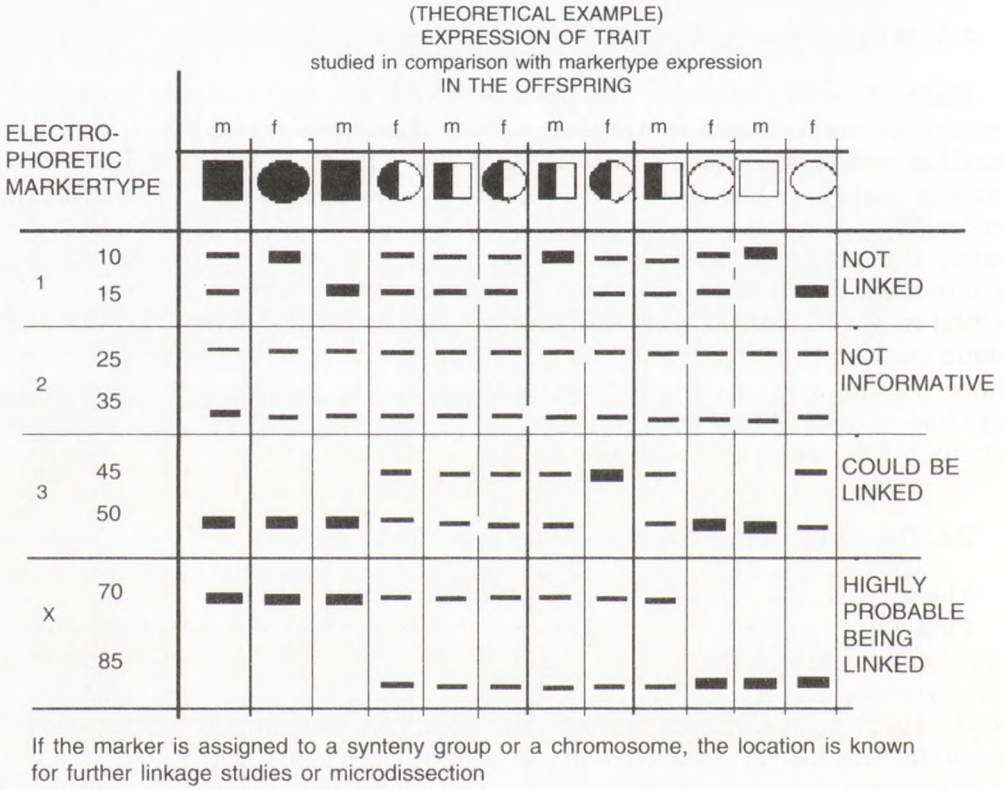


Fig. 3. Schematic demonstration of a linkage study with a family material using polymorphic molecular genetic markers.

2.7. Biotechnological applications, in combination with genetic engineering and transgenesis

Several molecular genetic techniques are used for sexing of sperm, genetic description of embryos, embryonic stem cell cultures in combination with cloning and homologue recombination for transgenic animals or knock-out experiments. In the future, the separation of X and Y chromosome bearing sperm to be able to produce the desired offsprings will be most important. For meat and milk production as well as for breeding purposes, the advantages of separated sperm are obvious. In addtion, one could also investigate sperm samples of males for estimation of the recombination frequency, using flanking markers on every chromosome.

3. Two examples as demonstration

For the sake of simplicity and for demonstration purpose, the two following figures will be shown in order to demonstrate the complex integration of molecular genetic techniques.

3.1. Linkage analysis

In Fig. 3 the linkage analysis and procedure for finding a marker or the gene for an important inherited trait are shown. As could be shown by the Zoo-FISH maps of cattle (Solinas et al., 1995) and pigs (Rettenberger et al., 1995), the homology of chromosome regions between different species are very much conserved and can be used in a wide range of extrapolations. In addition, the genome maps with type 1 and type 2 markers are very well developed and the markers are sufficient in number and distribution, that a successful linkage study for any given trait is advisable and should be successful. Most important is the polymorphism of the markers within the investigated population or family material and the evenly distributed location on the different autosomal chromosomes. For that reason the marker map has to be established further and as many informative markers as possible must be available. Microdissection techniques are now developed to further concentrate on defined regions of chromosomes to find the gene in question or closely linked markers for the recombination studies and gene detection.

3.2. Cloning application in animal production

Fig. 4 is more futuristic concerning the application of the ESC in using them for cloning of favourable animals, combining the fact that a conventional testing of the developed offspring of nuclear transfer animals have to be considered before the mass production of cloned animals can be realized. In

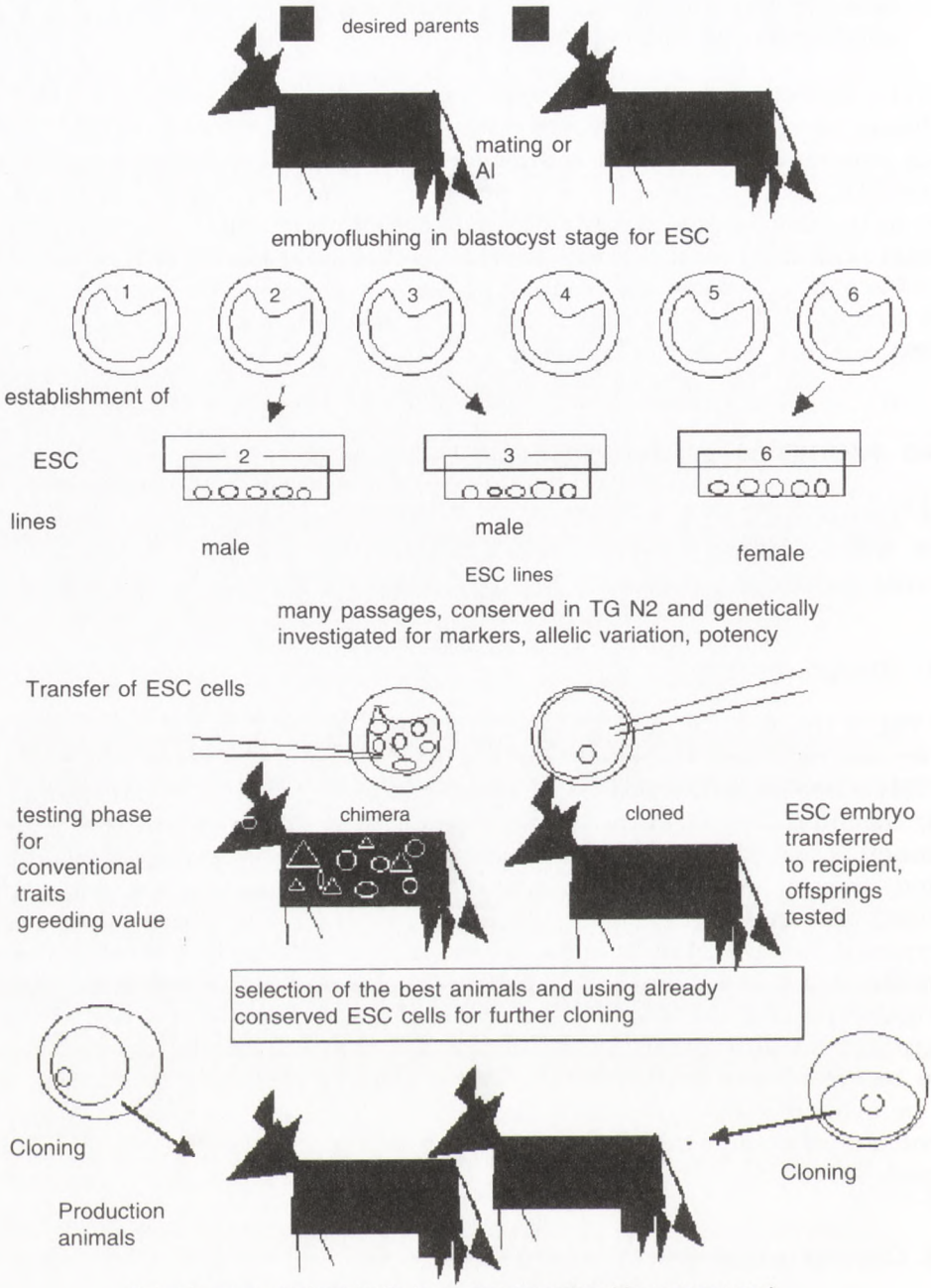


Fig. 4. Theoretical approach for the combination of biotechnology and molecular genetic diagnosis to produce cloned farm animals.

both figures, nearly in every step, the molecular genetic monitoring is necessary and without the above mentioned application not realistic.

4. Conclusion

The most likely application with molecular markers will be in genetic monitoring of animals and populations, the diagnostic of special traits in form of mutations and QTLs, contaminants, disease susceptibilities and inherited disorders. Monogenetic traits such as milk proteins, meat quality, growth pattern and disease resistance as well as inherited diseases will have priority. Secondly, the quantitative trait loci (QTLs) and allelic variation for special identified loci of interest will be used in animal production. The use of genetechnology in the way of producing transgenic animals for breeding purposes will have special limitations and might be reduced to very special cases. A more dramatic and powerful influence might have the technique of embryonic stem cells with regard to the cloning of favourable animals. This will be possible partly with molecular techniques and reproduction biotechnology applications. In general, there is a big hope that some of the mentioned new techniques will have significant influence on the future animal breeding strategies to make animal production more productive, predictable and safe for the animals and consumers. Animal welfare guidelines will become more important in combination with the intensified and powerful molecular techniques.

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Summary

The use of newly established molecular genetic informations gained by RFLPs, mini-and microsatellites, sequencing datas and the physical and genetic genemaps will have a significant influence on animal breeding strategies. Molecular pharming with farm animals is not considered as a part of animal breeding, since the aim of this production system is to concentrate on very specific products produced by very few animals under defined conditions.

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animal breeding, genemap.

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