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Importance of Molecular Markers in Livestock Improvement: A Review

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Abstract **– Molecular genetics investigates the genetic makeup of individuals at the DNA level. That includes the identification and mapping of molecular genetic markers and genetic polymorphisms. Molecular genetic markers (DNA markers) are one of the most powerful means for the genomic analysis and allow the connection of hereditary traits with genomic variation. They have proved to be more reliable than other forms of genetic markers. Molecular marker technology has developed rapidly over the last decade. Simple Sequence Repeats (SSRs), also known as microsatellites and the Single Nucleotide Polymorphisms (SNPs) are the two more common type of DNA based markers that have emerged. The properties of abundance, hypervariability and Mendelian inheritance make them very informative in the genome analysis. They have been used for a variety of purposes, including gene tagging, physical mapping, genome mapping, estimation of genetic diversity, phylogenetic and conservation genetic purposes in farm animal breeding. The ultimate use of SSRs markers is for mapping quantitative trait loci (QTL) and marker assisted selection (MAS) in order to practice genomic selection and improve the farm animal health. Genome-wide QTL scans with microsatellite markers have been used for identification of Marek's disease resistance genes. Developments in 'omics' technologies, such as genomic selection, may help overcome several of the limitations of traditional breeding programmes and will be especially beneficial in breeding for lowly heritable disease traits that only manifest themselves following exposure to pathogens or environmental stressors in adulthood.**

Keywords **– Molecular Genetic Markers, Marker Assisted S, Livestock Improvement.**

I. INTRODUCTION

Traditionally the animal improvement has relied on biometrical evaluation of breeding values from animals own performance and performance of parents, sibs and progenies. The past century was characterized by the development of quantitative genetic theory and methodologies towards the accurate selection and prediction of genetic response [1]. Although substantial genetic progress has been obtained and continue to be achieved using these quantitative genetic strategies, this approach has its own limitation because the environmental factors obscure phenotypic expression of trait there by reducing accuracy of evaluation or selection. Moreover, it is not clearly understood how many genes are actually involved in the expression of a particular quantitative trait or how each gene contributes to the trait and where it is located in the genome. Such knowledge is crucial for overall improvement of animals. Therefore, the endeavor of scientists over past few decades has been to locate and study the actual genes themselves.

Genetic marker can be defined as any stable and inherited variation that can be measured or detected by a suitable method, and can be used subsequently to detect the presence of a specific genotype or phenotype other than itself, which otherwise is non-measurable or very difficult to detect. Such variations occurring at different levels, i.e. at the morphological, chromosomal, biochemical or DNA level can serve as the genetic markers. The markers revealing variations at the DNA level are referred to as the molecular markers.

The developments in molecular biology during the past three decades have brought about revolutionary changes in the field of basic as well as applied genetics by providing several new approaches for genome analysis with greater genetic resolution. It is now possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for evaluation of the genetic basis for the observed phenotypic variability. Molecular genetics techniques allow direct genotyping of animals through molecular markers regardless of the age and sex of the animal. The expanding technologies of molecular genetics permit the investigation of variation in primary gene structure and cause animal breeders to approach selection decision in a new and improved way. Molecular marker that reveal polymorphism at gene level along with traditional selection method are now playing crucial role in selection of animals for improvement of milk and meat production at an early and in both sexes.

Molecular markers are sites where differences in DNA sequences occur among members of the same species, revealing polymorphism at the DNA level. A molecular marker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment. These markers have characteristic biological properties that can be detected and measured in parts of the body like the blood or tissue that are indicative of either normal or diseased processes in the body [2].

A molecular marker is any kind of landmark along the DNA molecules of organisms. Molecular markers are used in molecular biology and biotechnology experiments to identify a particular sequence of DNA along the chromosomes. [3]. Molecular genetic markers (DNA markers) are one of the most powerful means for the

genomic analysis and allow the connection of hereditary traits with genomic variation. They have proved to be more reliable than other forms of genetic markers [4].

II. Properties of Molecular Markers

The molecular markers, capable of detecting the genetic variation at the DNA sequence level possess unique genetic properties that make them more useful than other genetic markers. The properties of an ideal molecular marker are as follows:

- Highly polymorphic nature
- Co dominant inheritance (determination of homozygous and heterozygous status of diploid organisms)
- Frequent occurrence in genome
- Selective neutral behavior (the DNA sequences of any organism are neutral to environmental conditions or management practices)
- Easy access (availability)
- Easy and fast assay
- High reproducibility
- Easy exchange of data between laboratories

Types of Molecular Markers

Since the first demonstration of DNA-level polymorphism, known as the restriction fragment length polymorphism (RFLP), an almost unlimited number of molecular markers have accumulated. Currently, more powerful and less laborious techniques to uncover new types of DNA markers are steadily being introduced. The introduction of polymerase chain reaction (PCR) in conjunction with the constantly increasing DNA sequence data also represents a milestone in this endeavour. Various types of molecular markers utilized to evaluate DNA polymorphism in livestock are generally classified in three major groups on the basis of techniques used for their detection.

1. Hybridization-based DNA Markers

Hybridization–based marker technologies use cDNA, cloned DNA elements, or synthetic oligonucleotides as probes, which are labeled with radioisotope or with conjugated enzymes that catalyze a coloured reaction, to hybridize DNA. The DNA is either cleaved with restriction enzymes or amplified by PCR. Hybridization-based DNA markers are mainly of two types.

(i) Restriction Fragment Length Polymorphisms (RFLPs)
(ii) Oligonucleotide fingerprinting.

2. PCR-based DNA Markers

(i) Random Amplified Length Polymorphic DNAs (RAPDs)
(ii) Simple Sequence Repeats or microsatellites (SSRs),
(iii) Amplified Fragment Length Polymorphisms (AFLPs).

3. DNA Chip and Sequencing-based DNA Markers

(i) Single nucleotide Polymorphisms (SNPs)

1. Hybridization-based DNA Markers

(i) Restriction Fragment Length Polymorphisms (RFLPs):

Hybridization – based marker technologies use cDNA, cloned DNA elements, or synthetic oligonucleotides as probes, which are labeled with radioisotope or with conjugated enzymes that catalyze a coloured reaction, to hybridize DNA. The DNA is either cleaved with restriction enzymes or amplified by PCR. RFLP is representative of this type of technology and was first developed in 1980 to visualize the differences of the DNA structure based on the use of bacterial restriction enzymes that cut the DNA at sites with specific nucleotide sequences [5]. RFLPs are based on the analysis of patterns derived from a DNA sequence digested with known restriction enzymes. Differences are evident when the length of fragments are different, implying that the restriction enzyme cut the DNA at unrelated locations. Restriction polymorphism occurs when mutations remove existing restriction site or create a new restriction site. The alterations are detected by using a hybridization probe. The choice of the DNA probe is crucial in RFLP analysis.

The identification of RFLPs requires the use of gel electrophoresis to separate the DNA fragments of differing sizes followed by transfer of the fragments to a nylon membrane (Southern blot) and visualization of specific DNA sequences using radioactive or chemiluminescent probes exposed to an X-ray film [6].

Advantages

- Produces co-dominant (also known as semi-dominant) markers - this allows discrimination of homozygotic and heterozygotic states in diploid organisms.
- Stable and reproducible - gives constant results over time, and location.
- Selective neutrality.

Disadvantages

- Long methodology.
- Labour intensive.
- Requires high quality and large quantities of DNA.
- RFLPs limited the identification of the whole genome variation in animals.
- The reduced variability observed in domestic animals by inbreeding makes many RFLPs sites non-informative.

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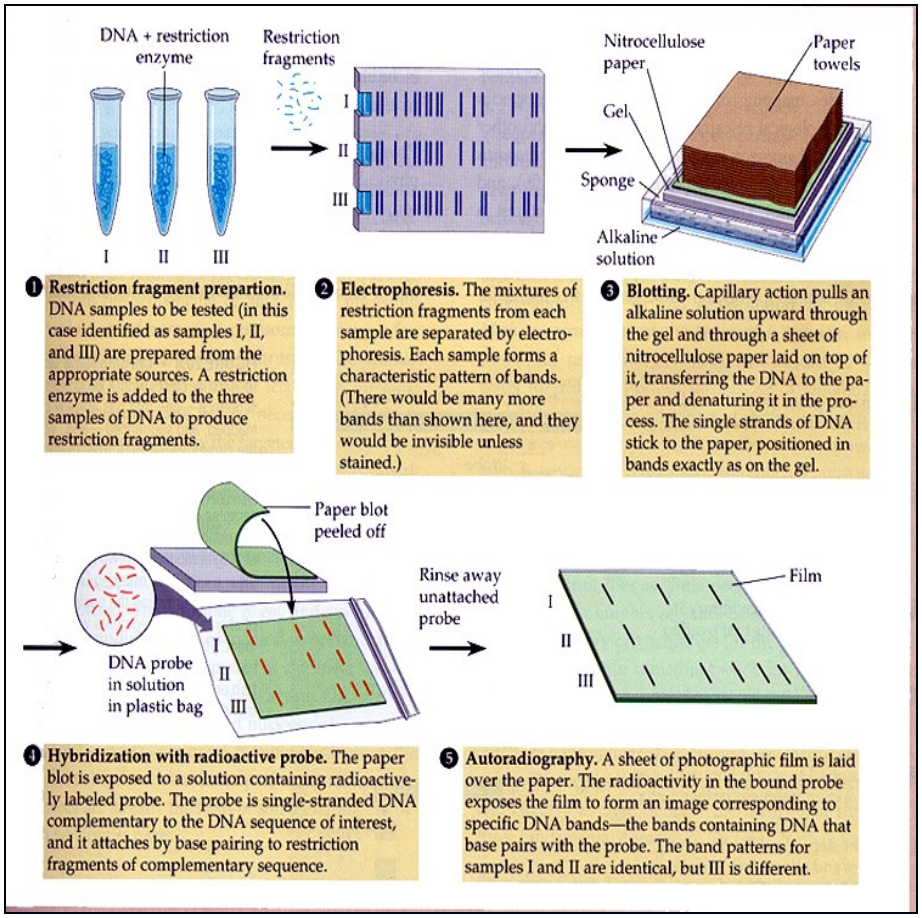

(ii) Oligonucleotide Fingerprinting

Hybridization can also be carried out with the probes (e.g. genomic or synthetic oligonucleotide) for the different families of hypervariable repetitive DNA sequences namely, minisatellite, simple repeats, variable number of tandem repeats (VNTR) and microsatellite to reveal highly polymorphic DNA fingerprinting patterns (DFP).

DNA fingerprinting is a way of identifying a specific individual, rather than simply identifying a species or some particular trait. It is also known as genetic fingerprinting or DNA profiling. As a technology, it has been around since at least 1985, when it was announced by its inventor, Sir Alec Jeffrey. DNA fingerprinting is currently used both for identifying paternity or maternity and for identifying criminals or victims. There is discussion of using DNA fingerprinting as a sort of personal identifier as well, although the viability of this is debatable. A DNA fingerprint of an individual is prepared by digesting its DNA with a restriction enzyme, subjecting the DNA digest to electrophoresis and Southern hybridization with a probe specific for a highly variable region so that a large amount of polymorphism is generated. In case of human beings, minisatellite DNAs are used as probes.DNA fingerprinting is a method for comparing the DNA sequences of any two individuals. Repetitive DNA is separated from bulk genomic DNA since it appears as a distinct peak during density gradient centrifugation.

Procedure

The DNA fingerprinting process is an elaborate one that can be conducted only in a laboratory. It involves six steps.

1. The DNA sample must be extracted, for which purpose even a tiny strand of hair or a piece of skin suffices.
2. The DNA is treated with various enzymes that work as biological sieves to cut and sort out the DNA strands according to their sizes.
3. This sample is placed on a thin nylon sheet and soaked overnight.
4. The nylon sheet is 'probed' by being exposed to radioactive radiation, which makes each strand of the DNA to stick only to certain places on the sheet.
5. Revealing a specific, unique pattern.
6. A set of 5-10 such 'probe' samples are examined together to arrive at the DNA fingerprint.

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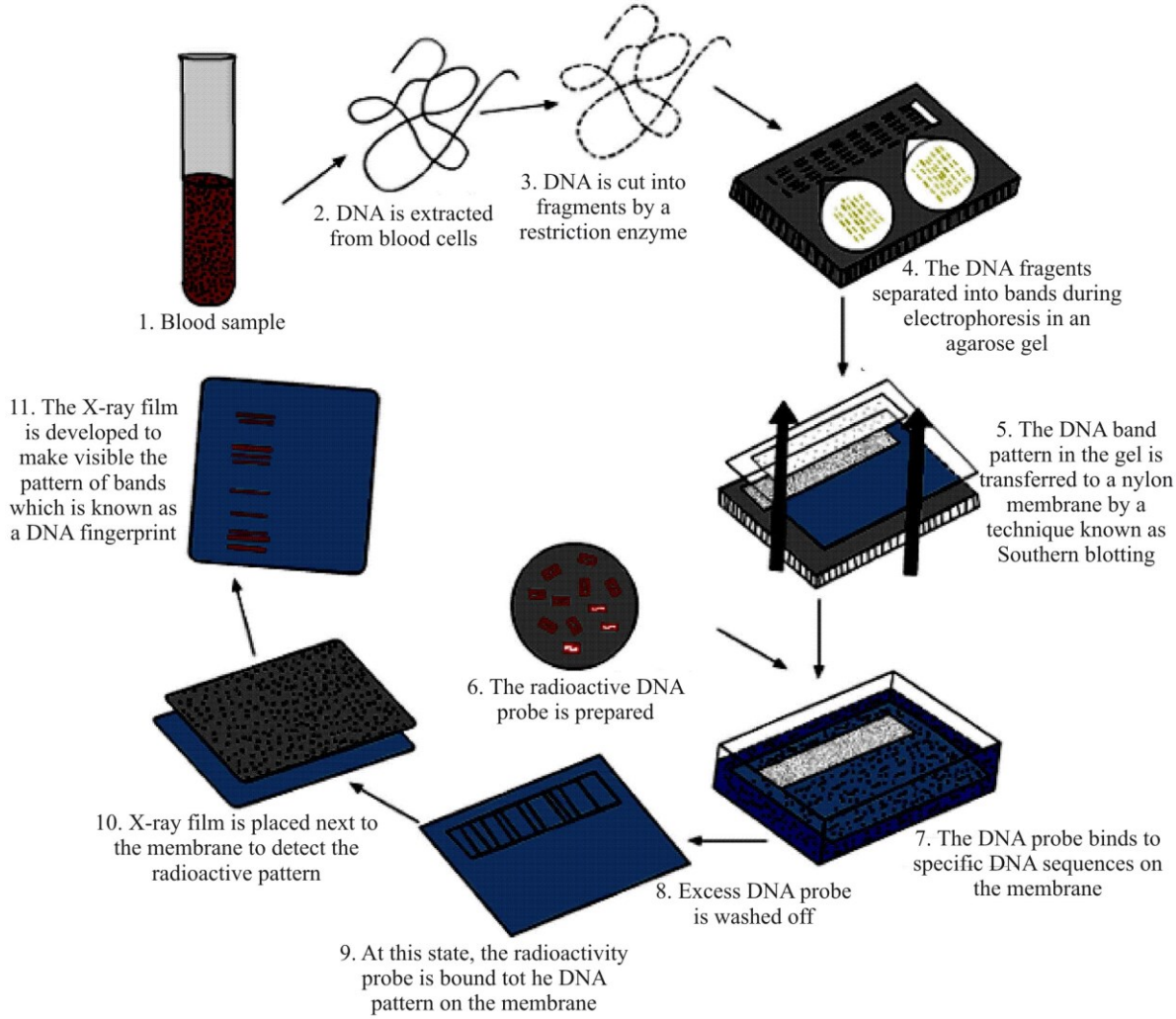


Advantages

- An advantage of DNA fingerprinting is that the results cannot be altered like fingerprints can.

Disadvantages

- The disadvantages of DNA fingerprinting it require standardization to be universally accepted as a reliable tool. Also, there aren't that many labs that can produce accurate results.

III. PCR-BASED DNA MARKERS

(i) Randomly Amplified Polymorphic DNA (RAPD):

Random Amplified Polymorphic DNA is also known as arbitrarily primed PCR (AP-PCR), or as a DNA amplification fingerprinting technique (DAF) and was described in 1990. This technique is based on the use of short, arbitrary primers in PCR reaction and can be used to produce relatively detailed and complex DNA profiles for detecting amplified fragment length polymorphisms between organisms. In the simplest format, only one short oligonucleotide, usually eight to 10 nucleotides in length, is used. Multiple primers are usually applied and a range of five to 21 nucleotides has proven successful if detection is coupled with polyacrylamide gel electrophoresis. Depending on the primer/template combination and ratios, amplified products range from less than 10 to over a 100. In this way, a spectrum of products characteristic for each template and primer combination is typically obtained and these can be adequately resolved and visualized using polyacrylamide gel electrophoresis and silver staining. Certain primers will produce unrelated patterns between unrelated animals and identical ones for very closely related animals. Presumably primer sites are randomly distributed along the target genome, and flank both conserved and highly variable regions. Wide variation in band intensity can be shown to be reproducible between experiments, which could be the result of multiple copies of the amplified regions in the template or the efficiency with which particular regions are amplified. The polymorphic bands obtained from a RAPD can also be cloned for further analysis. The polymorphisms are detected as the presence or absence of bands of a specific size [7].

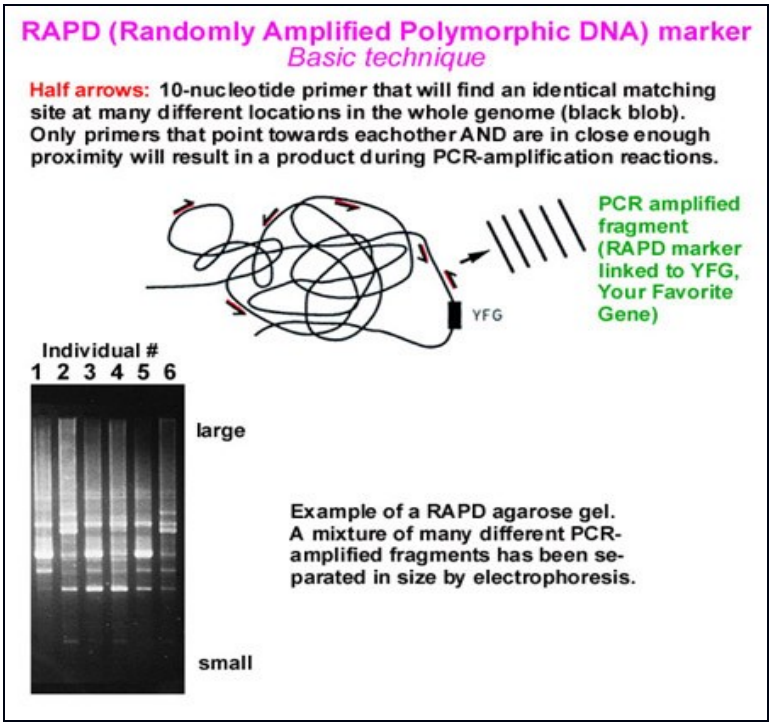
Advantages of RAPD Markers

- Cost effective.
- Simple and quick.
- Large number of bands are produced.
- No prior sequence knowledge is necessary.

- The required samples are very small because DNA will be amplified by PCR technique.

Disadvantages of RAPD

- Detection of polymorphism is limited, reproducibility of results may be inconsistent e.g. low annealing temperature may cause.
- Some unspecific non reproducible binding of primers.
- Dominant markers (homozygote cannot be discerned from heterozygote so allele frequencies cannot be estimated);
- The RAPD primers are very sensitive to PCR conditions and this may lead to poor reproducibility with respect to other methods.



(ii) Microsatellites or VNTRs

Microsatellites are the smallest and simplest repeated tandem repeat; They have core repeat units of 1-4 bases and general span less than 100bp. The tandem repeated sequences show a large distribution of the size of the repeat unit and the over length of the repeated locus. Other researchers have proposed the terms minisatellites and macrosatellites to describe regions with increasing larger repeat units and overall lengths. Together, microsatellites and minisatelites make up the variable number of tandem repeats (VNTRs). The lengths of particular microsatellite sequences tend to be highly variable among individuals. These differences make up molecular "alleles".

Microsatellites tend to be flanked by unique sequences; These unique sequences can be used to make primers for PCR. DNA can be extracted from an organism and many different microsatellites can be amplified. The amplified DNA can be run on a gel and labeled to generate a unique genetic fingerprint. Microsatellites consist of a stretch of DNA a few nucleotides long – 2 to 6 base pairs (bp) repeated several times in tandem (CACACACACACACACA) Microsatellites loci are also known as simple sequence repeats (*SSR's*), short tandem repeats (*STR's*), simple sequence tandem repeats (*SSTR*), variable number tandem repeats (*VNTR*), simple sequence length polymorphisms (*SSLP*), sequence tagged microsatellites (*STMS*). In the recent years, microsatellites are the most popular markers – marker of choice in livestock genetic characterization studies [8]. Microsatellites have been proven to be useful markers for a variety of purposes such as identification of animals, evaluation of genetic resources, parentage determination, disease research, determination of genetic variation within and among breeds, determining population substructure, reconstruction of phylogenetic relationships among populations and historical studies of domestication and migration of breeds because their high abundance in the genome, extremely high degree of polymorphism and easy detection [9]; [10]. Until recently, micro-satellites were the markers used for mapping quantitative trait loci for production and functional traits in farm animals and tightly linked markers are used for marker assisted selection in practice. In cattle microsatellite sequences were first described in 1990 and until year 2000 over than 2000 microsatellite sequences have been identified and mapped in this species. The properties of abundance, hypervariability and Mendelian inheritance make them very informative in the genome analysis [11].

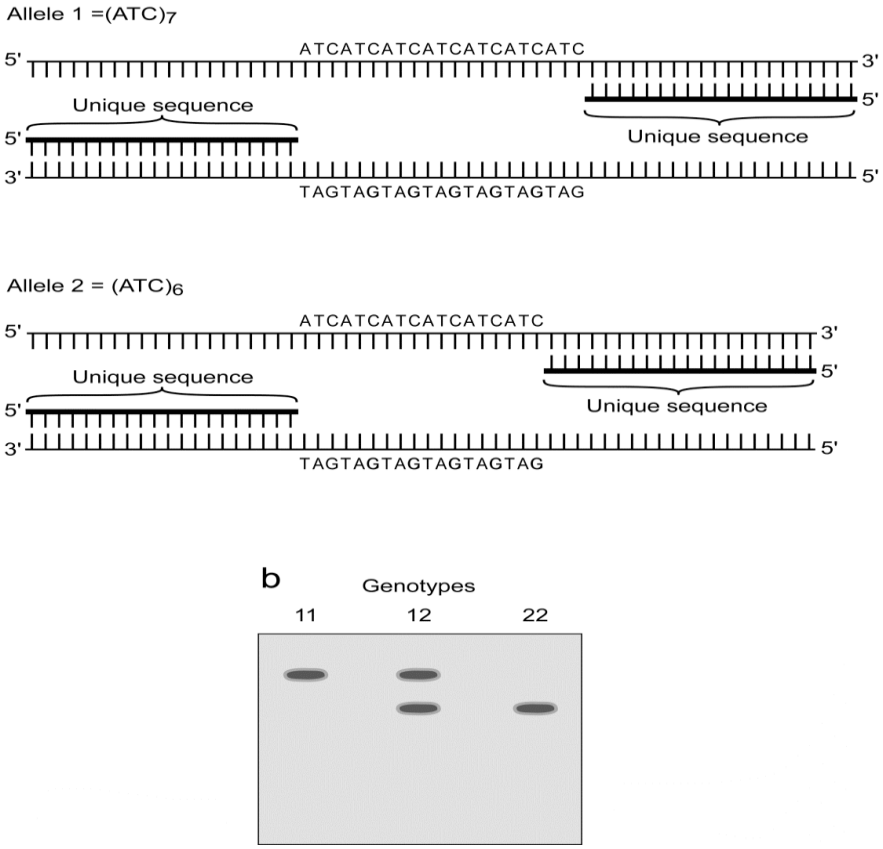


Fig. The simple sequence repeat (SSR) or microsatellite marker system

Various commercial kits are available for animal genotyping and animal parentage verifications for example StockMarks for Cattle® and StockMarks for Horse® parentage typing (http://www.appliedbiosystems.com).

Many authors show the advantages and disadvantages of SSR markers [5]; [12].

Advanatages

- Low quantities of template DNA required (10-100 ng), high genomic abundance, random distribution throughout the genome, high level of polymorphism, band profiles can be interpreted in terms of loci and alleles, codominant markers, allele sizes can be determined with high accuracy, comparison across different gels possible using size standard, high reproducibility, different microsatellites may be multiplexed in PCR, wide range of applications.

Disadvantages

- Initial high development costs, heterozygotes may be misclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites, stutter bands may complicate accurate scoring of polymorphisms, underlying mutation model (infinite alleles model or stepwise mutation model) largely unknown, homoplasy due to different forward and backward mutations may underestimate genetic divergence, time-consuming and expensive to develop, microsatellite markers help to identify neutral biodiversity but do not provide information on functional traits biodiversity.

(iii) Amplified fragment length polymorphism (AFLP)

AFLP is a common combination of RFLP and PCR techniques .DNA fingerprinting technique detects DNA restriction fragments by means of PCR amplification. Genomic DNA is first digested by appropriate restriction enzymes. AFLPs are dominant biallelic markers. Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions, in which a given mutation may be frequently present in undetermined functional genes. AFLP provides an effective, rapid and economical tool for detecting a large number of polymorphic genetic markers that are highly reliable and reproducible, and are able to be genotyped automatically. AFLP is considered as the "gold standard" for molecular epidemiological studies of pathogenic microorganisms and it is also widely used in forensic science. The AFLP technique has been used extensively to detect genetic polymorphisms, evaluate and characterize breed resources, assess the relationship between breeds, construct genetic maps and identify genes in the main livestock species - cattle, sheep, pigs [13]; [14], [15]; [16]; [17]; [18]; [19] AFLP is the ideal molecular approach for population genetics and genome typing except microsatellites.

AFLP Procedure Mainly Involves 3 Steps

(a) Restriction of DNA using a rare cutting and a commonly cutting restriction enzyme simultaneously (such as MseI and EcoRI) followed by ligation of

oligonucleotide adapters, of defined sequences including the respective restriction enzyme sites.

(b) Selective amplifications of sets of restriction fragments, using specifically designed primers. To achieve this, the 5' region of the primer is made such that it would contain both the restriction enzyme sites on either sides of the fragment complementary to the respective adapters, while the 3' ends extend for a few arbitrarily chosen nucleotides into the restriction fragments.

(c) Gel analysis of the amplified fragments.

AFLPs are extremely useful as tools for DNA fingerprinting and also for cloning and mapping of variety-specific genomic DNA sequences.

Advantages

- Fast
- Relatively inexpensive
- Highly variable

Disadvantage

- Markers are dominant
- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can't tell

IV. DNA Chip and Sequencing-based DNA Markers

(i) Single Nucleotide Polymorphisms (SNP)

Recently, DNA sequencing has allowed the discovery of single nucleotide polymorphism (SNPs). They represent one of the more interesting approaches in genotypization, because they are abundant in the genome, genetically stable and amenable to high-throughput automated analysis [7]. Single nucleotide polymorphisms (also referred to as "snip") are the most recent contribution to studying DNA sequence variation. A SNP is found where different nucleotides occur at the same position in the DNA sequence. They are found in both coding and non-coding regions of the genome and are present at one SNP in every 1000 bp [7].

They are bi-allelic markers, indicating a specific polymorphism in two alleles only of a population. SNP in coding regions can be directly associated with the protein function and as the inheritance pattern is more stable, they are more suitable markers for selection over time.

SNPs are becoming especially important as markers because they are very stable, i.e. have very low mutation rates and can be amplified with PCR for testing. Single nucleotide polymorphisms can be detected using Single Stranded Conformation Polymorphism (SSCP), Allele specific oligonucleotides (ASO), Single nucleotide polymorphic discrimination by an electronic dot blot assay, (ASO) on semiconductor microchips, Reverse dot blot on DNA chips, Dynamic allele specific hybridization (DASH), Allele-specific PCR (amplification refractory mutation system or ARMS test), Mutation detection the ARMS test in combination with the TaqmanTM 5'exonuclease assay (exploiting the 5'->3' exonuclease activity of Taq DNA polymerase), Minisequencing and analysis of the extension products by PAGE, Minisequencing and analysis of the extension products on DNA chips, Minisequencing and analysis of the extension products using matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry (MALDITOF), Pyrosequencing, OLA [5]. There are over two million of SNPs identified in cattle to date (more will be discovered) [20].

Advantage

- Detect level of variation within a species, Follow patterns of evolution, Mark genes, Distinguish alleles of "disease" genes, Create designer pharmaceuticals

Disadvantage

- The lower informational content compared with that of a highly polymorphic microsatellite, but it can be compensated by the use of a higher number of markers [21].

V. Conclusion

The genetic improvement of animals is a fundamental, incessant, and complex process. In recent years many methods have been developed and tested. The genetic polymorphism at the DNA sequence level has provided a large number of markers and revealed potential utility of application in animal breeding. Since the first demonstration of DNA-level polymorphism, known as the restriction fragment length polymorphism (RFLP), an almost unlimited number of molecular markers have accumulated. The invention of polymerase chain reaction (PCR) in accordance with the constantly increasing DNA sequence data also represents a milestone in this endeavour. The putting into practice of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application. Selection of markers for different applications are influenced by certain factors - the degree of polymorphism (PIC), the automation of the analysis, radioisotopes used, reproducibility of the technique, and the cost involved. Presently, the huge development of molecular markers will continue in the near future. It is expected that molecular markers will serve as an underlying tool to geneticists and breeders to create animals as desired and needed by the society.

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