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Popular Article

Molecular markers and their applications in livestock improvement

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Introduction

Molecular markers (also called DNA markers) are sites where differences in DNA sequences occur among members of the same species. Molecular markers have characteristic biological properties that can be detected and measured in parts of the body like the blood or tissue. A molecular marker is any kind of landmark along the DNA molecules of organisms (Deb *et al*., 2012; Ebegbulem and Ozung, 2013).

The properties of ideal molecular markers

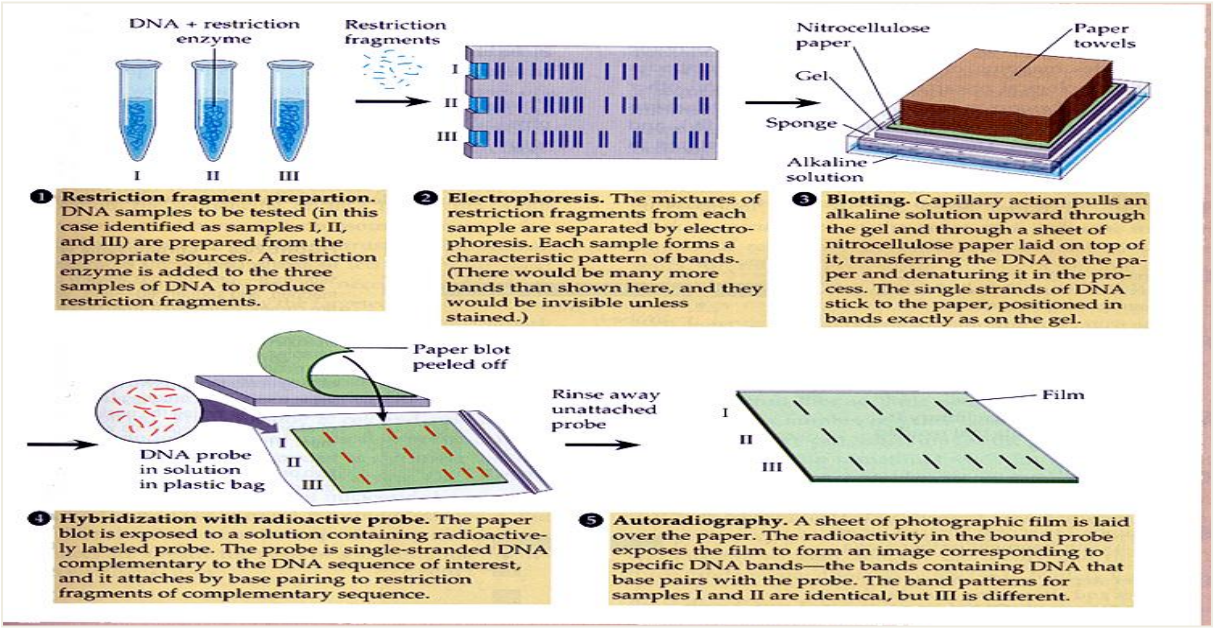
1. They should have highly polymorphic in nature.
2. They exhibit co-dominant inheritance.
3. Marker should evenly and frequently occur in genome.
4. Selective neutral behavior.
5. Their assay is easy, fast and inexpensive.
6. Easy exchange of data between laboratories.
7. Marker must show non-epistatic behavior

Types of Molecular Markers

S. No.	Base technique	Molecular marker
1.	Hybridization-based DNA markers	(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	Single Nucleotide Polymorphisms (SNPs)

1. Hybridization-based DNA markers: –

(i) Restriction Fragment Length Polymorphisms (RFLPs): - Genomic DNA digested with Restriction Enzymes. DNA fragments separated via electrophoresis and transfer to nylon membrane. Membranes exposed to probes labelled with P^{32} via southern hybridization. Film exposed to X-Ray (Ngo and Narinesing, 2007).

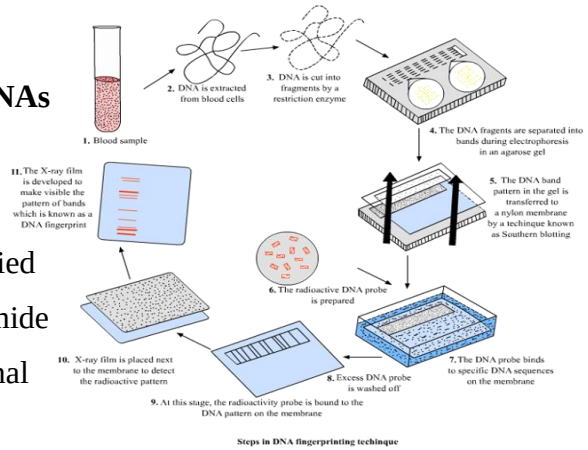


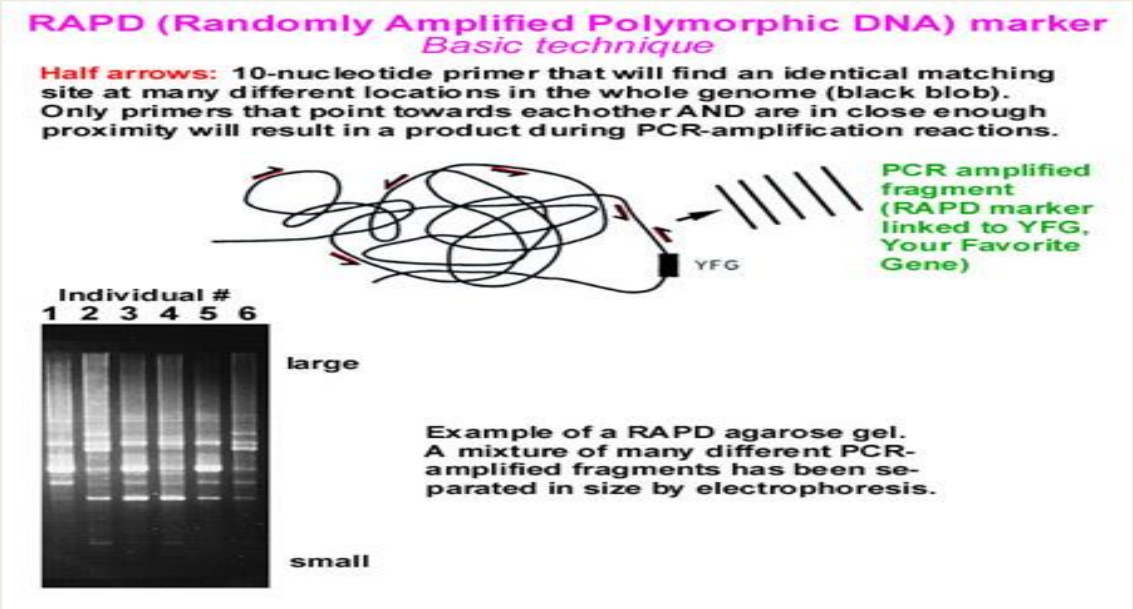
S. No.	Advantages of RFLP markers	Disadvantages of RFLP markers
1.	Produces co-dominant markers	Long methodology
2.	Stable and reproducible	Labour intensive
3.	Selective neutrality	Requires high quality and large quantities of DNA
4.		RFLPs limited the identification of the whole genome variation in animals

(ii) Oligonucleotide fingerprinting: - Also called genetic fingerprinting or DNA profiling. DNA fingerprinting is a way of identifying a specific individual. A DNA fingerprint of an individual is prepared by digesting its DNA.

2. PCR-based DNA markers: –

(i) Random Amplified Length Polymorphic DNAs (RAPDs):- Arbitrarily primed PCR (AP-PCR) or DAF used. PCR based marker with 10-12 base pairs. Random amplification of several short fragments of DNA. Amplified fragments run in agarose gel detected by ethidium bromide (EtBr). Spectrum of products resolved and visualized (Vignal *et al*., 2002).

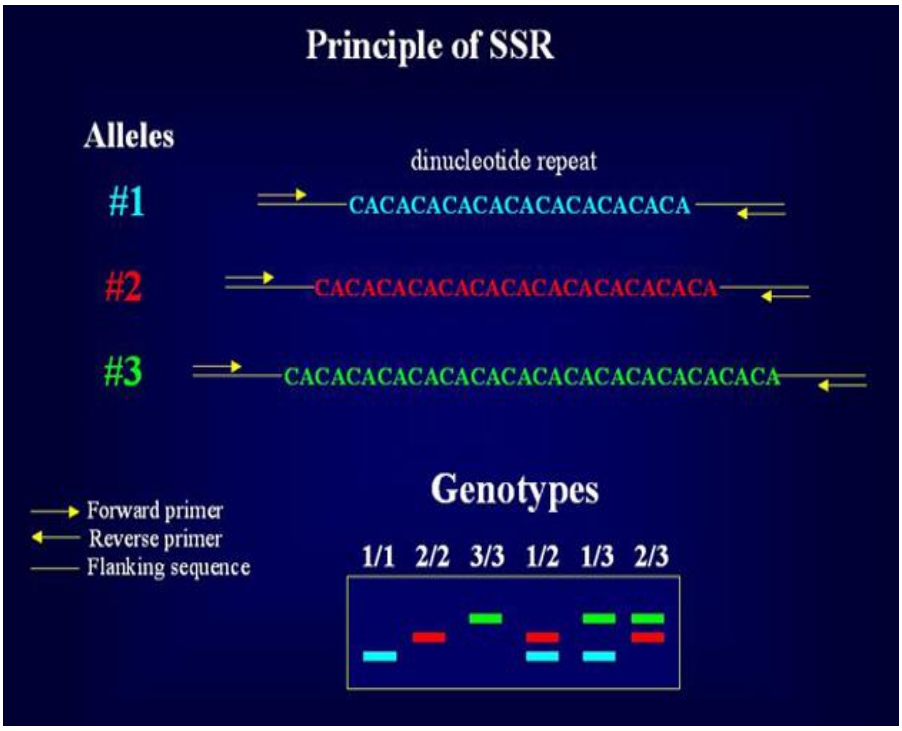




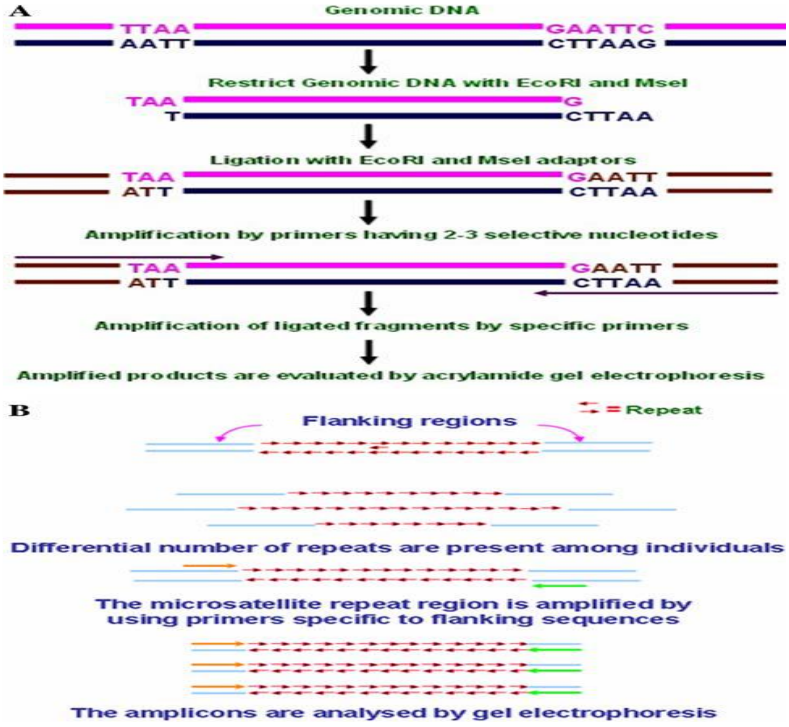
S. No.	Advantages of RAPD markers	Disadvantages of RAPD markers
1.	Cost effective	Detection of polymorphism is limited
2.	Simple and quick	Reproducibility of results may be inconsistent
3.	Large number of bands are produced	Dominant markers
4.	The required samples are very small	

(ii) Simple Sequence Repeats or microsatellites: Microsatellites also known as SSR's, STR's, SSTR, VNTR, SSLP, STMs. PCR based markers with 18-25 base pair primers. They map quantitative trait loci for production and functional traits. They are used for identification of animals, evaluation of genetic resources, parentage determination, disease research and determination of genetic variation within and among breeds (Adamov *et al*., 2011).

S. No.	Advantages of microsatellites	Disadvantages of microsatellites
1.	Low quantities of template DNA required	Initial high development costs
2.	High genomic abundance	Time-consuming and expensive to develop
3.	Random distribution throughout the genome	Microsatellite markers help to identify neutral biodiversity but do not provide infromation on functional traits biodiversity
4.	High level of polymorphism	
5.	Co-dominant markers	



(iii) Amplified Fragment Length Polymorphisms (AFLPs): - It is the restriction endonuclease digestion of DNA. Ligation of adaptors specific primers is practiced. Amplification of these ligated fragments. Separation of the amplified fragments via electrophoresis and visualization. They are considered as the "gold standard" for molecular epidemiological studies of pathogenic microorganisms (Ajmone-Marsan *et al.*, 2002).



S.No.	Advantages of AFLPs	Disadvantages of AFLPs
1.	Fast technique	Markers are dominant
2.	Relatively inexpensive	Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can't tell
3.	Highly variable	

3. DNA chip and sequencing-based DNA markers: -

(i) Single nucleotide Polymorphisms: - The "snip" are the most recent contribution to studying DNA sequence variation. SNP is found where different nucleotides occur at the same position in the DNA sequence. Single nucleotide polymorphisms can be detected using SSCP, ASO, Reverse dot blot on DNA chips, DASH (Mburu and Hanotte, 2005).

S.No.	Advantages of SNPs	Disadvantages of SNPs
1.	Detect level of variation within a species	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.
2.	Follow patterns of evolution	
3.	Mark genes	
4.	Distinguish alleles of "disease" genes	

Application of Molecular Markers

- ✓ Polymorphisms observed at the DNA sequence level have been playing a major role in animal genetics
- ✓ Gene mapping
- ✓ Pre and post natal diagnosis of genetic diseases
- ✓ Anthropological and molecular evolution studies
- ✓ Identification of animals carrying the transgenes
- ✓ DNA fingerprinting with oligoprobes (OAT18 and ONS1) has been used for determining the parentage of IVF buffalo calf
- ✓ The PCR-based RAPD fingerprinting assays are being used for characterization of zebu cattle breeds, highly inbred chicken lines and for detection of genetic variations in cattle and sheep
- ✓ DNA fingerprinting techniques and PCR-RFLP assay using sex-chromosome-specific primers, has enabled the identification of freemartin animal
- ✓ The use of multiplex PCR allows simultaneous genotyping for important loci like milk proteins, diseases carrier
- ✓ Genetic disorders caused by a single point mutation, BLAD, and DUMPS in cattle identified easily using PCR-RFLP assay
- ✓ A marker also helps in physical mapping of the genes using *in situ* hybridization
- ✓ Molecular markers are capable of unraveling genetic variations in both the coding and non-coding sequence regions

Conclusion

The genetic polymorphism at the DNA sequence level has provided a large number of markers and revealed potential utility of application in animal breeding. Selection of markers for different applications are influenced by certain factors - the degree of polymorphism, the automation of the analysis, radioisotopes used, reproducibility of the technique and the cost involved. The genetic improvement of animals is a fundamental and complex process. The putting into practice of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application. It is expected that molecular markers will serve as an underlying tool to geneticians and breeders to create animals as desired and needed by the society.

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