Lecture : 8

زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

MOLECULAR GENETICS

Subject : MOLECULAR MARKERS

Department: Animal Resources

Stage : 2

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Molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

Molecular markers are effective because they (1) identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. They can (2) identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. They (3) identify particular locations on a chromosome, allowing for physical maps to be created. Lastly they can (4) identify how many alleles an organism has for a particular trait (bi allelic or poly allelic).

Types of Markers

1. Morphological markers

Morphological markers can visually distinguish qualities. Main disadvantages of morphological markers are: they are limited in number, influenced by various environmental factors.

2. Cytological markers

Markers that are related with variations present in the numbers, banding patterns, size, shape, order and position of chromosomes are known as cytological markers. These variations reveal differences in the distributions of euchromatin and heterochromatin. For example, G bands are produced by Giemsa stain, Q bands are produced by quinacrine hydrochloride and R bands are the reversed G bands. These chromosome landmarks can be used in the differentiation of normal and mutated chromosomes.

3. Biochemical markers

Biochemical markers, or isozymes, are multi-molecular forms of enzymes which are coded by various genes, but have the same functions

4. Molecular markers/DNA markers

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Insertion, deletion, point mutations duplication and translocation are basis of these polymorphisms; however, they do not necessarily affect the activity of genes.

Classification of molecular markers

Molecular markers are classified into various groups on the basis of:

(1) Mode of gene action (co-dominant or dominant markers);

(2) Method of detection (hybridization-based molecular markers or polymerase chain reaction (PCR)- based markers);

(3) Mode of transmission (paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance or maternal nuclear inheritance)

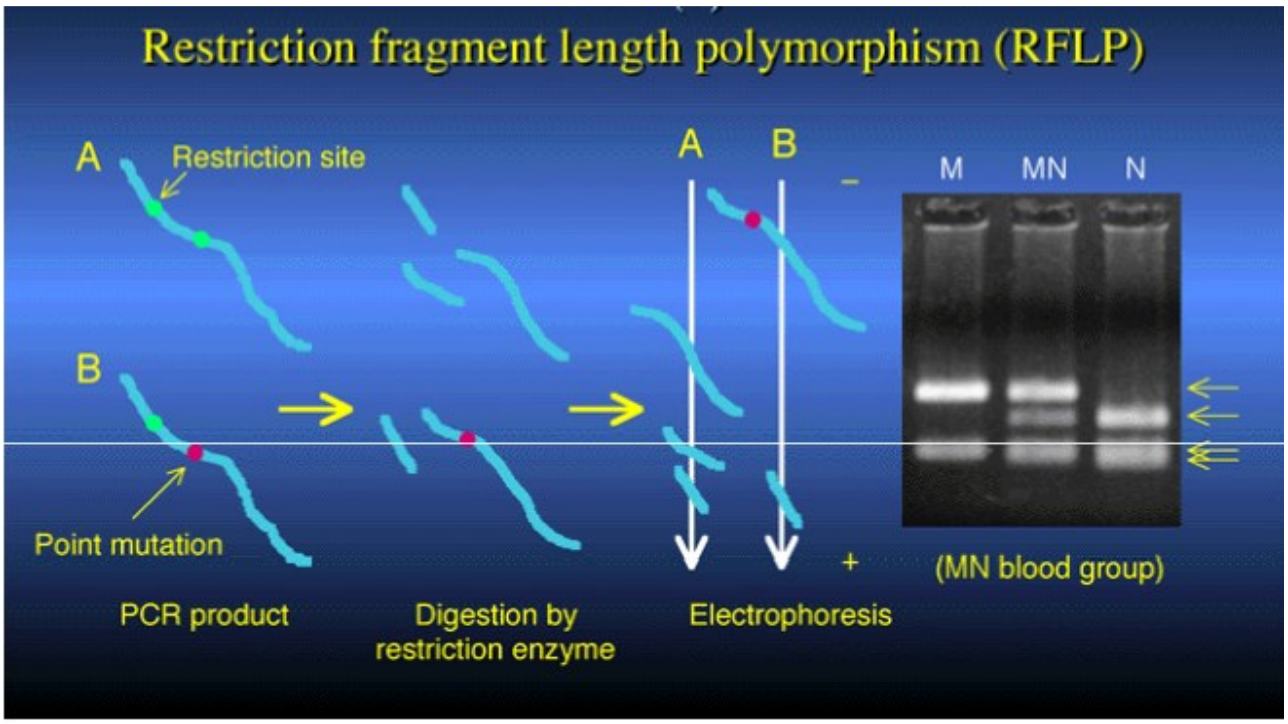
Table 1. Comparison of important characteristics of the most commonly used molecular markers.

Characteristics	RFLP	RAPD	AFLP	ISSR	SSR	SNP
Co-dominant/Dominant	Co-dominant	Dominant	Dominant	Dominant	Co-dominant	Co-dominant
Reproducibility	High	High	Intermediate	Medium–High	High	High
Polymorphism level	Medium	very high	High	High	High	High
Required DNA quality	High	High	High	Low	Low	High
Required DNA quantity	High	Medium	Low	Low	Low	Low
Marker index	Low	High	Medium	Medium	Medium	High
Genome abundance	High	Very high	Very high	Medium	Medium	Very high
Cost	High	Less	High	High	High	Variable
Sequencing	Yes	No	No	No	Yes	Yes
Status	Past	Past	Past	Present	Present	Present
PCR requirement	No	Yes	Yes	Yes	Yes	Yes
Visualization	Radioactive	Agarose gel	Agarose gel	Agarose gel	Agarose gel	SNP-VISTA

Types pf molecular marker techniques

1. RFLP

Restriction fragment length polymorphism (RFLP) is a type of polymorphism that results from variation in the DNA sequence recognized by restriction enzymes. These are bacterial enzymes used by scientists to cut DNA molecules at known locations. RFLPs (pronounced "rif lips") are used as markers on genetic maps. Typically, gel electrophoresis is used to visualize RFLPs.

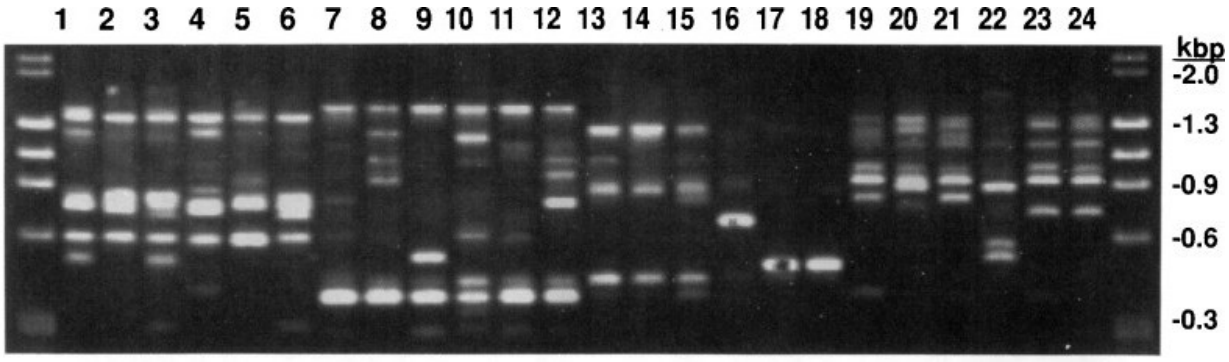


The main advantages of RFLPs include: 1) high reliability, because it is generated from specific sites via known restriction enzymes and the results are constant over time and location. 2) Co-dominance, which means investigators are able to distinguish heterozygotes from homozygotes.

2. RAPD

Randomly amplified polymorphic DNA, or RAPD, marker analysis utilizes short PCR primers consisting of random sequences usually in the size range of 8 to 15 nucleotides in length. Complex patterns of PCR products are generated as these random sequence primers anneal to various regions in an organism's

genome. RAPD suffers from poor reproducibility between laboratories largely because of the requirement of consistent PCR amplification conditions including thermal cycler ramp speeds. The complex patterns of RAPD also prevent mixture interpretation and provide challenges in consistent scoring of electrophoretic images even in single-source samples.



3. AFLPs

Amplified fragment length polymorphisms (AFLPs) provide an effective means of genotyping, particularly when little is known about the genome or genetics of an organism. Restriction enzymes cut the DNA and adaptors are attached to the ends of the fragments. Fragments are then amplified using PCR and their varying lengths can then be visualized on gel or capillary-based platforms. AFLP is very sensitive for detecting genetic polymorphisms but requires relatively large amounts of high-quality DNA and has difficulty with mixture analysis.

4. SNP

Single nucleotide polymorphisms, frequently called SNPs (pronounced “snips”), are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. (1) Single base change in DNA sequence. (2) Usually two alternative nucleotides at a single position (3) Least frequent allele present at 1% or greater