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 FORENSIC SCIENCE
 PAPER No. 12: Forensic Biology & Serology

 MODULE No. 32: Genetic Marker & its significance



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1. Learning Outcomes

After studying this module, you shall be able to know that:

- 1. About genetic marker, its types and uses.
- 2. Various techniques like RFLP,RAPD,AFLP.
- 3. Various applications of these techniques.

2. Genetic Marker & its uses

Genetic marker can be said as any visible character, or a gene or DNA sequence with a known location on chromosome.

The various uses or applications of genetic marker are as:

- □ Firstly, it is used to estimate the relationships,
- Secondly, it plays an important role to find the diseases,
- □ It is used to study the differences in the population,
- □ It is also used to describe the geographic patterns of genetic variation,
- Lastly, it is used to identify an individual or species in population.

3. Types of genetic marker

- \Box Now, let us learn about the types of genetic markers.
- □ The modern genetics shows two types of genetic marker:
- □ First is Biochemical Marker &
- □ Second is Molecular Marker

4. Biochemical marker

- □ The biochemical marker detects the changes or variation at the gene level such as amino acids.
- □ Biochemical marker can further be classified as: Allozymes & Isozymes.

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5. Allozymes & Isozymes

- □ Allozymes can be defined as an allelic form of an enzyme which can be distinguished by electrophoresis.
- \Box Also it is an enzyme that are coded by different alleles at the same locus.
- □ Isozymes can be identified as an enzyme that appear in two or more structural form but these can be identified and separated from each other by their activities.

6. Molecular marker

- Molecular marker detect the changes or variation at the DNA level such as the changes in nucleotide.
- □ Molecular marker consists of DNA marker which is categorized as:
- 1) Hybridization molecular based marker
- 2) PCR molecular based markers

7. Hybridisation molecular based marker

- □ The hybridization molecular based marker examines the differences in size of DNA fragments for which it requires high molecular weight DNA & probe, and is usually performed on cellular genome.
- □ The two techniques which comes under hybridization molecular based markers are:
- 1) DNA/DNA hybridization &
- 2) RFLP techniques

8. DNA/DNA hybridisation

- □ In actual hybridization the complementary single stranded DNA or RNA molecules combine together to form a single double stranded molecule through complementary base pairing.
- □ This process of hybridization is used in molecular biology laboratory to perform techniques like Southern blotting & PCR.



DNA/DNA hybridization technique is usually used to determine the genetic distance between two species.



9. RFLP

- RFLP is a technique in which DNA molecule is broken into pieces by restriction enzymes & then these pieces or fragments of DNA are separated by their length using agarose gel electrophoresis.
- Electrophoresis separate DNA by their size, then it is transferred to nylon membrane via Southern blot procedure.

Next hybridization takes place where radiolabelled DNA probes are made which hybridize with specific DNA sequences in the membrane which determines the length of the fragments which are complementary to the probe.

- □ An RFLP occurs when the length of detected fragment varies between individuals.
- **RFLP** technique is expensive & time consuming method.
- □ RFLP technique is a vital tool in determining genetic disease, gene mapping & most important to solve the paternity disputes.



10.PCR based marker

- First of let us learn what is PCR? It is a polymerase chain reaction in which double stranded DNA molecule is first denatured to single stranded DNA molecule, then annealing process occurs where primers are added, the complementary primers binds to the DNA strand, lastly extension of DNA strand takes place from where the single DNA molecule having two copies of double stranded DNA molecule exists. PCR is a powerful technique for amplifying DNA.
- □ The various PCR based markers which will be discussed are:
- 1) SNP's
- 2) SSR
- 3) AFLP
- 4) RAPD

11. SNP

- □ SNP is a DNA sequence variation.
- Any two unrelated individual differs; it occurs when changes occurs in nucleotide composition.
- Here in figure we can see that there are two DNA molecules with nucleotide composition, both DNA molecule differs at a single nucleotide composition that is C/G & A/T location, that's why it is said as single nucleotide polymorphism

12. SSR (Simple sequence repeats)

- □ SSR is simple sequence repeats which is often termed as microsatellites & STR that is short tandem repeats.
- □ It is a sequence where 2-6 base pairs of nucleotide are repeated.
- □ These repeat units are generally di, tri, tetra or penta nucleotides.
- □ One common example of microsatellite is (CA)n repeat where C and A are nucleotide which is repeated variable number of times.
- □ The variability of microsatellite is due to higher rate of mutation.



13. AFLP

- □ AFLP is the most complex of marker technologies, it is a PCR based tool used in genetics research & DNA fingerprinting.
- □ The procedure of AFLP technique is as follows:
- □ It involves digestion of DNA with restriction enzymes, followed by ligation of adaptors to the sticky end of restriction fragments.
- □ Selected fragments is then amplified using two PCR primers.
- □ The amplified primers are then separated & visualised using gel matrix.
- \Box AFLP is used in:
- □ Molecular phylogeny studies,
- □ Creation of genetic maps,
- □ Used in criminal & paternity tests to determine slight differences within populations.
- Establishment of linkage groups in crosses.

14. RAPD (Random amplified polymorphic DNA)

- □ RAPD (Random amplified Polymorphic DNA) is a type of PCR reaction but the segments of DNA amplified are random.
- DNA markers are developed by using random sequence of specific markers through the use of random primers.
- **RAPD** is inexpensive, fast and easy method for detecting polymorphisms.
- Application of RAPD are as follows:
- □ It is used in genetic mapping,
- **T**o develop genetic markers linked to a trait in question,
- □ To study population & evolutionary genetics.



15. Genetic marker characteristics

Characteristics	Morphological Markers	Protein Markers	RFLP Markers	RAPD Markers	SSR markers
No. of loci	Limited	Limited	Unlimited	Unlimited	High
Inheritance	Dominant	Co-dominant	Co-dominant	Dominant	Co-dominant
Positive features	Visible	Easy to detect	Utilized before the latest technologies available	Quick assays	Well distributed
Negative features	Possibly negative	Possibly tissue specific	Expensive	High basic investment	Expensive
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16. Forensic significance

- ✓ In forensic science hybridization probes are used for the detection of short tandem repeats & restriction fragment length polymorphism are used in DNA profiling of an individual.
- ✓ To solve paternity disputes.
- ✓ Genetic markers are useful in gene mapping.
- ✓ Also helps in the characterization of genetic diversity or breeding patterns in animals.

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17.Summary

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- > The modern genetics show two types of genetic marker:
- □ First is Biochemical Marker &
- □ Second is Molecular Marker.
- The biochemical marker detect the changes or variation at the gene level such as amino acids.
- Biochemical marker can further be classified as: Allozymes & Isozymes.
- > Molecular marker detect the changes or variation at their DNA level such as the changes in nucleotide.
- □ Molecular marker consists of DNA marker which is categorized as :
- 1) Hybridization molecular based marker
- 2) PCR molecular based markers
- > The two techniques which comes under hybridization molecular based markers are:
- 1) DNA/DNA hybridization &
- 2) **RFLP** techniques



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- □ To solve paternity disputes.
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