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Molecular Markers: Tools Revolutionizing Modern Genetics

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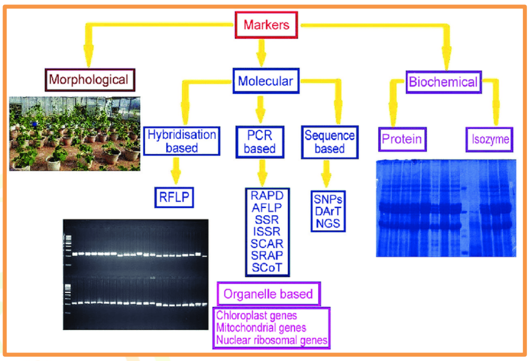
A molecular marker is a DNA sequence that can be identified at a specific location on a chromosome, making it useful for chromosome mapping.

- A molecular marker is a genetic marker
- A molecular marker is a DNA or gene sequence located at a known chromosomal position, used as an identification tool.
- In a pool of unknown DNA or an entire chromosome, molecular markers assist in identifying specific DNA sequences at defined locations.



Applications

- It is essential in gene mapping by determining the locations of related genes on the chromosome that are inherited together.
- It also finds any genetic anomaly or change in a DNA sequence. It determines which genes are implicated in genetic illnesses.
- It is used to identify distinct features in a DNA sequence that are utilized to differentiate across species, populations, or individuals.
- Molecular markers are distinguished by methods like southern hybridization and PCR and have special genetic characteristics (i.e., they are heritable DNA sequences and phenotypically neutral).

PCR based genetic markers: RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat), STR (Single Tendem Repeats), VNTR (Variable Number Tendem Repeat), STS (Sequence Tag Size), SNP (Single Nucleotide Polymorphism), EST (Expressed Sequence Tagged)

Hybridization based genetic markers: RFLP (Restriction Fragment Length Polymorphism)

Molecular markers also can be classified as

- 1st generation markers (at the time of 1980-1990): RFLP, RAPD
- 2nd generation markers (1990-2000): AFLP, SSR, STR, VNTR, STS
- 3rd generation markers (After 2000): SNP, EST

Quality for a good genetic marker

Molecular marker must possess the following desirable properties-

- It needs to be polymorphic in order to measure diversity.
- It ought to be dispersed uniformly across the genome.
- It ought to be quickly and easily identified.
- It needs to differentiate between heterozygotes and homozygotes.

Random Amplified Polymorphic DNA (RAPD)

- Since it is based on the PCR assay, no prior DNA sequencing is necessary. This process amplifies the DNA region at random using a short, arbitrary primer that is 8–12 bp long. Both forward and reverse primer functions are provided by this primer.
- When a single primer anneals to the genomic DNA at two different locations on the complementary strand of the DNA template, this reaction is initiated. The sites complementary to the primers' sequence determine whether a DNA fragment is amplified.
- The RAPD fragments range in size from 0.2 to 5.0 kb and can be seen using polyacrylamide gel electrophoresis or agarose gel electrophoresis stained with ethidium bromide. A unique pattern of amplified DNA segments will appear on the gel if there is a mutation in the primer binding site, which will prevent the production of any PCR product.

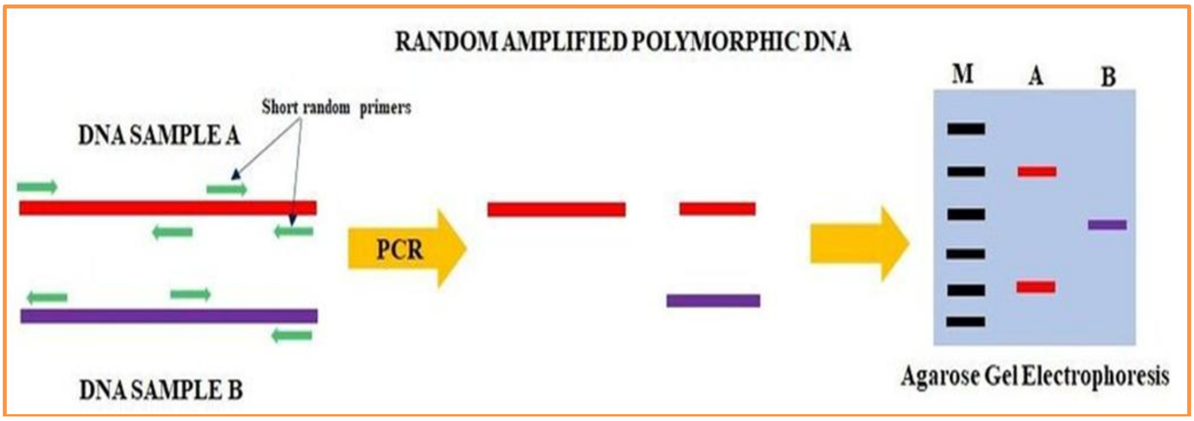


Fig 1: Basic procedure of Random Amplified Polymorphic DNA (RAPD) technique

Application:

- Various samples exhibit distinct amplification patterns. For this reason, polymorphism can be studied using RAPD.
- RAPID can be used for individual-specific genotyping, linkage analysis, and genomic mapping.
- RAPID markers have limitations for mapping purposes because they are dominating in nature.
- RAPID necessitates sensitivity because it is solely laboratory dependant.

Demerits: Its drawbacks include low repeatability, hazy results, and issues with band scoring

Restriction Fragment Length Polymorphism (RFLP)

It is a hybridization-based approach that was among the first to be utilized for DNA analysis in a variety of domains, including forensic science. Alec Jeffreys, an English scientist, created it in 1984 while studying geneticillnesses. The restriction endonuclease enzymes used in RFLP cut at a specified

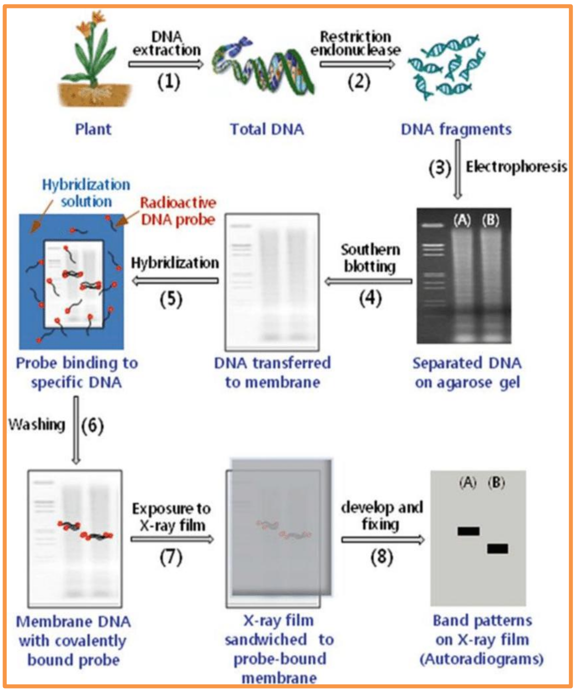


Fig 2: Restriction fragment length polymorphism (RFLP) procedure.

location, producing fragments of different lengths in addition to the fragment of interest. Blotting, which has now been supplanted by sequencing, is used to measure the length of the discrete pieces. Because restriction endonuclease is utilized, RFLP markers are co-dominant and primarily locus-specific.

Applications:

- One of the earliest methods used for genetic fingerprinting or profiling was RFLP, which is utilized for heterozygous detection, genetic mapping, and the identification of inherited disorders and their carriers.
- According to the RFLP's molecular foundation, any point mutations in the genome, such as deletions, substitutions, and insertions, or changes like duplications or inversions, might create new restriction sites or remove existing ones.
- By examining pieces of varying length that have been digested by the restriction endonuclease enzyme, these changes in the genome can be found.

Demerits: Need a comparatively big DNA sample, a time-consuming and difficult technique, sensitivity, and additional contamination prevention measures.

Amplified Fragment Length Polymorphism (AFLP)

The AFLP approach was created in 1993 by Zabeau and Vos. KeyGene was the original developer of AFLP in 1990. It is a fingerprinting method based on PCR. Both PCR and RFLP are included. The amplification of specific segments and restriction digestion of the entire genomic DNA of a particular organism form the basis of AFLP.

The steps for the AFLP are as follows: DNA is extracted, digested by restriction, and then ligated using short adaptor sequences. PCR amplification of limited fragments Examination of gel electrophoresis or PAGE findings, followed by autoradiography.

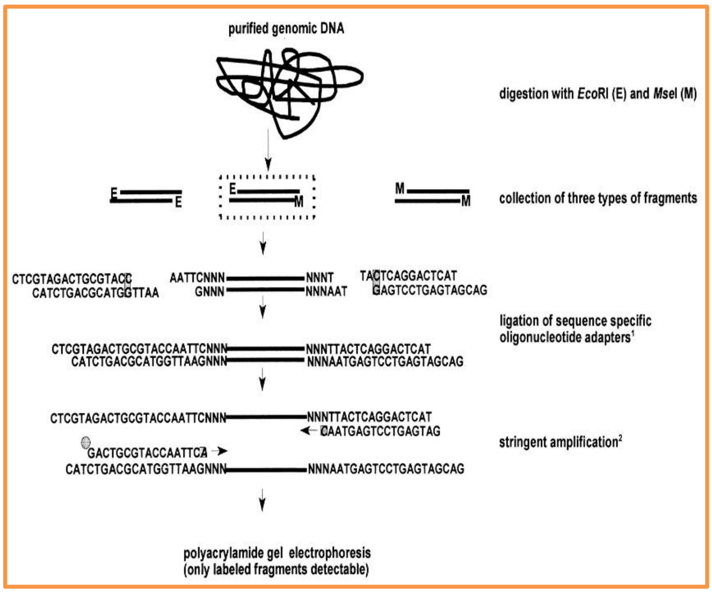


Fig. 3: Schematic representation of the AFLP analysis

Applications: AFLP can quickly create marker fragments for any organism without requiring DNA sequencing beforehand. Additionally, compared to RAPD and ISSR (inter-simple sequence repeats), it requires only little pieces of initial template DNA and is far more reproducible. Crop enhancement initiatives, parentage, and genomic interpretation of different crop species are the main applications of AFLP.

Demerits: AFLP require large DNA samples and require purification

Inter Simple Sequence Repeat (ISSR) markers

PCR is the foundation of the inter simple sequence repeat (ISSR) technology. In 1994, Ztetikiewicz et al. reported about it. Using a single primer made up of microsatellite core sections, DNA segments between two similar microsatellite repeat areas are amplified by PCR to create the ISSR markers. Primers typically range in length from 16 to 25 bp and can be either detached or attached at the 3′ or 5′ end.

Applications: Because of its larger primer length, it offers higher repeatability than RAPD and is just as easy, quick, and affordable. ISSR is a highly polymorphic marker that may be analyzed without prior knowledge of the genome. ISSRs are employed in phylogenetic analysis, genetic diversity identification, cultivar proximity detection, and somaclonal variation determination in plants. ISSR can be used to examine gene mapping, gene tagging, distinct strain identification, and parental recognition because of its straightforward setup.

Demerits: Because of its multilocus nature, ISSR is less reproducible and does not have homology of identically sized pieces.

Microsatellites or simple sequence length polymorphisms (SSLPs)

Jeffrey et al. coined the term "microsatellite" in 1985. Simple sequence repeating (SSR) loci, often known as microsatellites, are PCR-based markers that need prior knowledge of the gene sequence. It is known in the literature as sequence tagged microsatellites (STMS), simple sequence length polymorphisms (SSLPs), or variable number of tandem repeats (VNTRs). They are scattered throughout prokaryotic and eukaryotic nuclear genomes to varying degrees. Microsatellite primers are simple sequence repeats (SSRs) or short tandem repeats (STRs), which are sequences of one to six base pairs that are repeated several times. Microsatellites are typically produced less than 100 times. By building a small-insert genomic library, screening the library, and sequencing the positive clones, microsatellites can be identified. Microsatellites serve as markers for research on population genetics, gene mapping, and species proximity. Tandem array amplification and gel visualization aid in the detection of DNA length variation. Slipped strand mispairing, or polymerase reduction during DNA replication, is the primary source of the variance in DNA length.

Applications: Co-dominance of alleles characterizes microsatellite, which uses minimal amounts of DNA templates. It is highly reproducible and cost-effective. It is possible to automate the screening of microsatellite variation. Cons: If there aren't enough primer sequences for the species of interest, the assay can be expensive. If changes are observed in the primer annealing sites, the genotype score is incorrect. chances of homoplasy (some characters are present in more than one.

Expressed Sequence Tags (ESTs)

Random cDNA clones are partially sequenced to create ESTs, which are molecular markers. Two types of ESTs are produced by sequencing a few hundred nucleotides (500–800 bp) from either the 3’ or 5’ ends of cDNA after it has been generated. A 5′ EST, which often codes for a protein, is produced when the 5' end of cDNA is sequenced. Within a gene family, these areas seldom alter and are typically preserved across species. Untranslated regions (UTR), or the 3′ EST, are non-coding and less conserved across species when the 3' end of cDNA is sequenced. As a result, ESTs are a cDNA subsequence that serves as a tag for the full cDNA.

Applications: Whole genome sequencing and gene of interest research are done using EST. It is also utilized for gene mapping and gene cloning of interest.

Single nucleotide polymorphism (SNP)

Lander came up with SNP in 1996. Any change or mutation in a single nucleotide (A, T, C, or G) results in the formation of an SNP. SNP is represented by point mutations, which are substitutions, insertions or deletions in a single nucleotide. SNPs are based on the hybridization of identified DNA fragments using SNP chips, which are arrays of DNA probes. The hybridization results are used to name the SNP allele.

Applications: In biomedical research, SNPs are frequently utilized to compare illness case and control groups. Additionally, it is employed in the research of genetic variation, phylogenetics, etc.

Demerits: Since the amount of information gathered is less than that of microsatellites, a significant number of markers and full genome sequencing are required.

Conclusion

In order to address global issues like environmental sustainability, water shortage, and food security, plant breeding is essential. Crop improvement has been transformed by molecular markers, which provide precise, repeatable instruments for assessing genetic variation. These markers are crucial to contemporary plant breeding efforts because they help identify important genes, comprehend complex features, and find novel genetic variations. Molecular markers play a crucial role in crop development by supporting marker-assisted breeding, parental selection, cultivar identification, and genetic diversity study. They encourage the creation of hardy, high-yield crops to help feed the world's expanding population.

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