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Paper Authors
Dr Dhirendra Kumar, Aadya Jha, Sunita Patnaik, M.Krishnaveni

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# Application of Molecular Markers in Biotechnology

[1]**Dr Dhirendra Kumar**
Department of Botany, Chaudhry Bansi Lal University Bhiwani, Haryana,
dhirencblu@gmail.com
[2]**Aadya Jha**
Research Scholar, Department of Botany, University of Kalyani, West Bengal, India.
jhaaadya5@gmail.com
[3]**Sunita Patnaik**
Asst. Professor, Roland Institute of Pharmaceutical Sciences, Berhampur
sunita.patnaik01@gmail.com
[4]**M.Krishnaveni**
Assistant Professor in Biochemistry, Nadar Saraswathi College of Arts & Science, Theni, Tamilnadu, India.
venibio87@gmail.com

**Abstract**

Plant biotechnology and genetics have increasingly used molecular markers to detect DNA-level variation. Genetic investigations use morphological, biochemical, and DNA-based molecular markers. DNA-based markers are either PCR-based (RAPD, AFLP, SSR, SNP, etc.) or non-PCR-based (RFLP). Due to their simplicity, microsatellite DNA markers have become widespread. PCR amplification and denaturing gel electrophoresis determine allele size. Due to their high allele count, microsatellite markers are rich in information. SNP markers, although being bi-allelic, have gained attention. SNP markers' abundance and genome coverage have transformed genetic investigations. SNP markers are cost-effective, high-throughput, and compatible with several genotyping technologies. These markers are essential for studying genetic variability and diversity in plants. This thorough overview covers biochemical and molecular markers used in plant genetics investigations. We discuss marker type pros and cons and their uses. We compare markers to understand their advantages and applicability for diverse study situations. We can better comprehend genetic variability and variety in plant species thanks to the fast development of innovative and specialised markers.

**Keywords:** biochemical markers, molecular markers, genetic research, polymorphism, microsatellite markers, SNP markers, genotyping, plant biotechnology, genomic variability, genetic diversity.

## Introduction

DNA markers, also known as DNA methylation markers or signatures, are DNA methylation patterns that may indicate biological processes, illnesses, or situations. DNA methylation, an epigenetic alteration, adds methyl groups to cytosine residues in CpG dinucleotides. Bisulfite sequencing or array-based genome-wide DNA methylation profiling approaches identify DNA markers. DNA markers may illuminate gene expression regulation, cellular differentiation, aging, and disease. They are helpful in personalized medicine for diagnosing, prognosing, and predicting. Researchers and physicians may use DNA markers to detect illness risk, track disease development, predict therapy response, and guide treatment.

DNA markers are identified and validated by comparing DNA methylation patterns across groups or situations, such as healthy people vs patients, various illness stages, or therapy responders against non-responders. Differentially methylated regions or sites are identified using statistical and bioinformatics methods. DNA markers may improve early identification, understanding, and tailored therapy of complicated illnesses. Cancer, neurological, cardiovascular, and developmental illnesses have been intensively explored. DNA markers may become more significant in precision medicine and patient-specific therapeutics as epigenetics advances[1].

## Biomarkers

### Allozymes

Allozymes are biochemical indicators for enzyme variations encoded by distinct gene alleles. During gel electrophoresis, these variations' electrophoretic mobility distinguishes them. Allozymes give genetic variation data for population genetics and evolutionary biology investigations. Researchers may learn about population genetic organization, diversity, and evolution by examining allozymes in individuals. Extracting enzymes from tissues or cells, separating them by gel electrophoresis, then labelling them to identify allozymes. The gel shows allozyme bands for each gene's alleles. Researchers estimate allele frequencies and genetic differentiation by comparing allozyme patternsv[2].

Allozyme analysis has studied plants, animals, and microbes. It illuminated population genetics, evolutionary history, mating systems, and gene flow.

Conservation biology uses allozymes to evaluate endangered species' genetic diversity, monitor conservation efforts, and influence management options. DNA sequencing has mostly supplanted allozymes as markers. Allozymes lack resolution, precision, and simplicity of analysis compared to DNA-based markers like microsatellites, SNPs, and GBS. Allozyme analysis has helped us comprehend genetic variation and evolution.

**Molecular markers**

Molecular markers are genetically variable DNA sequences that can be readily discovered and tracked. These markers use naturally existing DNA polymorphism to build solutions for varied applications. A marker must be polymorphic to identify mutant genes from normal genes on chromosomes. Genetic polymorphism occurs when a population has two or more genotypes or trait variations. Polymorphic DNA markers help evaluate and choose plant resources. DNA markers, unlike protein markers, segregate as single genes and are unaffected by environmental variables. DNA extraction from plant materials is simple, and DNA marker analysis is cost-effective and labor-efficient. RFLP markers, which analysed restriction digestion fragments, were among the first DNA markers. Since then, other marker systems have expanded the breadth of DNA markers for diverse uses. These molecular markers transformed plant breeding, genetic mapping, genetic diversity analyses, and other agricultural and biological studies. They let researchers locate and monitor genes or genomic areas of interest, measure genetic diversity within populations, study trait inheritance, and choose desirable characteristics in breeding programs. DNA markers have been used in plant genetics and other domains for studying and managing genetic variation[3]

**DNA marker**

Finding a molecular marker that fits all requirements is difficult. Many molecular methods may identify DNA polymorphism. Depending on the approach, these markers are hybridization-based or PCR-based. Hybridization-based markers visualize DNA profiles by hybridizing restriction enzyme-digested DNA with a tagged probe, a known DNA fragment. This approach detects DNA sequences of interest. Hybridization patterns reveal DNA polymorphism.PCR-based markers amplify particular DNA sequences or locations. This method employs heat-stable DNA polymerase and primers. Target DNA fragment

amplification detects polymorphisms. Electrophoresis separates amplified fragments, and staining or autoradiography shows banding patterns.

Since the mid-1980s, PCR has been revolutionary. Heat-stable DNA polymerase improved its usefulness in 1988. Research and clinical labs use versatile PCR-based markers. Primer sequences enable focused DNA amplification by attaching to template DNA. Different marker systems may meet certain research needs. The study's goals, resources, and results determine the marker system[4].

**Restriction fragment length polymorphism (RFLP)**

RFLP molecularly detects genetic variation in DNA samples. It analyses restriction enzyme-cleaved DNA patterns. Genetics, genomics, and other biological sciences employ RFLP to research genetic diversity, population genetics, and genetic mapping.

**DNA Digestion:** The organism's genomic DNA is extracted and digested using restriction enzymes. These enzymes break DNA at their recognition sites to produce DNA pieces of various lengths.

**Gel electrophoresis**: separates digested DNA fragments by size. DNA fragments are electrophoresed on an agarose gel. Larger particles travel slowly through the gel.

**Southern Blotting:** After electrophoresis, Southern blotting transfers DNA fragments from the gel to a solid substrate, usually nitrocellulose or nylon. This transfer immobilizes DNA fragments in their original gel locations.

**Probe Hybridization:** A tagged probe hybridizes the membrane with the transferred DNA fragments. A target sequence-complementary DNA fragment is the probe. The probe forms stable DNA-DNA hybrids with its corresponding membrane sequence.

**Detection:** Different labelling techniques detect the tagged probe. Autoradiography may see tagged DNA fragments if the probe is radioactive. Enzymes and fluorescent dyes may be used to identify non-radioactive labels.

Analyse the membrane's hybridized DNA fragments. Fragment sizes and patterns show genetic diversity. These changes may indicate links, genetic diversity, or disease-associated alleles. RFLP analysis was one of the earliest molecular methods for DNA fingerprinting, genetic mapping, and genetic diversity. PCR and DNA sequencing have mostly supplanted it.



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However, RFLP analysis led to additional molecular marker methods and improved our knowledge of genetics and genomics [5].

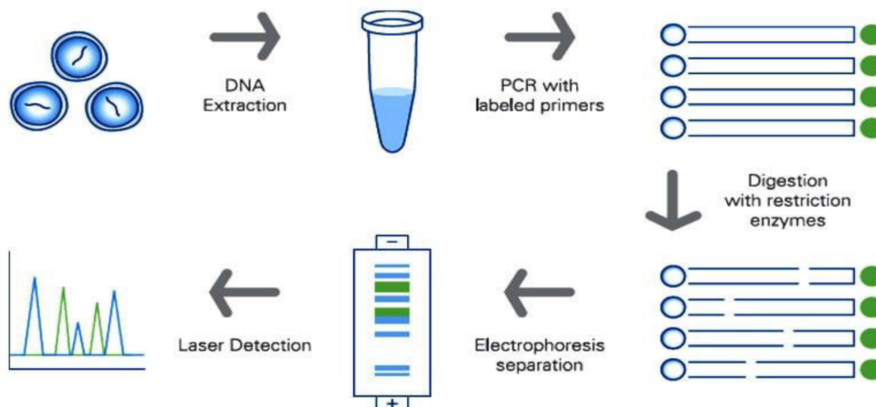


Fig.1Steps involved in RFLP

**Random Amplified Polymorphic DNA (RAPD)**

RAPD, a PCR-based molecular approach, detects genetic variation in DNA samples without previous knowledge of DNA sequences. RAPD produces DNA fingerprints or profiles unique to people or groups.

**Primer Selection:** RAPD analysis PCR amplification employs short, random oligonucleotide primers (usually 10-mer). These primers anneal to random genomic locations, amplifying DNA fragments of various sizes.

**PCR Amplification:** Random primers amplify genomic DNA. Using a heat-stable DNA polymerase enzyme, PCR cycles through denaturation, primer annealing, and DNA synthesis. Amplification produces DNA fragments from different genomic locations.

**Agarose gel electrophoresis:** sizes the amplified DNA fragments. The gel is charged with DNA fragments. Larger pieces travel slower through the gel than smaller ones.

**Visualization:** DNA stains or fluorescent dyes detect DNA fragments in the gel after electrophoresis. DNA bands form the sample's RAPD profile.

**Analysis:** RAPD profiles are evaluated for genetic polymorphisms and demographic variances. DNA bands reveal genetic differences. RAPD markers are dominant markers, meaning the presence of a band denotes a certain allele and the lack of a band shows its absence.

Population genetics, genetic diversity, phylogenetic, and plant and animal breeding require RAPD analysis. It may quickly reveal genetic linkages, population organization, and genetic differences between groups. RAPD markers have technical variability and repeatability and transferability issues across labs. RAPD can generate genetic profiles and detect genetic variation, especially when sequence information is scarce. Its simplicity and low cost make it popular in genetic investigations [6].

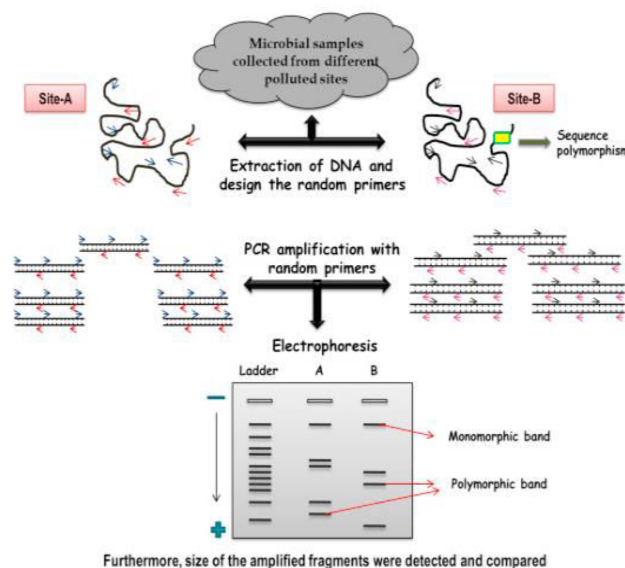


Fig.2 Steps involved in RAPD

**PCR sequencing**

PCR-based DNA sequencing uses PCR amplification to identify the nucleotide sequence of a DNA fragment. Targeted DNA sequencing is common in molecular biology, genetics, and genomics.

**PCR amplification:** First, particular primers amplify the DNA area of interest. These primers anneal to target DNA flanking regions. PCR involves denaturation, primer annealing, and DNA synthesis using a DNA polymerase enzyme. Millions of target DNA fragments are generated[7].

**PCR Product Purification:** After amplification, the PCR product is purified to eliminate primers, nucleotides, enzymes, and other reaction components that may interfere with sequencing.

**DNA Sequencing Reaction:** The purified PCR result is sequenced. DNA polymerase, DNA sequencing primers, fluorescently tagged ddNTPs, and dNTPs are used in this procedure. Sequencing

primers anneal to PCR product complementary strands.

**DNA Sequencing:** A DNA sequencer detects the fluorescence signals from tagged ddNTPs integrated into the developing DNA strand. The DNA sequencer generates fluorescence peaks by reading the nucleotides along the template DNA strand.

**Data Analysis:** Specialized software interprets the sequence and determines nucleotide order. Chromatograms depict the nucleotide peaks in the sequence. [8]

PCR-sequencing accurately determines a DNA fragment's nucleotide sequence. It is ideal for sequencing genes or genetic markers. Genetic diagnostics, mutation detection, genetic profiling, and genome sequencing have relied on PCR-sequencing.PCR-sequencing is one of numerous DNA sequencing technologies. Sanger sequencing and next-generation sequencing (NGS) both have benefits and uses depending on research aims and sequencing size [9].

## Conclusion

Finally, biotechnology applications depend on molecular markers. These markers detect and monitor genes or DNA sequences, revealing genetic diversity, gene expression, and genome mapping. Key biotechnological molecular marker applications include:

**Genetic variety Studies:** Molecular markers measure genetic variety within and across species. This information aids conservation, breeding, and evolutionary research.

**Marker-Assisted Breeding:** Molecular markers help select plant and animal characteristics. Breeders may use markers to improve efficiency and accuracy.

**Genomic Mapping:** Molecular markers locate genes and DNA sequences to create accurate genome maps. These maps enable gene function, genetic linkage, and genome evolution research. Genetic illness diagnostics uses molecular markers. Patient samples may discover disease indicators for early identification and targeted therapy.

**Forensic Analysis:** STRs are utilized in forensic science to profile and identify DNA. These markers may connect people to biological samples using distinct genetic signatures.

**Transgenic Organism Identification:** Molecular markers identify and monitor GMOs. They assure GMO labelling, traceability, and regulatory compliance [10].

Evolutionary research: Molecular markers aid evolutionary studies. Researchers may rebuild phylogenetic trees and understand evolution by comparing and analysing DNA sequences. Molecular markers have improved genetic analysis, breeding programs, illness diagnosis, and evolutionary research. New marker technologies and genomic tools broaden their applicability, promising biotechnology and associated sector advances.

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