Received: 24 Apr 24 | Revised: 23 May 24 | Accepted: 13 June 24
DOI: https://doi.org/10.62460/TBPS/2024.022

REVIEW ARTICLE

Trends in Biotechnology and Plant Sciences

# Use of Molecular Markers for Diagnostic purposes

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## Abstract

Molecular markers, encompassing DNA sequences, serve as instrumental tools for the precise identification and targeted elucidation of nucleotide sequences or specific genes within the genomic framework. In the past, numerous conventional techniques such as biotyping, ribotyping, and protein analyses were used for the diagnosis and monitoring of infectious diseases, but these techniques lacked precision and reliability. Thus, the discovery of molecular markers led to the use of these molecular markers in the early stages of disease detection. The article covers various molecular markers, such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter-simple sequence repeat amplification (ISSR), cleaved amplified polymorphic sequences (CAPS), and sequence-characterized amplified region (SCAR). Quantitative polymerase chain reaction (qPCR), and enzyme-linked immunosorbent assay (ELISA).

KEYWORDS
Nucleotide sequences, Hybridization, Polymerase chain reaction,

## 1 | INTRODUCTION

The development and application of molecular markers over the past few years have completely changed the way diseases are diagnosed and identified. Prior to this, the diseases were identified using the following techniques. These include protein analysis, bio typing, ribotyping, antibiogram, resist gram, bacteriocin (Tang et al., 1997; Kamal et al., 2024). These techniques weren't as effective at detecting diseases, so the focus is shifting to molecular marker diagnostic techniques. A.S. Serebrovskii was the first person who gave the background about the molecular marker as a diagnostic tool (Khlestkina, 2014).

There are following types of genetic markers**(i)** Morphological markers that identify the characters based on the visual bases of the characters such as plant height, color, seed shape, color, flower color, and shape, etc. (Kordrostami & Rahimi, 2015; Ali et al., 2023). **(ii)** Biochemical markers, these are also known as the isozymes that are the allelic alternatives of enzymes and these can be easily detected by using gel electrophoresis. These markers have the ability to identify the variation among species at the gene level which is functional and these markers show a small level of inheritance. **(iii)** DNA marker that identifies the variation among species at the DNA level (Kordrostami & Rahimi, 2015). The identification and the diagnosis of the disease by the use of these markers are easy and accurate.

The use of molecular markers improves detection accuracy. Due to a lack of reliability and handling issues, the earlier conventional methods are no longer used in favour of molecular markers. These markers are used to detect complex traits (Tavares et al., 2011; Ali et al., 2023).

## 2. CONVENTIONAL METHODS

The traditional methods of microbe identification were based on the physical characteristics of the microbes, such as their growth, shape, and food consumption. The following techniques were employed to identify diseases.

### 2.1. Biotyping

This method identifies the microbes on the basis of their physical appearance such as their growth mechanism, consumption of the food, shape, etc. to construct a profile of any organisms is known as biogram (de La Higuera et al., 1999). So, the identification of different organisms based on their profile is known as biotyping. The organisms whose biogram comes same this shows the different strain of the same organism (Tenover et al.,

  

1994; Tang et al., 1997). The biogram method uses physical characteristics of microbes, such as their growth mechanism, food consumption, shape, etc., to identify them and create a profile of any organisms[5]. Bio typing is the process of identifying various organisms based on their profiles. The organisms whose bio grams are identical demonstrate various strains of the same organism (Tenover et al., 1994; Tang et al., 1997).

### 2.2. Ribotyping

Ribotyping is the method which identifies the bacteria based on the formation of the rRNA (Alaluusua et al., 1994). In this method we take the DNA and then cut with the restriction enzymes and then separate on the gel electrophoresis that is then transferred to the nylon sheet and then that is dipped in the probes the probe will bind to their complementary sequence and then we compare the sequence with the data stored in the databases (Tenover et al., 1994; Tang et al., 1997).

Ribotyping is a technique that uses the rRNA7's formation to identify the bacteria. This method involves taking DNA, cutting it with restriction enzymes, separating it on a gel electrophoresis, transferring it to a nylon sheet, dipping it in probes, which bind to their complementary sequences, and then comparing the sequence to information stored in databases (Alaluusua et al., 1994).

### 2.3. Protein Analysis

We can identify the various antigenic proteins that are made by microbes by using proteins. This technique involves the application of various monoclonal antibodies to various organisms, followed by the detection of those antibodies. After that, the protein is extracted, separated using SDS PAGE, and detected (Tang et al., 1997; Fernández-Álvarez et al., 2018).

## 3. TYPES OF MOLECULAR MARKERS

There are mainly two types of marker PCR based and non-PCR based methods. Non-PCR base methods or Hybridization base Methods include Restriction fragment length polymorphism (RFLP) (Finger & Klank, 2010; Tharachand et al., 2012) and PCR base methods are Random amplification of polymorphic DNA (RAPD) (Bardakci, 2001; Kumar & Gurusubramanian, 2011), Amplified fragment length polymorphism (AFLP) (Agarwal et al., 2008), Microsatellite polymorphism Simple sequence repeat (SSR ) (Shehata et al., 2009; Vieira et al., 2016) Variable number tandem repeat (VNTR) (Jonah et al., 2011), Sequence Tagged Site (STS) (Alhasnawi et al., 2015), Sequence Characterized Amplified Region (SCAR) (Adinolfi et al., 2007), Inter-Simple Sequence Repeat Amplification (ISSR) (Datta et al., 2011), Cleaved Amplified Polymorphic Sequences (CAPS) (Jiang, 2013; Elangbam & Misra, 2016), Single nucleotide polymorphism (SNP) (Lateef, 2015; Grover & Sharma, 2016), Short tandem repeat (STR) (Tharachand et al., 2012), Retrotransposon Microsatellite Amplified Polymorphism (REMAP) (Schulman, 2007; Kalendar & Schulman, 2006), Simple sequence length polymorphism (SSLP) (Semagn et al., 2006), Diversity Arrays Technology (DArT) (Appleby et al., 2009), Anchored Microsatellite Primed PCR (AMP-PCR) (Pradeep-Reddy et al., 2002), Aritrarily Primed Polymerase Chain Reaction (AP-PCR) (Kumar, 1999), DNA Amplification Fingerprinting (DAF) (Kumar, 1999), Inverse PCR (IPCR) (Silva et al., 2017), Inverse Sequence-Tagged Repeats (ISTR) (Semagn et al., 2006). Some of these markers are similar to each other and some work on the same methods.

### 3.1. Non-Pcr Base Methods or Hybridization Base Methods

Non-PCR based techniques and hybridization-based techniques have proven to be extremely useful molecular markers in the field of diagnostic applications. These methods allow for the quick and precise identification of particular genetic sequences, assisting in the diagnosis of diseases and the practice of personalized medicine. The direct visualization of target DNA or RNA sequences within cells or tissues is made possible by hybridization techniques like fluorescence in situ hybridization (FISH), which helps identify genetic abnormalities linked to various diseases like cancer. On-PCR techniques like next-generation sequencing (NGS) give a complete picture of a person's genetic makeup and make it possible to find mutations and variants that are linked to specific diseases.

#### *3.1.1. Restriction Fragment Length Polymorphism (Rflp)*

This hybridization-based marker for polymorphism finds the difference. Utilizing this kind of marker, polymorphism between two species or individuals can be found. Due to mutation, even a small change in nucleotides of about 1, 2 base pairs results in different types of bands (Tharachand et al., 2012). Therefore, the mutation may cause the restriction site to gain or lose. The following is how the RFLP marker works:

The DNA of the organisms is first extracted, and then it is cut with molecular scissors using restriction enzymes that are found in bacteria and used as their defense mechanism by this enzyme to break down the DNA at restriction sites. Then, based on their size to mass ratio, DNA fragments are separated on the agarose gel. Then, through the process of blotting, these DNA bands are transferred to the nylon or nitrocellulose membrane. With the aid of a sponge, the

gel is added to an alkaline buffer bath in this method. The apparatus's top is covered with the stack of towels. The denatured double-stranded DNA is sucked up by the towel along with the buffer solution, which causes the single-stranded DNA to move along the nylon sheet and stick firmly to it. The nylon sheet is then removed from there and submerged in a bucket of radioactively marked probes. The complement sequence of the target will bind the probe. Then take the sheet and wash it, the DNA that is not attached with the probes will wash out. At the end, gel is place under UV light and observe the bands (Semagn et al., 2006; Asif Raheem et al., 2019).

### 3.2. Pcr Based Methods

In the field of molecular biology, Polymerase Chain Reaction (PCR) is a revolutionary technique that has completely changed the way diagnostic purposes are approached. Since its inception, PCR has established itself as a key technique for amplifying particular DNA sequences, making it possible to detect minute genetic components in biological samples. Its unmatched sensitivity, specificity, and capacity to operate with small sample sizes have opened the door for a wide range of diagnostic applications (Zafar et al., 2020).

Scientists and clinicians can identify, measure, and analyze genetic markers linked to a variety of diseases, pathogens, and genetic conditions by utilizing the principles of DNA replication through PCR. This introduction explores the essential function of PCR as a tool for molecular markers, highlighting its importance in contemporary diagnostic methods and its advancement of personalized medicine.

#### *3.2.1 Random Amplification of Polymorphic Dna (RAPD)*

As the name indicates this type of primer is applied which randomly amplify the DNA strand. This is a PCR based marker in which we get amplification by using PCR. We use short (2–6 bp) primers. the following requirements should be met by the primer we used The GC content must be at least 40% and must not include any palindromic sequences. The primer must be annealed at a higher temperature if it contains more percentage, while the RAPD must anneal at a lower temperature. We only use one primer in RAPD, and it serves as both a forward and a reverse primer. The protocol of RAPD is designed to short oligonucleotide primer add in the PCR the primer then will anneal at low temperature (Zafar et al., 2022).

If the reverse and forward primers are not more than 3000 nucleotides apart, a portion of the DNA will be amplified (Kumar & Gurusubramanian, 2011). The band will then be amplified by the primer. This marker has significant drawbacks because it is not reproducible, which means that if we repeat the sample, the outcome will differ. Since it only detects dominant traits, it is a dominant marker. It is primarily used to determine whether a trait is present or not. In addition, it aids in plant and animal breeding and paternity tests (Bardakci, 2001).

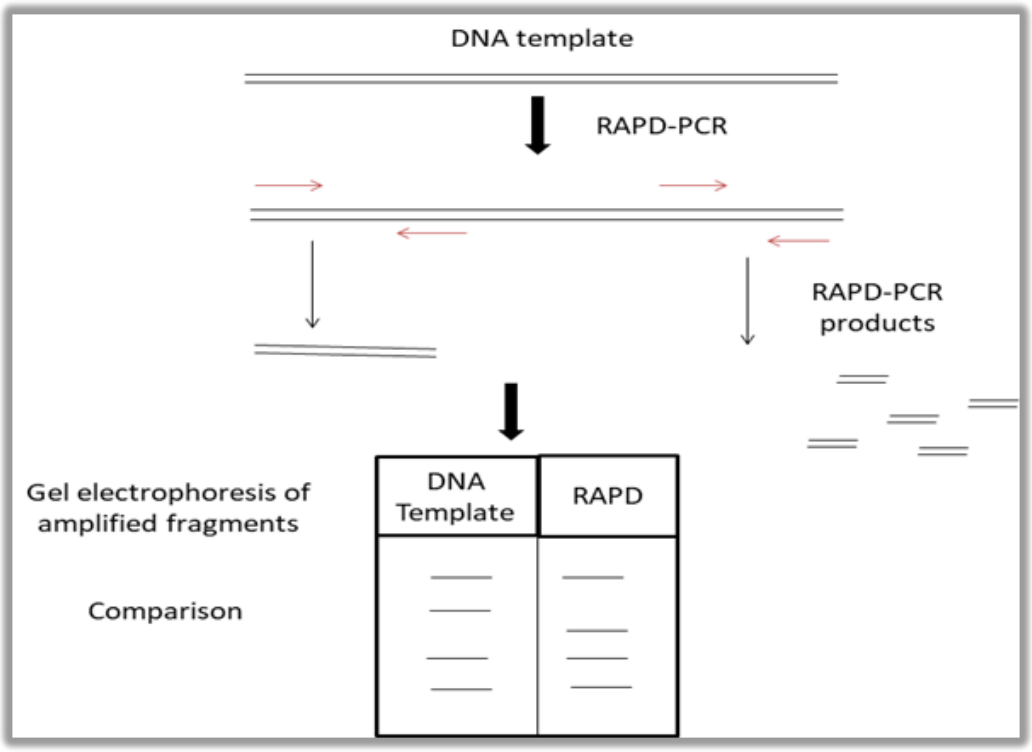


**Fig. 1:** DNA template is taken and design primers of short base pairs up to 10. Then PCR the DNA template the RAPD will attach to the sites complementary to the primer. Run on gel and comparison the Marker with the template DNA.

### *3.2.2.* Amplified Fragment Length Polymorphism (AFLP)

The RFLP and RAPD markers are combined to create this marker. In this, the DNA is first cut with a restriction enzyme before being amplified with PCR (Agarwal et al., 2008). The marker procedure involves removing the genomic DNA and then slicing the DNA with restriction enzymes. Next, we design particular adapters that will bind with the sticky ends of the DNA in order to prevent the ligation of the restriction sites of the DNA. During the PCR, this adopter will signal amplification. In this procedure, we perform a first pre-amplification step in which we increase the adopter length by one base pair. Then, following pre-amplification, we perform selective amplification, extending the adopter up to 3 bp in order to produce specific bands as opposed to the 1 bp extension that occurs frequently in the genome (Haroon et al., 2022).

As a result, the obtained amplified region is separated using gel electrophoresis, stained using silver staining, and detected using autoradiography (Semagn et al., 2006).

int of polymorphism in the genomes of plants and animals is this distinction. The SSR can be found in both coding and non-coding regions of the genome. Due to the various repeat counts, they exhibit polymorphism in nature. The polymerase reaction (PCR) can be used to detect them and make them easily reproducible. They exhibit high polymorphism and can reproduce, which makes them useful for paternity tests, building large genome maps, mapping beneficial genes, marker-assisted selection, evolutionary relationship analysis, and other diagnostic procedures (Vieira et al., 2016).

SSR markers can be divided into two categories. The first is based on the repeats' sizes. These have 2, 3, or 4 repeats each and are called di, tri, tetra, and penta. The second classification is based on the presence or absence of repeats. These may be compound imperfects, which are interrupted by a pair of base pairs rather than by another base pair, or perfects with the same repeated base pairs in all three cases. The names of the microsatellites are based on where they appear in the genomes. If they are present in nucleus they are termed as nuclear SSR and termed the same if they present in mitochondria chloroplast etc. (Asif Raheem et al., 2019).

It is seen that the SSR are mostly present as nuclear SSR. The SSR is found in coding and non-coding regions of the genome. But it is demonstrated that the SSR is located in the coding regions of the genome[34]. The SSR is developed by first isolating them from the genome's coding or non-coding regions, and then, following isolations, cloning them. Their ligation in the vector results in the formation of their clone, from which copies are produced. Following the hybridization of these clones with repeat-containing probes, which can then bind to nylon sheets and be viewed under UV light, the SSR are isolated and gathered in libraries (Varshney et al., 2005; Tharachand et al., 2012).

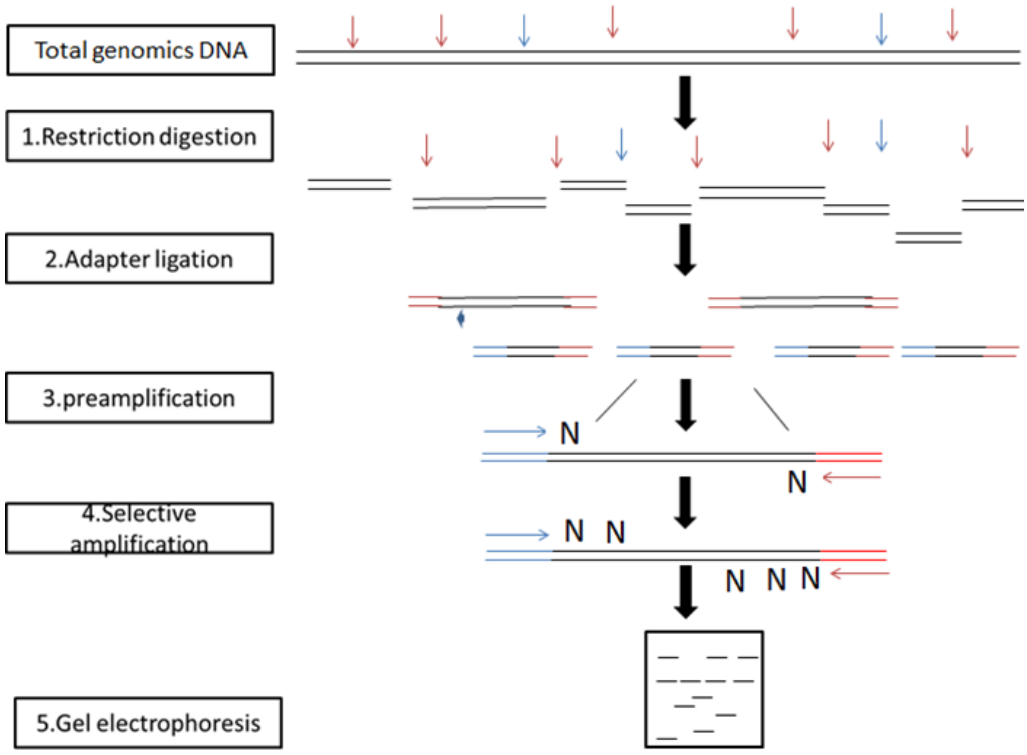


**Fig. 2:** Take the template DNA digest it by the help of restriction enzymes. Design adopter to prevent the attachment of the sequences of DNA. Start the preamplification process in the PCR run another PCR for selective amplification in which 1 or 2 base pairs of adaptors are changed. Then run on the gel and read it.

### *3.2.3.* Simple Sequence Repeats (SSR)

SSR is abbreviated as short tandem repeats. They are also known as microsatellites. These tandem repeats, which are common in many eukaryotes and prokaryotes, range in length from 1-6 base pairs. While AC repeats are mostly found in animals, AT repeats are mostly found in plants. The general po

### *3.2.4.* Inter-Simple Sequence Repeat Amplification (ISSR)

The genomic region between two SSR repeats that are present in the opposite direction of the genome 28 is amplified using ISSR. The region between the repeats can be amplified using this technique because it used simple sequence repeats as a primer in the PCR reaction. Di, tri, or tetra base pair repeats of the SSR can be used as primers. The length of the primer, which is between 15 and 20 bp, necessitated a high annealing temperature, which is largely dependent on the concentration of GC. It demonstrates a high degree of individual polymorphism. It can be detected using silver staining and PAGE. It has any limitations that it is of dominant inheritance, it is not reproducible (Haroon et al., 2023).

### *3.2.5.* Cleaved Amplified Polymorphic Sequences (CAPS)

The term is shortened to sequence tagged sites. This marker is the result of combining two procedures: PCR and RFLP20. For this marker, we first extract the DNA and then use PCR to amplify it while providing all the prerequisites. The obtained DNA is then used in the following step, which involves using a restriction enzyme to cut the amplified DNA. Therefore, by comparing the restriction sites, we can assess the level of polymorphism among individuals. The STS has the benefit of being a co-dominant marker, allowing it to identify both dominant and recessive traits. Due to the amplification in PCR, this method has the additional benefit of being very time-consuming. It is also known as PCR-RFLP (Haroon et al., 2022).

### *3.2.6.* Sequence Characterized Amplified Region (SCAR)

With the aid of PCR, a specific genomic sequence can be identified by using two pairs of primers. It was created with the aid of the cloning of the RAPD marker, which is used for a particular type of diagnostic purpose. The scar's ability to transform into a co-dominant nature is one of its advantages. it is used to detect specific locus of any trait, and the amplification is not very sensitive (Zafar et al., 2022).

### *3.2.2.* Quantitative Polymerase Chain Reaction (QPCR)

The important molecular marker technique known as quantitative polymerase chain reaction (qPCR) is frequently used for diagnostic purposes. Pathogens, mutations, and levels of gene expression can all be precisely detected thanks to its accuracy in quantifying nucleic acids. qPCR assists in the highly sensitive and specific identification of disease-causing agents, such as viruses and bacteria, by amplifying and quantifying DNA or RNA molecules. This method enables early diagnosis and treatment modifications by tracking viral loads in infections like HIV and SARS-CoV-2. In addition, qPCR makes it easier to evaluate genetic changes linked to inherited diseases, diagnose cancer using oncogenes, and monitor treatment outcomes. Modern molecular diagnostics cannot function without qPCR due to its speed, accuracy, and capacity to analyze small sample sizes.

### *3.2.3.* Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay, also known as ELISA, is a crucial molecular marker technique that is frequently used for diagnostic purposes. ELISA is essential in the diagnosis of diseases because it can identify and measure particular proteins or antigens in samples. It assists in the detection of a number of medical conditions, including autoimmune disorders, cancer biomarkers, and infectious diseases (like HIV and hepatitis). The high sensitivity and specificity of ELISA allow for precise target molecule detection even at low concentrations. Additionally, due to its adaptability, it can be used with a variety of sample types, including tissue extracts, blood, serum, and urine. ELISA has transformed diagnostic medicine by enabling early disease detection and tracking treatment efficacy. It is a non-invasive, economical method (Zafar et al., 2022).

## 4. CONCLUSION

The best method used for diagnostic purposes is the use of molecular markers. Similar to before, traditional techniques like bio typing, ribotyping, and protein analysis were used to find infections. But these methods' outcomes are not definite enough to be relied upon. Thus, the development of molecular markers has made detection methods very simple and clear. Every molecular marker has a specific use and a variety of detection techniques.

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