

**Course – M.Sc. Botany Part – II Paper – XVI**  
**Topic – DNA Fingerprinting – A Detailed Study**

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**Introduction:**

Every cell of our body contains DNA and approximately 99.9% of DNA is similar between two humans. Only 0.1% difference is responsible to make someone unique (except identical twins) and this 0.1% of DNA plays an important role in DNA Fingerprinting.

As we know, only 3 % of our genome are coded and act for protein synthesis i.e. called *gene* and rest of 97% are non-coding, repetitive and junk; this junk DNA are utilized to perform DNA fingerprinting. The sequence structure and number of repeats varies between individuals and organisms. On this basis, the DNA print can be prepared.

DNA fingerprinting technique was first developed by British Professor *Sir Alec Jeffrey* in 1984. He realized that we can detect variations in human DNA, in the form of minisatellites. *Jeffrey* created the first DNA profile using the restriction digestion length polymorphism. His method was actually a combination of RFLP and autoradiography.

**Definition**

**“It is a molecular method to identify an individual or any living organism from their DNA sample by looking at unique patterns in their DNA.”**

**Satellites DNA:**

The satellites DNA are non-coding, repetitive DNA regions, located on telomeres and centromeres. It helps in proper replication, whereas mutation in those sequences causes replication errors.

It is of two types present in human genome based on their repeat sequence nature:

1. *Minisatellite*
2. *Microsatellites*.

*Minisatellite* DNA sequences are 10 to 60 base pair long and repeated 5 to 50 times in a genome. They are highly variable (polymorphic), unique, and GC-rich sequences. It is generally present in telomeric regions. *e.g.* VNTRs.

*Microsatellites* are smaller than minisatellites. It contains repeated DNA sequence of 1-6 base pair and 5 to 10 times in a genome. *e.g.* STR and SSR. These regions are also hypervariable, non-coding and telomeric.

### **Process of DNA Fingerprinting:**

1. Collection of biological samples.
2. DNA extraction
3. Restriction digestion or PCR amplification
4. Agarose gel electrophoresis, capillary electrophoresis or DNA sequencing
5. Interpreting results

#### 1. Sample collection:

DNA can be obtained from any biological samples. *e.g.* any part of plant or animals.

#### 2. DNA extraction:

DNA can be extracted with different methods like CTAB DNA extraction methods, Proteinase K DNA extraction methods and Phenol-chloroform DNA extraction method or may be with any DNA extraction kit.

#### 3. Restriction digestion or PCR amplifications :

This process can be performed by three different techniques.

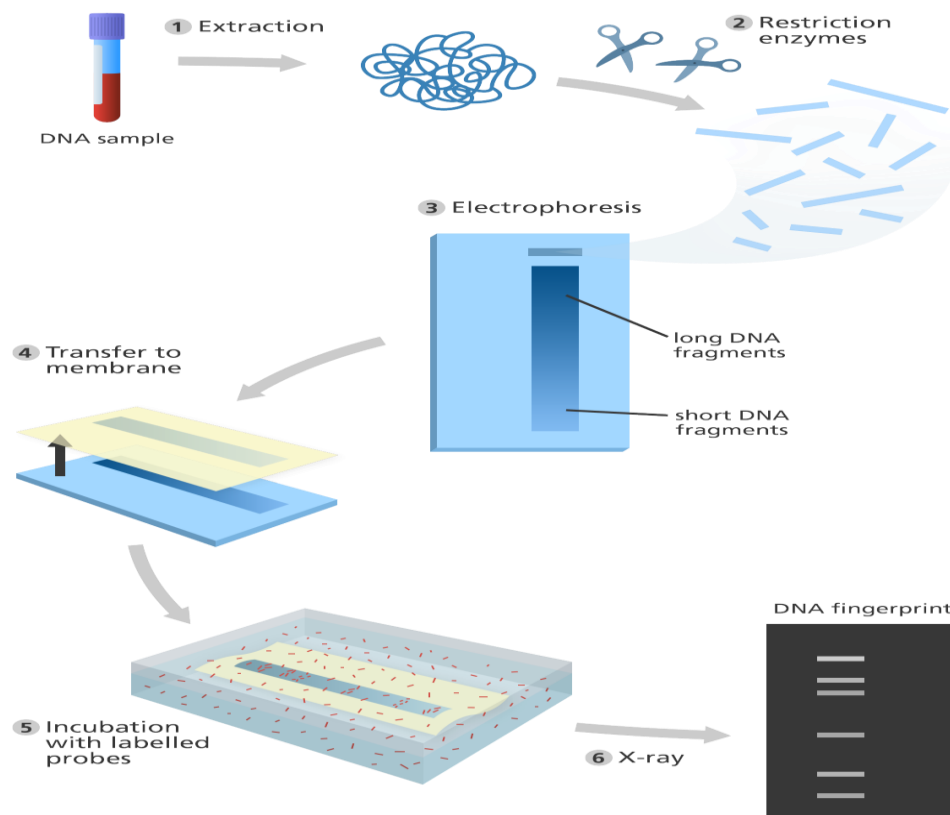
- (i) RFLP analysis.
- (ii) PCR analysis.
- (iii) Real Time PCR analysis.



**Fig 1: Showing Different DNA fingerprinting techniques**

## RFLP analysis:

RFLP is the first method used for DNA fingerprinting. It has been performed with restriction enzymes, which is used to cut the DNA. This resulted in thousands of pieces of DNA with a variety of different lengths. With the help of agarose gel electrophoresis these DNA has been separated on the basis of their size. Further the piece of DNA has been transferred to nylon membrane. The nylon membrane was incubated with radioactive probes (Probes are small fragments of minisatellite DNA tagged with radioactive phosphorous). The probes only attach to the complementary pieces of DNA and here, they attached to the minisatellites in the genome. The minisatellites attached with probes were then visualised by exposing the nylon membrane to X-ray film. When exposed to radioactivity a pattern of dark bands appeared on the film at the sight of the labelled DNA. This pattern is called the DNA fingerprint. To compare two or more different DNA fingerprints the different DNA samples were run side-by-side on the same electrophoresis gel.



**Fig 2: Showing RFLP analysis with the help of X-ray film**

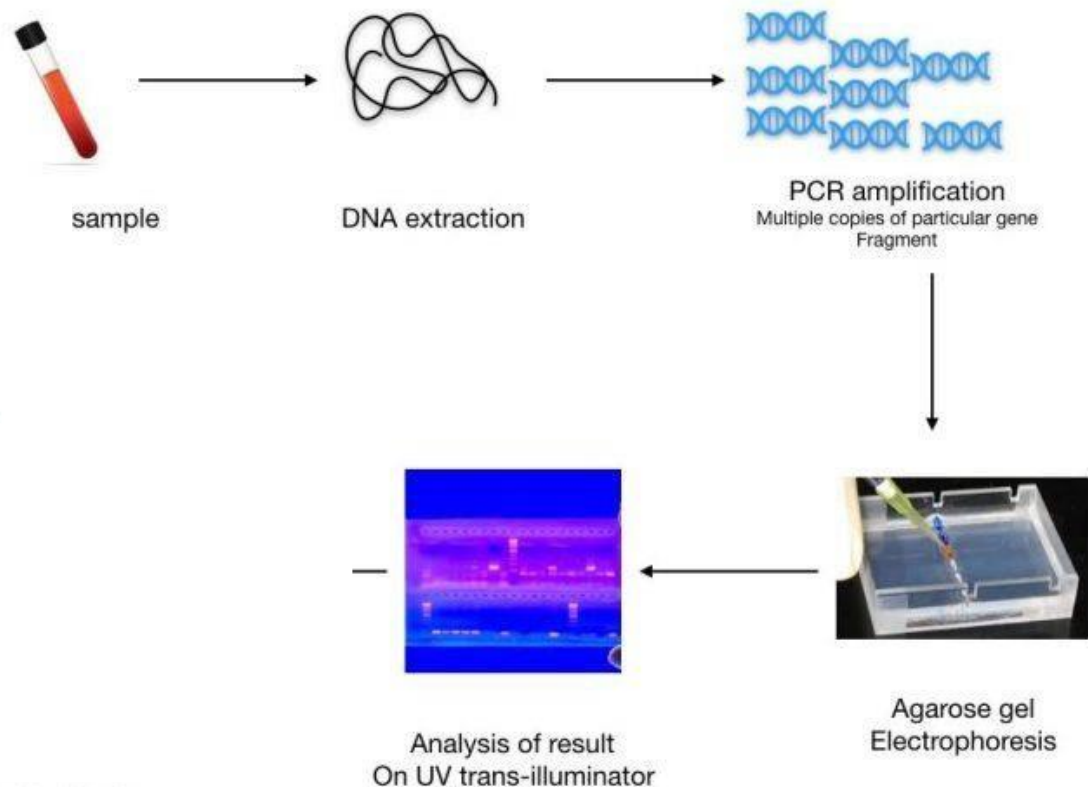
### PCR based analysis:

PCR based DNA fingerprinting technique is most popular among all. It is simpler than RFLP-autoradiography and gives reliable results than traditional techniques. It is faster and more accurate.

PCR based DNA fingerprinting relies on microsatellites like STRs (Short tandem repeats) and VNTRs. Unlike the RFLP DNA fingerprinting method, it does not use restriction enzymes to cut the DNA. Polymerase chain reaction (PCR) has been used to produce many copies of specific STR and VNTR sequences.

Many STRs and VNTRs sequences of our interest are available on NCBI database and primers can be designed on basis of these sequenced data. PCR has been performed with the set of prepared primers and further agarose gel electrophoresis set up to obtain results. On the basis of size of DNA fragments, different DNA bands appear in a gel.

However, PCR based gel electrophoresis is mostly used for VNTR analysis. STRs are small in length so, it difficult to distinguish in agarose gel. For this purposes, RT PCR based analysis performed.

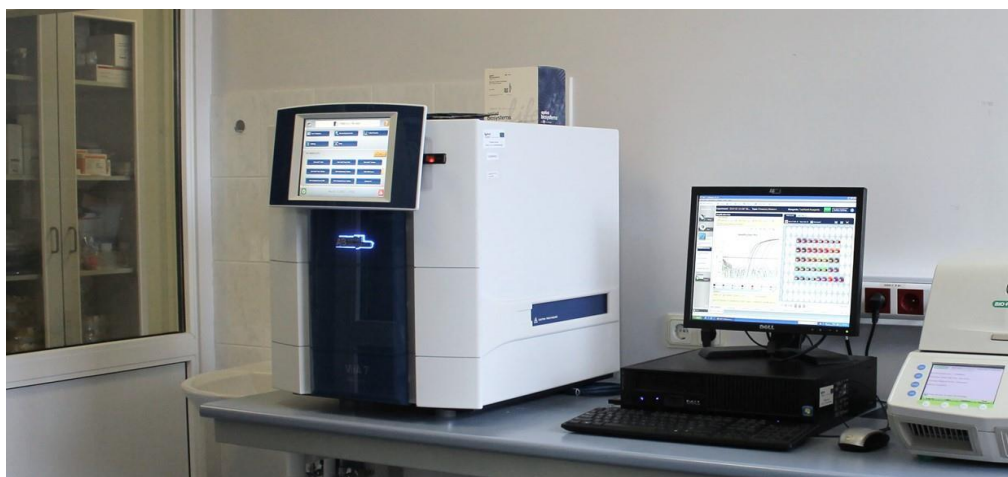
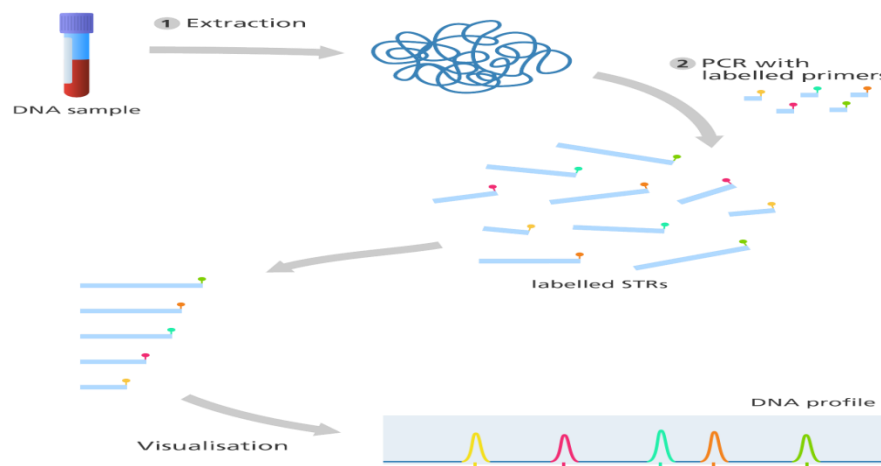


**Fig 3: Showing PCR technique for DNA fingerprinting with Agarose gel electrophoresis.**

### RT-PCR based analysis:

As we know, STRs are small in length so, it is very difficult to visualize on agarose gel. Real-time PCR is able to amplify DNA as well as counts the number of amplicons. Therefore the number of repeats present in a sample can be calculated. It is accurate, quick, reliable, and cheaper than other methods. Moreover, it can even quantify a smaller amount of DNA from any sample.

Fluoro-labeled probes are used in RT-PCR. Once the fluoro-labeled probe is paired with complementary sequence, it signals the machine to monitor the amplification process of that particular fragment. Based on the fluorescent signals received, it creates different peaks for different repeats on the monitor (therefore Gel electrophoresis doesn't require in this technique). We can calculate the number of repeats present in different samples.



**Fig 4: (a) RT- PCR with fluorescent labeled primer  
(b) RT-PCR machine (No use of electrophoresis) result show on screen**

### **DNA fingerprinting in Plants:**

DNA fingerprints have great value in plant breeding due to its somatic stability and high variability, which can be used to identify individuals. Its databases act as important tools for plant molecular research because it provide important technical and information support for variety quality control, crop breeding and molecular marker-assisted breeding. DNA fingerprinting plays an essential role in determining the genetic character of particular species in a broader way.

### **Other applications of DNA fingerprinting:**

DNA fingerprinting has applications in **paleontology, archaeology, various fields of biology, medical diagnostics** and **forensics science**.

In criminal investigations, the DNA fingerprint of a suspect's blood or other body material is compared to that of the evidence from the crime scene to see how closely they match.

- (i) The technique can also be used to establish paternity.
- (ii) Identify a dead body that's too old or damaged to be recognizable.
- (iii) Match tissues of organ donors with those of people who need transplants.
- (iv) Identify diseases that are passed down through your family.
- (v) Help find cures for those diseases, called hereditary conditions.
- (vi) DNA fingerprinting is extremely accurate. Most countries now keep DNA records on file in much the same way police keep copies of actual fingerprints.

Scientists and researchers are using DNA fingerprinting for various applications. It has been updated time to time, to make the technique more accurate. PCR is a powerful tool and gives sensitivity, accuracy, precision, and low-cost assay to DNA.

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