# Types of DNA Markers:

1. Restriction Fragment Length Polymorphism (RFLP)
2. Amplified Fragment Length Polymorphism (AFLP)
3. Random Amplified Polymorphic DNA (RAPD)
4. Cleaved Amplified Polymorphic Sequences (CAPS)
5. Simple Sequence Repeat (SSR) Length Polymorphism
6. Single Strand Conformational Polymorphism (SSCP)
7. Heteroduplex Analysis (HA)
8. Single Nucleotide Polymorphism (SNP)
9. Expressed Sequence Tags (EST)
10. Sequence Tagged Sites (STS)

***Random Amplified Polymorphic DNA (RAPDs):***

RAPD refers to polymorphism found within a species in the randomly amplified DNA generated by restriction endonuclease enzyme. RAPDs are PCR based DNA markers. RAPD marker assays are performed using single DNA primer of arbitrary sequence.

RAPD primers are readily available being universal. They provide moderately high genotyping throughput. This technique is simple PCR assay (no blotting and no radioactivity). It does not require special equipment. Only PCR is needed. The start-up cost is low.

RAPD marker assays can be performed using very small DNA samples (5 to 25 ng per sample). RAPD primers are universal and can be commercially purchased. RAPD markers can be easily shared between laboratories. Locus-specific, co-dominant PCR-based markers can be developed from RAPD markers. It provides more polymorphism than RFLPs.

**Disadvantages:**

The detection of polymorphism is limited. The maximum polymorphic information content for any bi-allelic marker is 0.5. This technique only detects dominant markers. The reproducibility of RAPD assays across laboratories is often low. The homology of fragments across genotypes cannot be ascertained without mapping. It is not applicable in marker assisted breeding programme.

**Uses:**

This technique can be used in various ways such as for varietal identification, DNA fingerprinting, gene tagging and construction of linkage maps. It can also be used to study phylogenetic relationship among species and sub-species and assessment of variability in breeding populations.

***Restriction Fragment Length Polymorphisms (RFLPs):***

RFLPs refer to variations found within a species in the length of DNA fragments generated by specific endonuclease. RFLPs are first type of DNA markers developed to distinguish individuals at the DNA level. RFLP technique was developed before the discovery of Polymerase Chain Reaction (PCR).

**Advantages:**

RFLP technique has several advantages. It is a cheaper and simple technique of DNA sequencing. It does not require special instrumentation. The majority of RFLP markers are co-dominant and highly locus specific. These are powerful tools for comparative and synteny mapping.

It is useful in developing other markers such as CAPS and INDEL. Several samples can be screened simultaneously by this technique using different probes. RFLP genotypes for single copy or low copy number genes can be easily scored and interpreted.

**Disadvantages:**

Developing sets of RFLP probes and markers is labour intensive. This technique requires large amount of high quality DNA. The multiplex ratio is low, typically one per gel. The genotyping throughput is low. It involves use of radioactive chemicals. RFLP finger prints for multi-gene families are often complex and difficult to score. RFLP probes cannot be shared between laboratories.

**Uses:**

They can be used in determining paternity cases. In criminal cases, they can be used in determining source of DNA sample. They can be used to determine the disease status of an individual. They are useful in gene mapping, germplasm characterization and marker assisted selection. They are useful in detection of pathogen in plants even if it is in latent stage.

***Amplified Fragment Length Polymorphism (AFLP):***

AFLPs are differences in restriction fragment lengths caused by SNPs or INDELs that create or abolish restriction endonuclease recognition sites. AFLP assays are performed by selectively amplifying a pool of restriction fragments using PCR. RFLP technique was originally known as selective restriction fragment amplification.

**Advantages:**

It provides very high multiplex ratio and genotyping throughput. These are highly reproducible across laboratories. No marker development work is needed; however, AFLP primer screening is often necessary to identify optimal primer specificities and combinations.

No special instrumentation is needed for performing AFLP assays; however, special instrumentation is needed for co-dominant scoring.

Start-up costs are moderately low. AFLP assays can be performed using very small DNA samples (typically 0.2 to 2.5 pg per individual). The technology can be applied to virtually any organism with minimal initial development.

**Disadvantages:**

The maximum polymorphic information content for any bi-allelic marker is 0.5. High quality DNA is needed to ensure complete restriction enzyme digestion. DNA quality may or may not be a weakness depending on the species. Rapid methods for isolating DNA may not produce sufficiently clean template DNA for AFLP analysis.

Proprietary technology is needed to score heterozygotes and ++ homozygotes. Otherwise, AFLPs must be dominantly scored. Dominance may or may not be a weakness depending on the application.

The homology of a restriction fragment cannot be unequivocally ascertained across genotypes or mapping populations. Developing locus specific markers from individual fragments can be difficult and does not seem to be widely done.

The switch to non-radioactive assays has not been rapid. Chemiluminescent AFLP fingerprinting methods have been developed and seem to work well.

The fingerprints produced by fluorescent AFLP assay methods are often difficult to interpret and score and thus do not seem to be widely used. AFLP markers often densely cluster in centromeric regions in species with large genomes, e.g., barley (Hordeum vulgare L.) and sunflower (Helianthus annuus L.).

**Uses:**

This technique has been widely used in the construction of genetic maps containing high densities of DNA marker. In plant breeding and genetics, AFLP markers are used in varietal identification, germplasm characterization, gene tagging and marker assisted selection.