

DNA Fingerprinting

- It is a technique used to identify people using sample of their DNA.
- Dr. Alec John Jeffreys in 1984/1985 invented this technique.

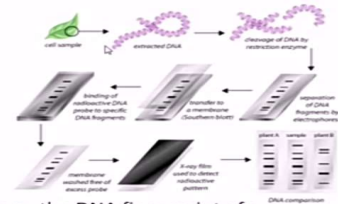
Steps of DNA Fingerprinting

1. Sample collection.
2. PCR.
3. Restriction digestion.
4. Gel electrophoresis.
5. Transfer of DNA to membrane.
6. Addition of probes.
7. Detection.

Application

- It is the basis of paternity testing in case of disputes.
- Useful as identification tool in forensic applications.
- Helps in diagnosis of inherited disorder.
- Used to determine population.
- To know genetic diversity in evolutionary biology.

DNA Fingerprinting



Applications:

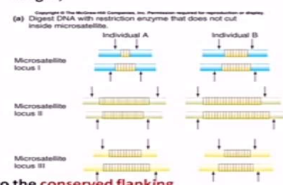
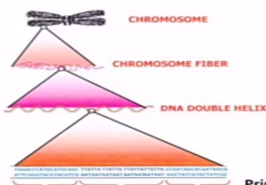
1. In criminal investigations, the DNA fingerprint of a suspect's blood or other body material is compared to that of the evidence from the crime scene to see how closely they match. The technique can also be used to establish paternity.
2. Cataloging and exploring the livestock
3. Screening of germplasm.

Simple sequence length polymorphisms (SSLPs)

SSLPs are arrays of repeat sequences that display length variations. Unlike RFLPs that can have only two alleles, **SSLPs can be multi-allelic** as each SSLP can have a number of different length variants.

- **Microsatellites or simple tandem repeats (STRs)**, whose repeats are shorter, usually dinucleotide or tetranucleotide units.

Minisatellites, also known as variable number of tandem repeats (**VNTRs**), in which the repeat unit is up to 25 bp in length;



Primers to the conserved flanking regions of VNTR locus were developed

A – 8 repeats

Forward primer →
 ...GCTCCAGGCTTAGACTTCTTCTTCTTCTTCTTCTTCGCACTTTAACGATACGG...
 ...CGAGGTCGGAATCTGAAGAAGAAAGAAAGAAAGAAAGCGTGAATTTGCTATGCC...
 ← Reverse primer

B – 7 repeats

Forward primer →
 ...GCTCCAGGCTTAGACTTCTTCTTCTTCTTCTTCTTCGCACTTTAACGATACGG...
 ...CGAGGTCGGAATCTGAAGAAGAAAGAAAGAAAGAAAGCGTGAATTTGCTATGCC...
 ← Reverse primer

C – 9 repeats

Forward primer →
 ...GCTCCAGGCTTAGACTTCTTCTTCTTCTTCTTCTTCTTCGCACTTTAACGATACGG...
 ...CGAGGTCGGAATCTGAAGAAGAAAGAAAGAAAGAAAGCGTGAATTTGCTATGCC...
 ← Reverse primer

Simple sequence repeats (SSR)

- Simple sequence repeats are present in the genomes of all eukaryotes.

The number of SSRs is highly variable among individuals

unique flanking regions

A dinucleotide repeat such as (dC-dA)n . (dG-dT)n was reported to occur in the human genome as many as 50,000 times with n varying from 10 to 60.

Principle of ISSR

Primers can be designed based on a microsatellite repeats exclusively

PCR product 3'-anchored primer

PCR product 5'-anchored primer

Inter simple sequence repeats (ISSR)

primers can be extended outside or inside the ISSR in which case a unique region most likely will be amplified.

Designing primers for ISSR polymorphism

Gel with products of different primers

Advantages:

- do not require sequence information
- variation may be found at several loci simultaneously
- microsatellite sequence-specific
- reliable DNA profiling, especially for closely related species

Disadvantages:

- dominant markers
- band staining can be weak

Single nucleotide polymorphism (SNP)

These are positions in a genome where some individuals have one nucleotide (e.g. a G) and others have a different nucleotide (e.g. a C).

Polymorphism "Poly" many "morpho" form

General population 94%

Single nucleotide polymorphism (SNP) 6%

Normal: TCTAAGTCGATATAA, AGATTCAAGCATATT, AGATTCAAGCATATT, TCTAAGTCGATATAA (Green)

Carrier: TCTAAGTCGATATAA, AGATTCAAGCATATT, AGATTCAAGCATATT, TCTAAGTCGATATAA (Yellow)

Disease: TCTAAGTCGATATAA, AGATTCAAGCATATT, AGATTCAAGCATATT, TCTAAGTCGATATAA (Red)

Advantage and disadvantages of SNP

Advantages:

- their abundant numbers
- they can be typed by methods that do not involve gel electrophoresis.
- SNP detection is more rapid because it is based on oligonucleotide hybridization analysis.

Disadvantages:

- there is a high possibility that a SNP does not display any variability in the family that is being studied.

SNP Profiles

DNA molecule

Version 1 C T A A G T A

Version 2 C T A C G T A

Version 3 C T A G G T A

Version 4 C T A T G T A

SNP

- Genome of each individual contains distinct SNP pattern.
- People can be grouped based on the SNP profile.
- SNPs Profiles important for identifying response to Drug Therapy.