DEPARTMENT OF BIOTECHNOLOGY

MARKERS

By-
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DEFINATION

- Small stretch of nucleotide sequences present at different location in a genome in a repeated manner.
- Also called as genetic markers.

Properties

- Repeat in genome
- Non coding sequences
- They are heritable in nature(Parent → offsprings)
- Mark the DNA segments.

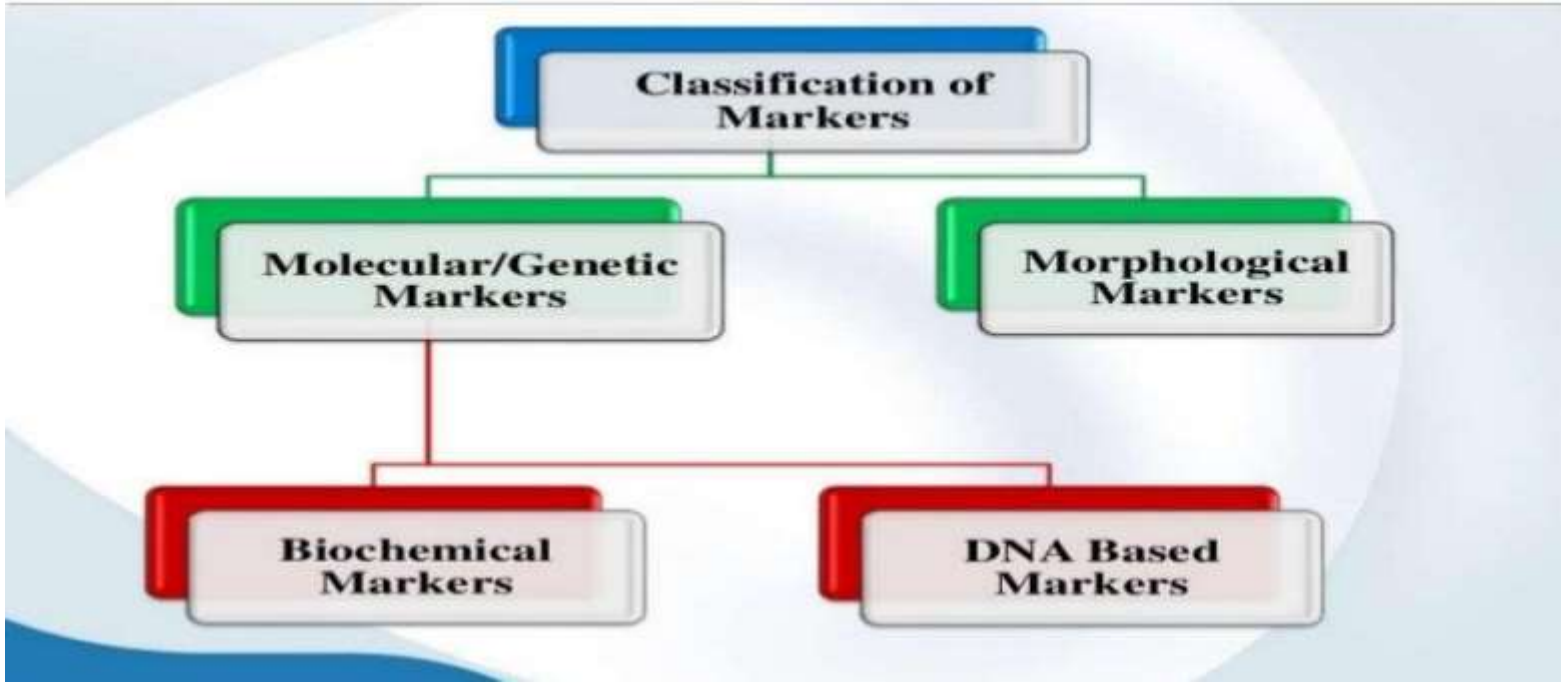
Applications:

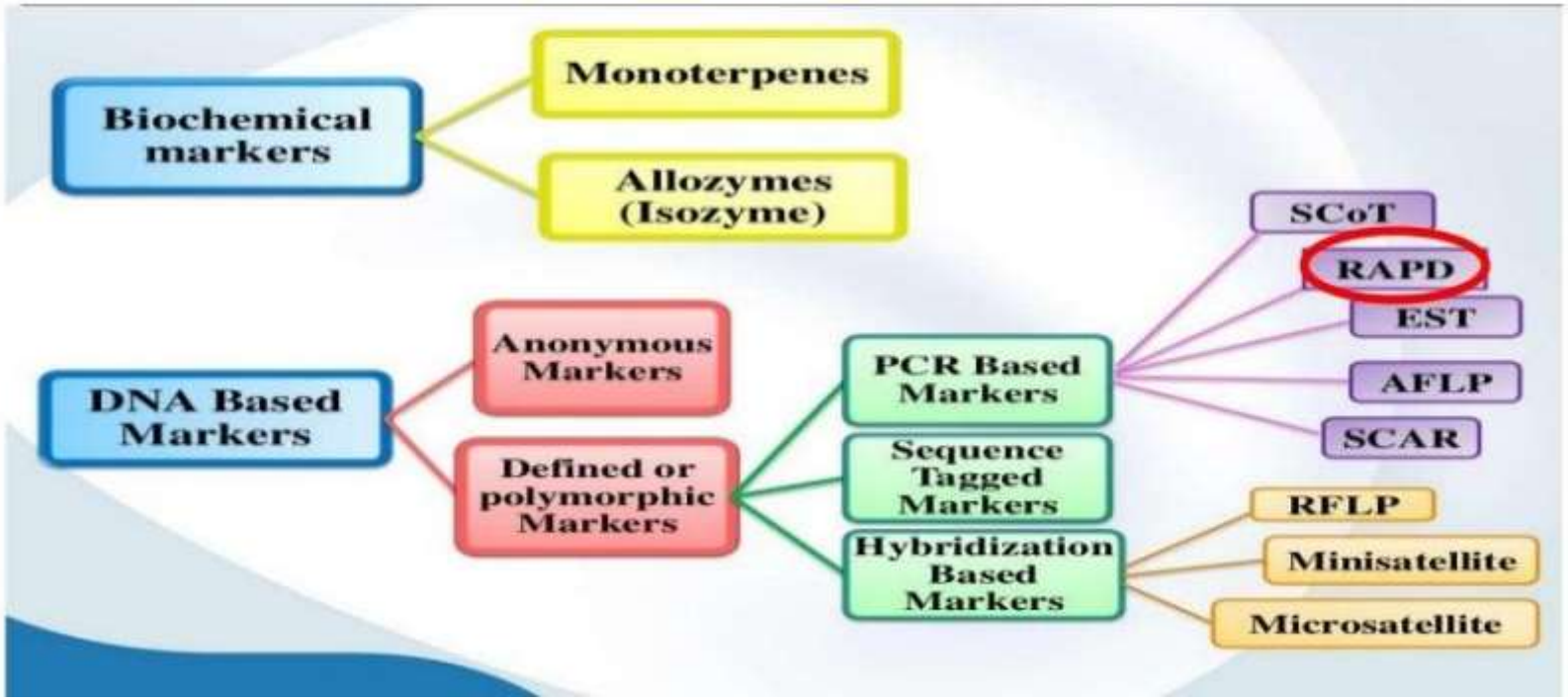
Forensic science

Identification of Genetic Disease.

Differ from one person to another.

Types of Markers.




Biochemical markers
Monoterpenes
Allozymes (Isozyme)
DNA Based Markers
Anonymous Markers
Defined or polymorphic Markers
PCR Based Markers
Sequence Tagged Markers
Hybridization Based Markers
SCoT
RAPD
EST
AFLP
SCAR
RFLP
Minisatellite
Microsatellite

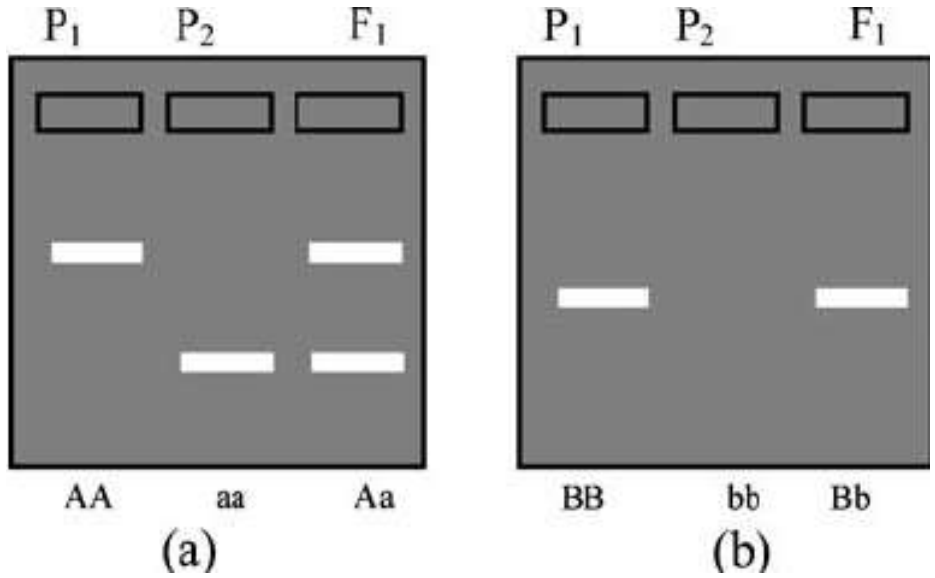
Markers on Basis of Inheritance.

- **DOMINANT MARKER:**

  ISSR,RAPD,AFLP

- **CO-DOMINANT MARKER:**

  SSR,RFLP,STR



Restriction fragment length polymorphism (RFLP)

Hybridization based Marker.

DEFINATION

The variation in the restriction DNA fragments length between the individuals of a species is called restriction fragment length polymorphism (RFLP).

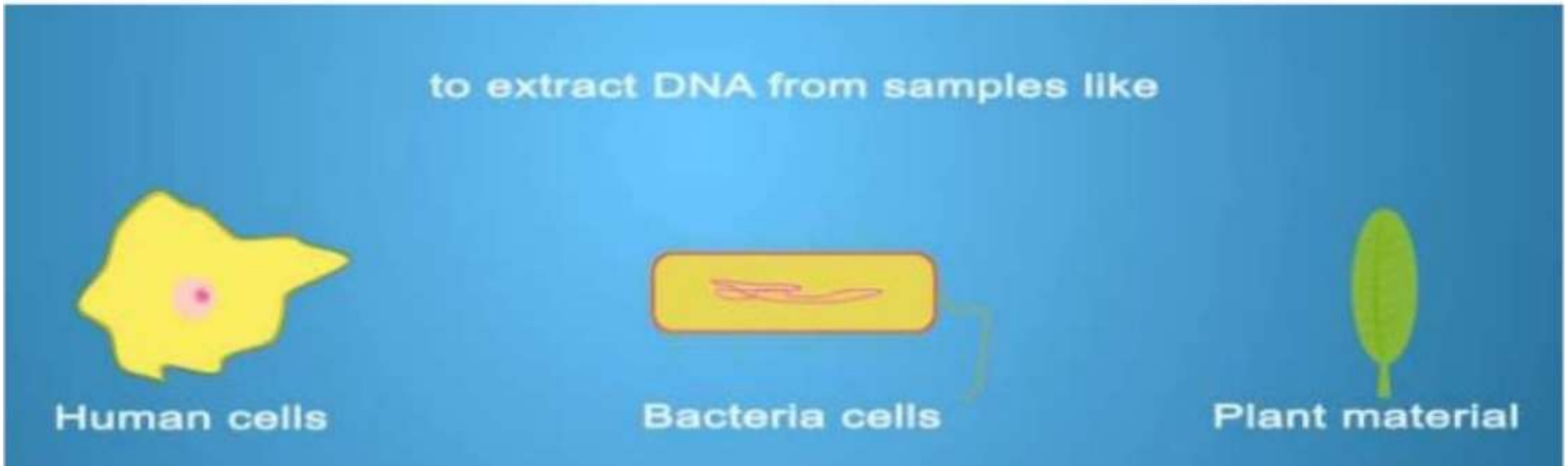
Laboratory techniques to analyze & compare DNA of two or more individuals of a species or of different species.

Principle of RFLP

- RFLP is an enzymatic procedure for separation & identification of desired fragments of DNA.
- Using Restriction endonuclease enzymes fragments of DNA is obtained & the desired fragments is detected by using restriction probes.
- May be used to differentiated two organism by analysis of pattern derive from cleavage of their DNA.

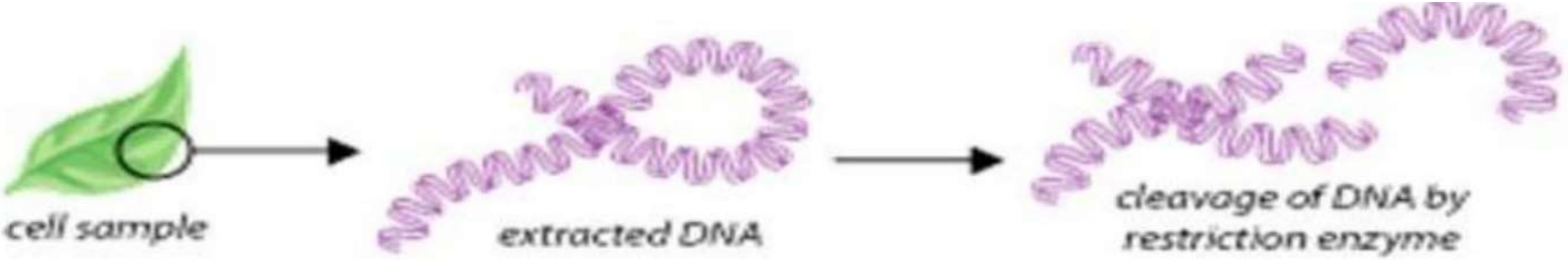
collection of sample

- DNA is extracted from tissue sample



Step II: Restriction digestion

- The DNA in each sample is digested with same restriction enzymes.
- The enzymes RE has specific restriction sites on the DNA, so it cut DNA into fragments.
- Different size of fragments are generated along with specific desired fragments.



Step III: Gel Electrophoresis

The Desired fragments are run in polyacrylamide gel electrophoresis or Agarose gel electrophoresis to separate the fragments on the basis of length or size or molecular weight.

STEP IV: DENATURATION

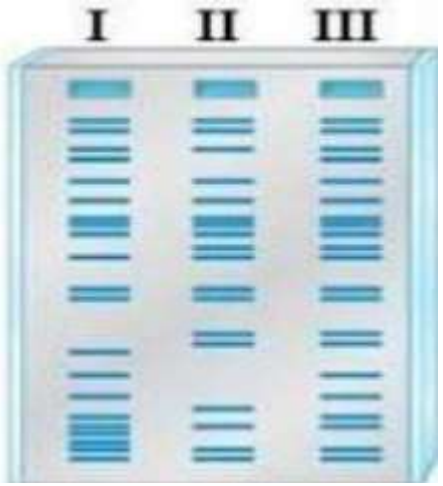
The gel is placed in sodium hydroxide solution for denaturation so that single stranded DNA are formed.

STEP V : Blotting

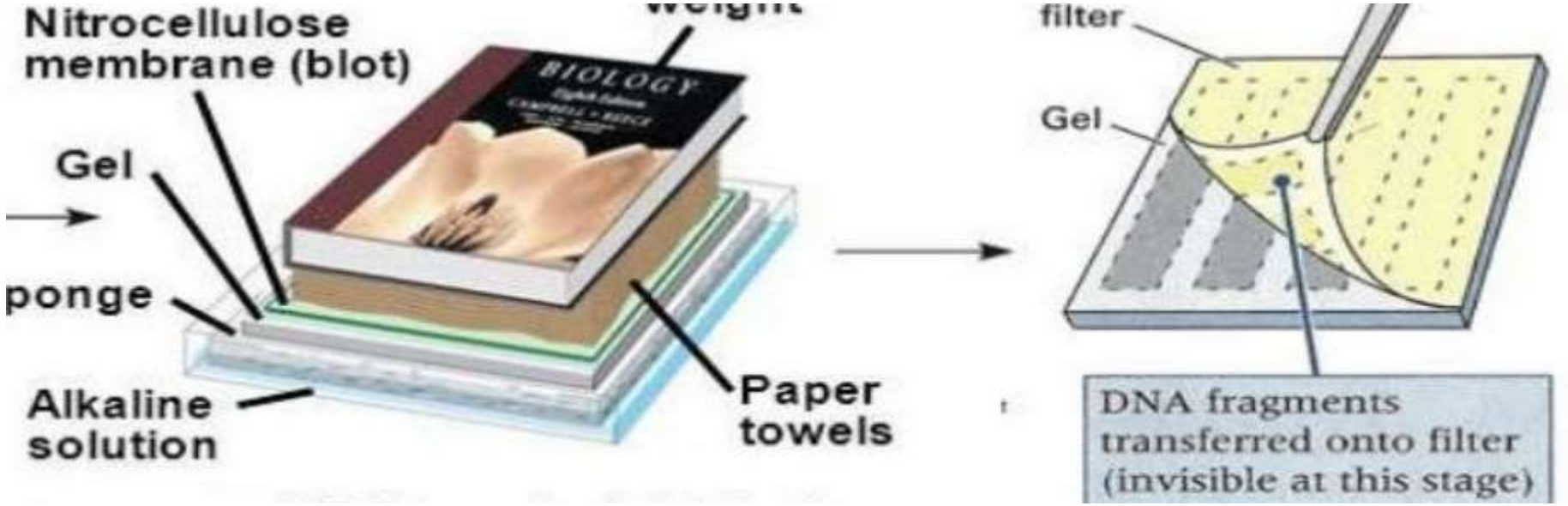
The single stranded DNA obtained are transferred into charge membrane i.e Nitrocellulose paper by the process called capillary blotting (southern blotting) or electro-blotting

STEP VI: Hybridization & visualization.

- The nitrocellulose paper transferred with DNA is fixed by autoclaving.
- Then the membrane is blocked by using bovine serum albuminor caeisin to prevent binding or labelled probe non specifically to the chardged membrane.



GEL ELECTROPHORESIS

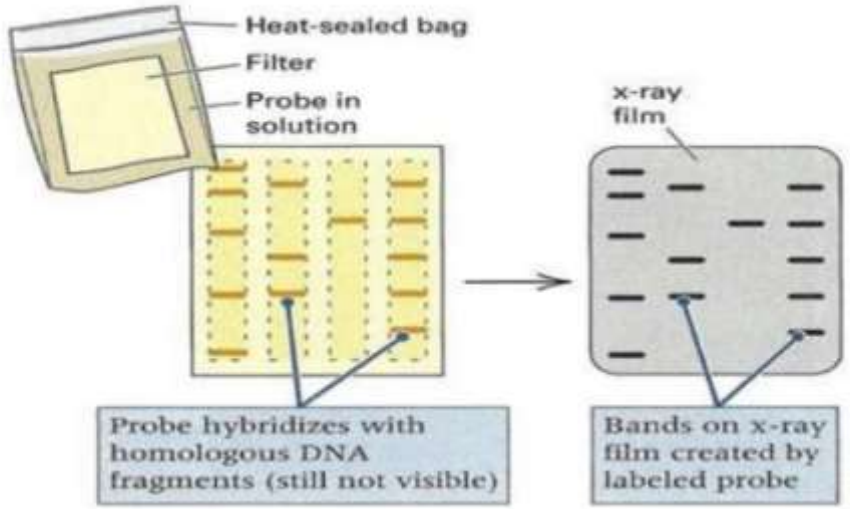


DNA BLOTTING

STEP VI: HYBRIDIZATION & VISUALIZATION

The labelled RFLP probe is hybridized with DNA on the nitrocellulose paper.

The RFLP probes are complimentary as well as labelled with with radioactive isotopes so they form color band under visualization by autoradiography.

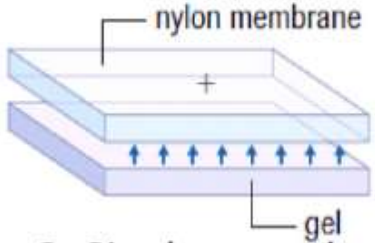


RFLP are identified by Southern blots

1. Sample of DNA **extracted** and put on **gel**.

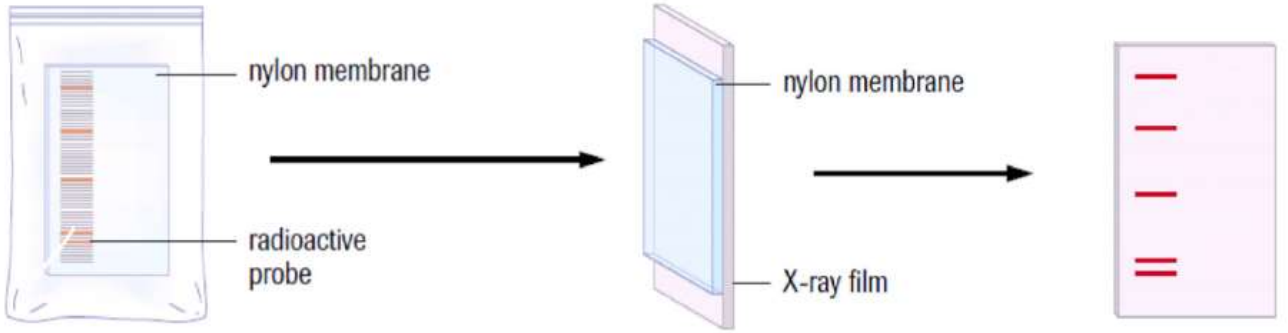
2. Fragments appear as smear.

Gel placed against **nylon membrane** and put in a **denaturing solution**.



3. Single stranded DNA's migrate from gel to nylon membrane. (**Southern Blotting**)

- Nylon membrane is soaked in solution with **radioactive complementary probes.**



4. Radioactive probes **anneal** at specific locations.

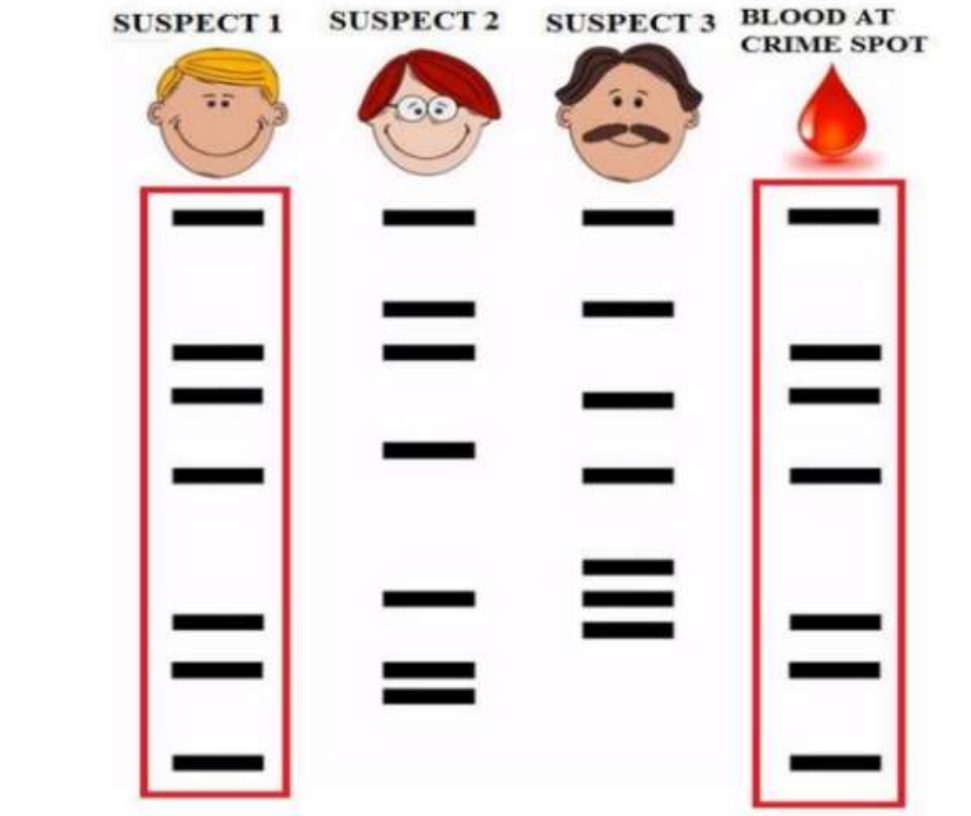
Nylon membrane against an **X-ray**

5. Radioactive probes burn the image in the X-ray film

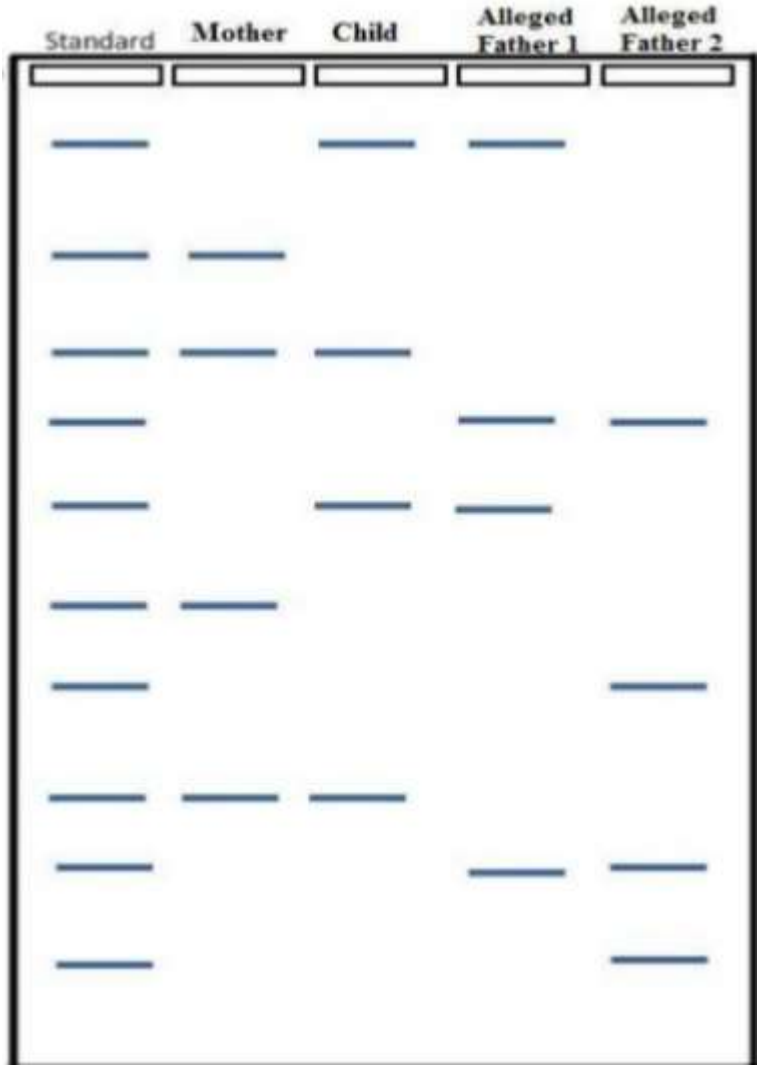
6. Now you have an **autoradiogram**

APPLICATION OF RFLP

1. CRIMINAL TEST



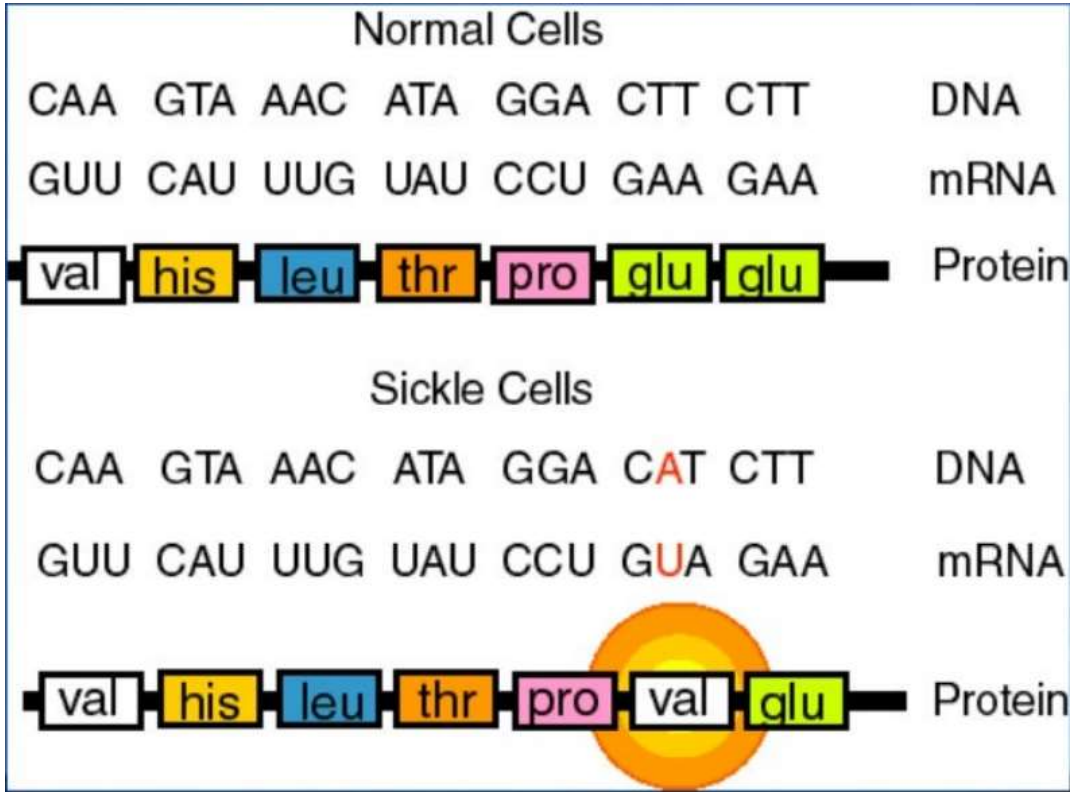
2. PATERNITY TEST



3. DISEASE PROGNOSIS

- RFLP allows investigator to detect defective DNA sequences.

- It can be used effectively in the diagnosis of disease in which specific mutational site.



4. Genetic counselling

- If a particular RFLP is usually associated with a particular genetic diseases, then the presence or absence of that RFLP can be used to counsel people about their risk of developing or transmiting the disease.

APPLICATIONS:

- Very long methodology before results are gained.
- High labour requirements.
- High quality, and large quantities of DNA must be used.
- Must frequently work with radio isotopes.
- Many probes are not available depending on species.
- Too many polymorphisms may be present for a short probe.
- Cost of development is very high due to time, and labour requirements.
- Low frequency of desired polymorphisms in polyploid plants (eg. Wheat).

2. Random Amplified Polymorphic DNA (RAPD)

- Randomly Amplified Polymorphic DNA (RAPD) are genetic markers resulting from PCR amplification of genomic DNA sequences recognized by ten-mer random primer of arbiratary nucleotide sequences.
- RAPD are dominant markers that require no prior knowledge of the DNA sequences, which makes them very suitable for investigation of species that are not well known.
- Set of DNA generated by random PCR is called RAPD

RAPD

- It is a PCR based technology.
- This procedure detects nucleotide sequences polymorphism in DNA
- It is used to analyze genetic diversity of an individual by random primer.
- In RAPD the decamer primer will or will not amplify a segment of DNA depending on the position that are complimentary to a primer sequences.
- If the priming sites are in the amplifiable region a discrete DNA product is formed through cyclic amplification.
- Amplified product are then separated on agarose gel electrophoresis & view under UV.

STEPS:

Genomic DNA Isolation / Cells or tissues .

↓

Keep the tubes in PCR thermo cycler.

↓

Denature DNA

↓

94^{0C} 1 minutes

↓

DNA Strands separated.

↓

Annealing of primers.

↓

Complementary DNA Synthesis (35 -45 cycles).

↓

Amplified product separated by Gel Electrophoresis

Application

- **RAPD** is used as Genetic markers for constructing genetic maps of higher organisms.
- **Example** – *pines, rice* etc.
- **RAPD** analysis help as to identify genes of high economic values through comparison of RAPD fragments .
- **Example**- *rice, wheat, maize , pea* etc.
- **RAPD** Markers help to determine specific genes in chromosomes.
- **RAPD** can be used for identification of somatic Hybrid among the developing regenerates .
- **RAPD** can be used for evaluation &character of genomic resources .

SSR (Simple sequence repeat) /Microsatellite

- Known as Simple Sequence Repeats.
- Microsatellites sequences are especially suited to distinguished closely related genotypes, because of their high degree of variability, they are, therefore favoured in population studies.
- Microsatellite polymorphisms can be detected by Southern hybridisation or PCR
- If nucleotide sequence in the flanking regions of the microsatellites are known, specific primers can be designed to amplify the microsatellite by PCR
- Microsatellites may be identified by screening sequences databases, polymorphism can be detected by gel electrophoresis.

ADVANTAGES.

- Low quantities of template DNA
- The strength of microsatellites is high & analyses do not require high quality DNA.