FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

IV. RESTRICTION ENDONUCLEASE DIGESTION

•Also called as molecular scissors

•DNA is cut into fragments with restriction enzyme(RE) based- on nucleotide sequence

•Nowadays many artificial RE are also used

How does it work????

•Restriction enzymes attach to DNA and are activated by restriction sequences in the DNA. Once activated, the restriction enzymes hydrolyse and destroy the bonds between nucleotides. The restriction sequences along the DNA are inherited, thus, people who are related have similar restriction sequences along their DNA.

V. SEPARATION OF DNA SEQUENCE

•Fragments are separated according to molecular size using agarose gel electrophoresis

•DNA fragments are injected into wells and an electric current is applied along the gel.

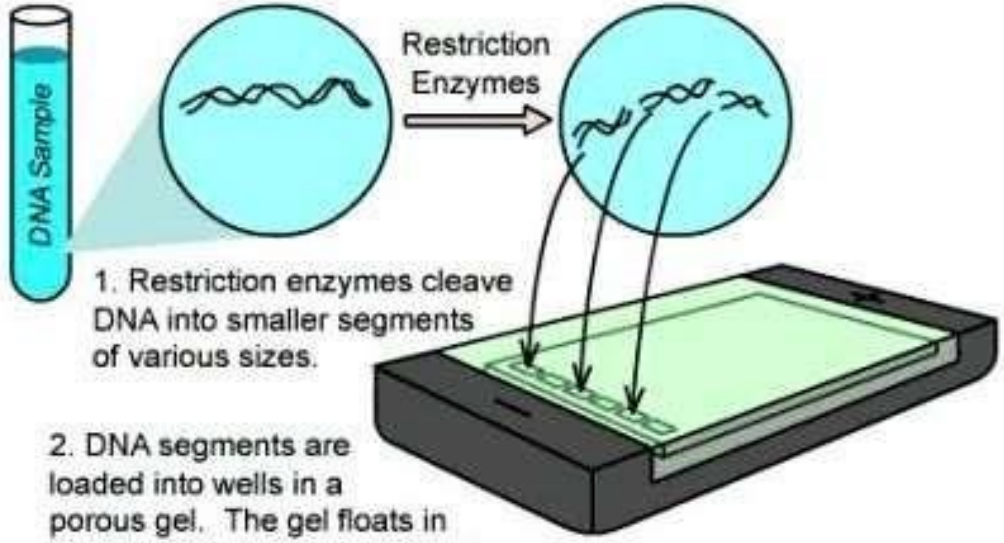
•Electrical current carries negatively-charged DNA through gel towards positive (red) electrode

•Distance moved in given time will depend on mass of molecule of fragment

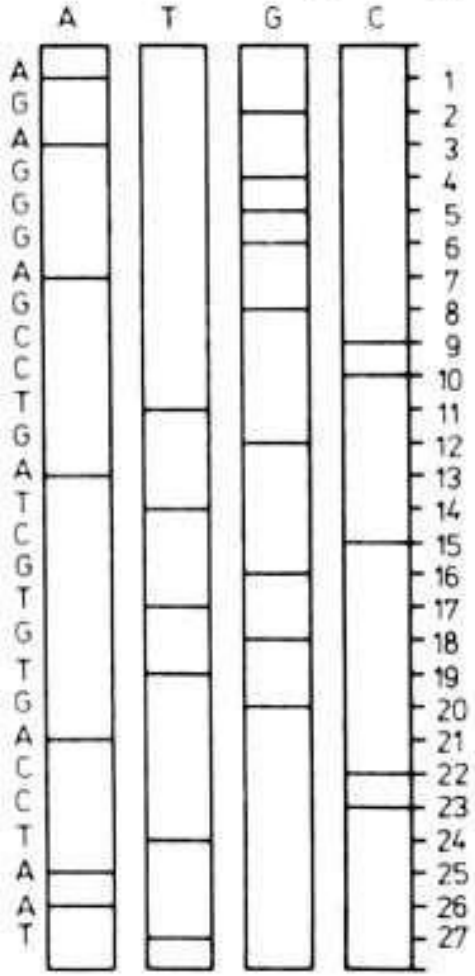
•The separated fragments are visualized by staining them(Ethylene bromide, methylene blue, nile blue A) or by using complementary gene probe

{Gene probe:- single stranded piece of DNA with abase sequence complementary to the DNA that you wish to identify and it must be labelled}

Gel Electrophorsis Procedure



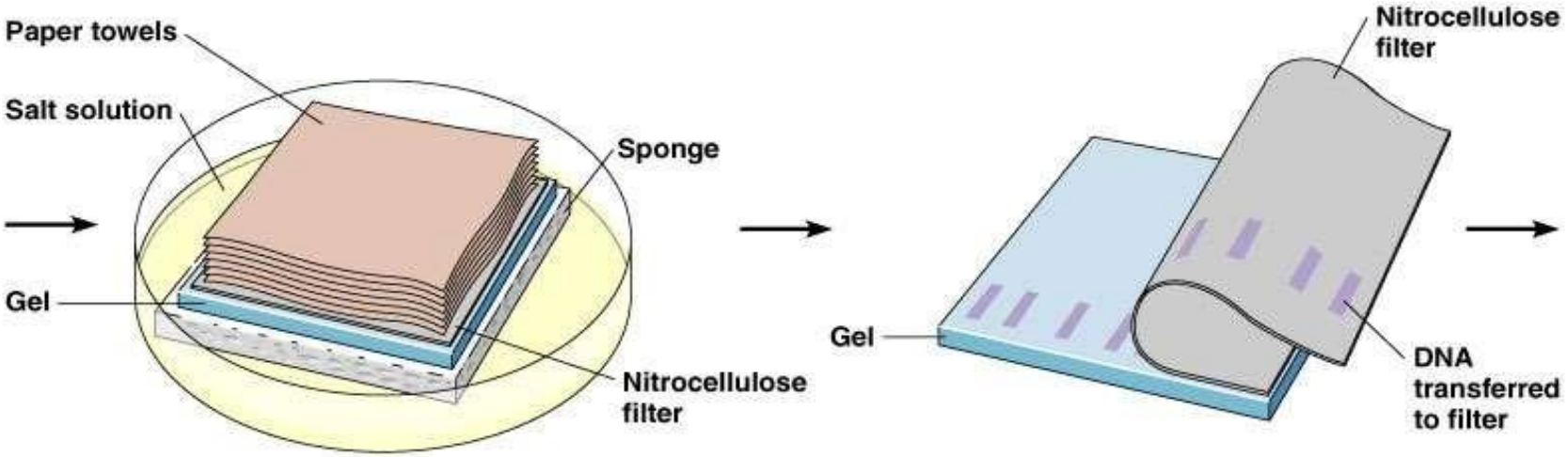
Maxam-Gilbert Sequencing



VI. SOUTHERN BLOTTING

•Separated DNA sequences are transferred on a nitro cellulose or nylon membrane

•They stay immobile on the sheet. The s

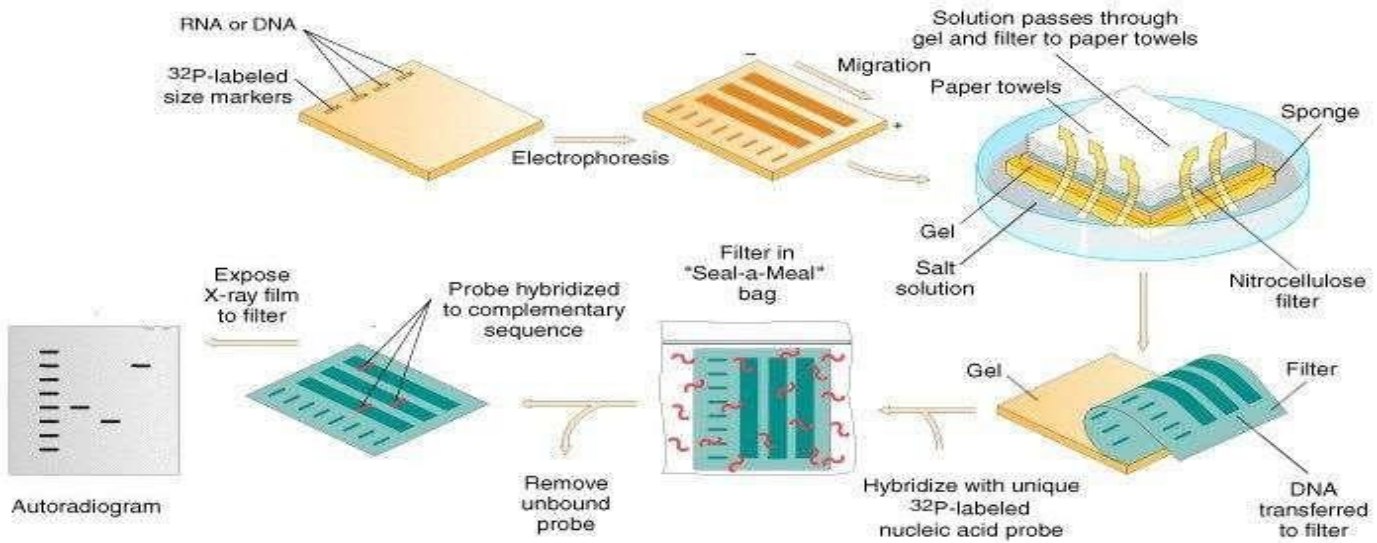


3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.

4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.

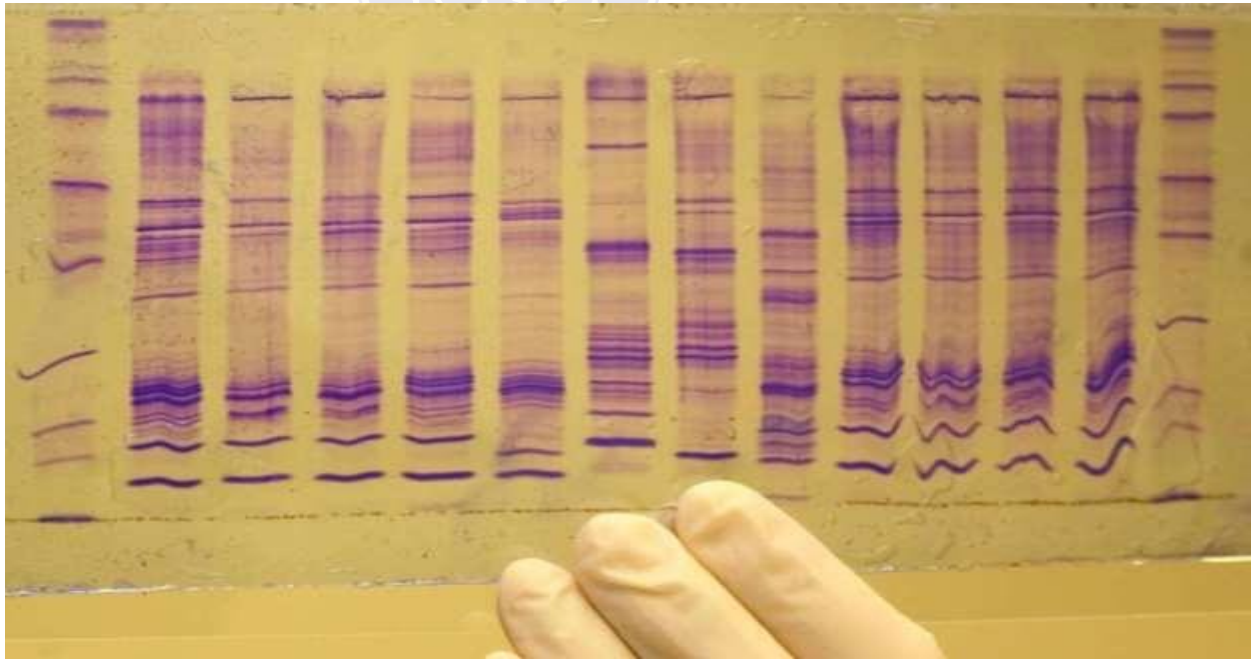
HYBRIDIZATION

- The nylon membrane is immersed in a bath
- Radioactive probes are added
- These probes target a specific nucleotide sequence of the single DNA strand on the membrane that is complimentary to them
- When the sequences of the target DNA probe are determined then it is labelled so that it can be identified. The tagging is done by a fluo[illegible] disti[illegible]



AUTORADIOGRAPHY

- When the probe is labelled, now it can be taken out of the nylon membrane and can be viewed through autoradiography on the x-ray film.
- The nylon membrane is pressed on the x ray film
- Dark bands are developed at the probe site which resembles the bar codes



DIFFERENT METHODS OF DNA FINGERPRINTING

1.Restriction fragment length polymorphism (RFLP)

2.Randomly amplified polymorphic DNA (RAPD)

3.Amplified fragment length polymorphism

(AFLP) 4.Simple sequence repeats (SSR)

- Forensic science
- Paternity and Maternity determination
- Personal identification
- Application to both plants and animals
- To identify genetic diversity with in breeding populations
- To differentiate between plant species cultivars
- To identify plants containing gene of interest
- To detect genetically modified organism in agriculture
- used for identify species or population
- used for estimating genetic distance and finger printing of wheat
- biological parentage
- used for characterization & determination of genetic diversity of tea germplasm

- Confirms the morphology and growth behavior of suspected hybrid .
- Effect of habitat fragmentation
- Extend of gene flow within and among populations.
- Reveal the extend of clonal growth
- Spontaneously occurring somatic mutation