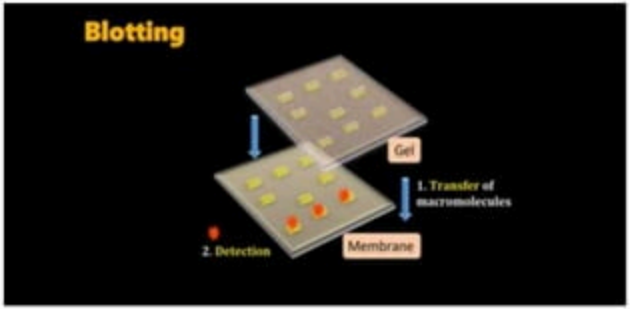
SOUTHERN BLOTTING

Harini.V
2nd MSC Zoology,
Queen Mary's College.

BLOTTING

- Technique for transferring DNA, RNA & PROTEINS onto a carrier so they can be separated , and often follows the use of a gel electrophoresis.



TYPES OF BLOTTING

Blotting technique

Southern Blot

It is used to detect DNA.

Northern Blot

It is used to detect RNA.

Western blot

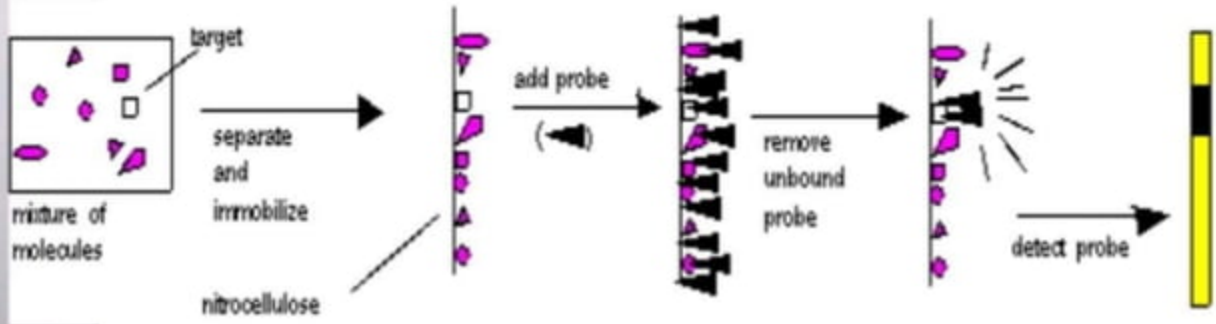
It is used to detect protein.

SOUTHERN BLOTTING

- The Technique was developed by E.M. SOUTHERN in 1975.
- The southern blot is used to detect the presence of a particular DNA fragment in a sample.

PRINCIPLE

- The key to this method is HYBRIDIZATION.
- It is a process of forming a double stranded DNA molecule between a single stranded DNA probe and a single stranded target DNA.


target
mixture of
molecules
separate
and
immobilize
nitrocellulose
add probe
remove
unbound
probe
detect probe

STEPS

1st isolation and purification of dna from cells

2nd Restriction digestion

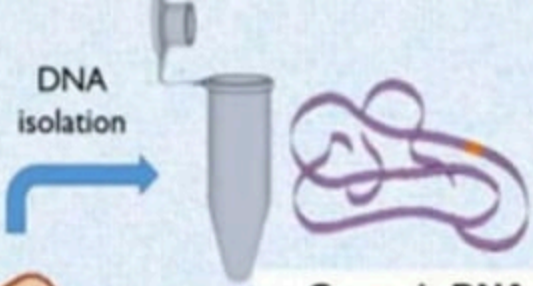
3rd Gel electrophoresis

steps

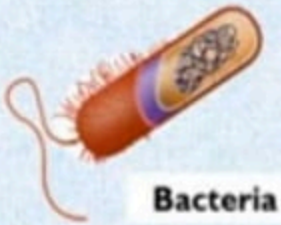
- 4th and 5th denaturation and blotting
- 6th hybridization
- 7 and 8th wash and autoradiography

1) DNA ISOLATION AND PURIFICATION

- ISOLATION AND PURIFICATION OF DNA FROM CELLS
  - incubate cells with detergent to promote cell lysis.
  - lysis free cellular proteins and DNA.
- proteins are enzymatically degraded by incubation with proteinase.
- DNA is purified from solution by alcohol precipitation'
- Visible DNA fibers are removed and suspended in buffer.



Genomic DNA
(of bacteria)



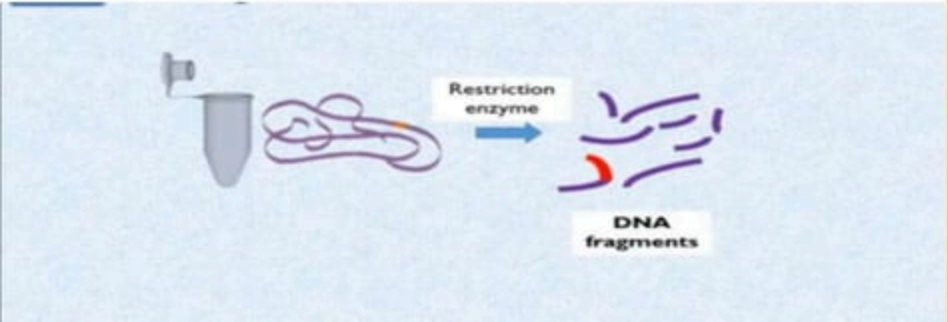
Bacteria

Find out the presence of a specific DNA sequence in the bacterial genome.

?

2) RESTRICTION DIGESTION

- Cut the DNA into different sized fragments using Restriction endonucleases. Eg: *EcoRI*

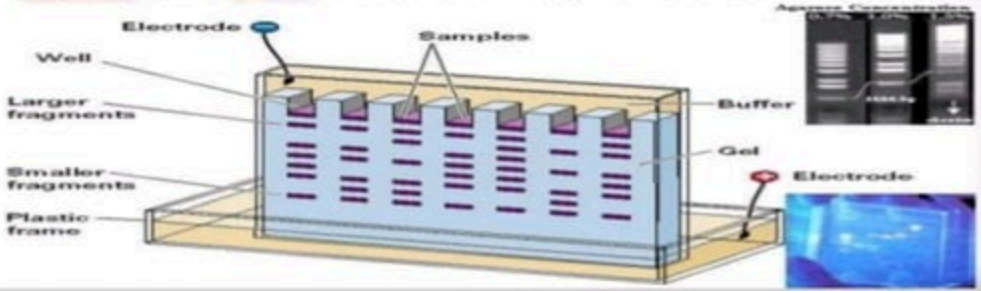


3) GEL ELECTROPHORESIS

- Technique for separation of DNA fragments by size.
- Gel- AGAROSE

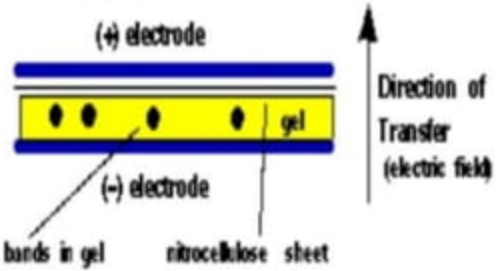
3. Separated by electrophorosis.

- The complex mixture of fragments is subjected to gel electrophoresis to separate the fragments according to size.

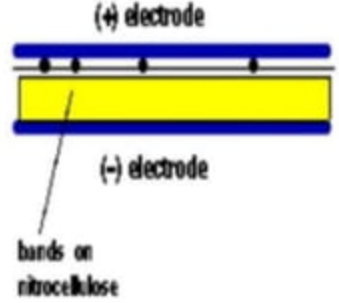


Side View:

Before Transfer:



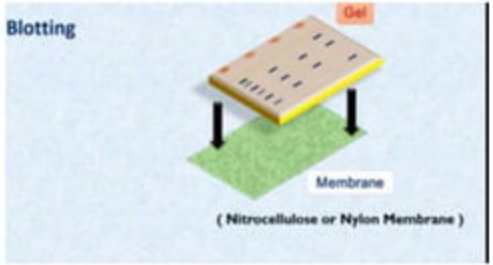
After Transfer:



Note: All the layers are pressed tightly together.

STEP 4& 5 – DENATURATION AND BLOTTING

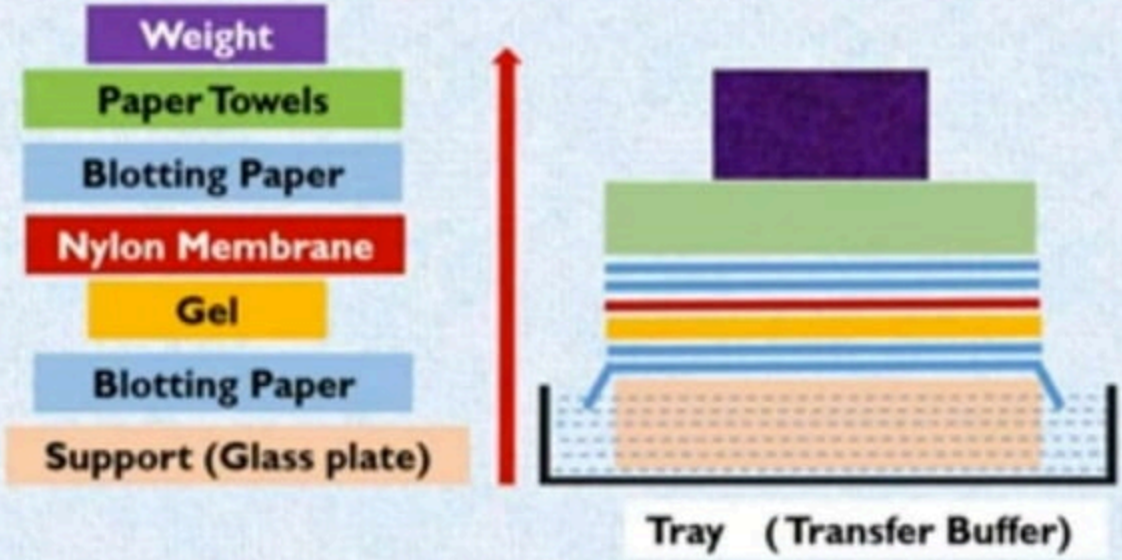
- DNA is denatured with an alkaline solution such as NAOH.
- DNA is then neutralized with NaCl to prevent re-hybridization before adding the probe.



- BLOTTING- transfer of DNA bands from the gel to a nitrocellulose membrane.

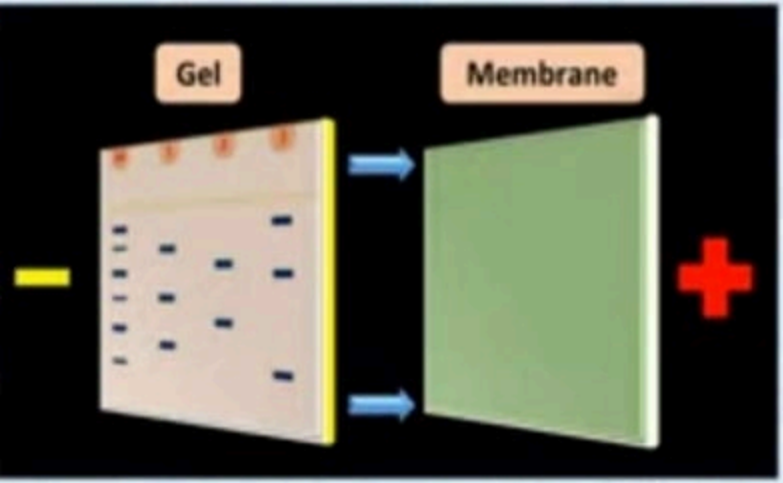
Blotting

Traditional Method (Capillary Action)



Blotting

Electrophoretic Transfer



6) HYBRIDIZATION

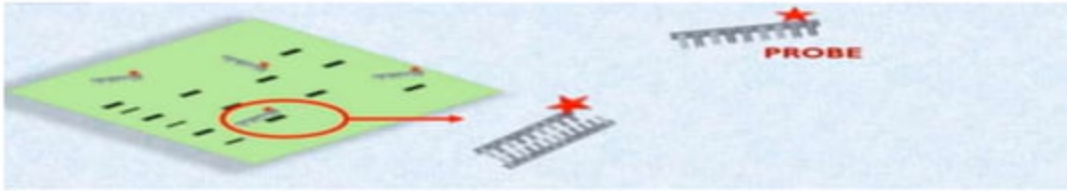
- The labelled probe is added to the membrane in buffer and incubated for several hours to allow the probe molecules to find their targets.

- PROBE – small piece of labelled DNA used to find complementary DNA fragment.

7th & 8th WASH AND AUTORADIOGRAPHY

❑WASHING

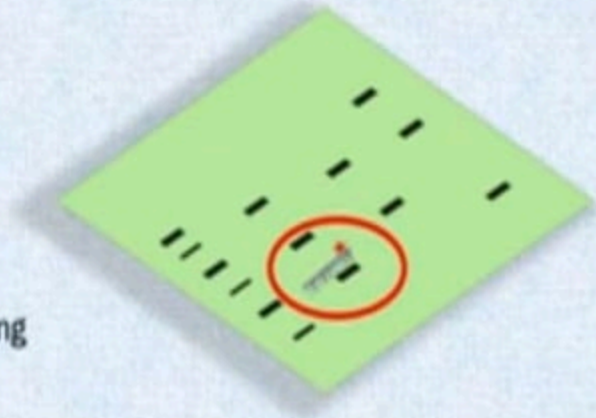
Unbound probes are washed out



- For radioactive probes , x-ray FILM is placed over the membrane.
- After development, there will be dark bands on the film wherever the probe bound.

Detection

- Autoradiography
- Fluorescence
- Colour or Light forming reactions



SUMMARY

extract and purify DNA from cells

DNA is restricted with enzymes.

separated by electrophoresis

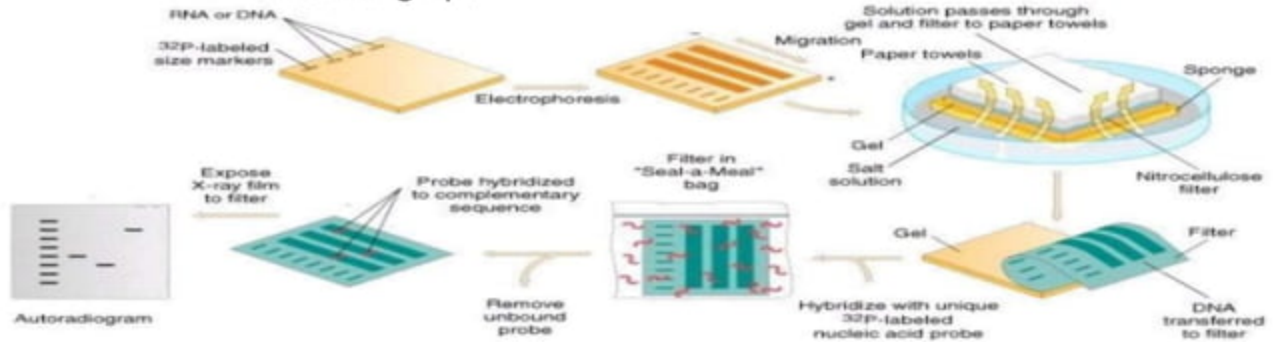
denature DNA

transfer to nitrocellulose paper (blotting)

add labelled probe for hybridization to take place

wash off unbound probe

autoradiograph



APPLICATIONS

- TO identify specific DNA in a sample
- Isolate desired DNA for making rDNA
- Diagnosis
- Used in phylogenetic analysis
- DNA Fingerprinting like personal identification, criminal identification, forensics, …

ADVANTAGES

- Effective way to detect a specific DNA sequence in a large, complex sample of DNA.
- Can be used to quantify the amount of the present DNA.
- Cheaper than DNA sequencing.

DISADVANTAGES

- More expansive than most other tests.
- Complex and labor-intensive.
- Time consuming and cumbersome.

THANK YOU