BHARATHIDASAN UNIVERSITY

Program: M.Sc., Biomedical Science

Course Code : 18BMS47C10

Course Title : Genetic Engineering

Southern Blot

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Dept. of Biomedical Science

Northern, Southern, Western

Ed Southern:

Possibly regrets this photo

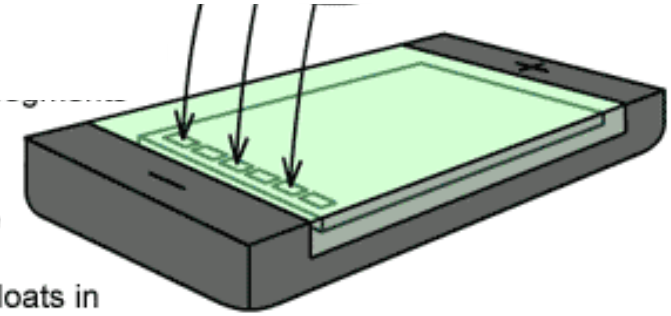
In the 1970s Ed Southern of Oxford University invented a revolutionary DNA blotting technique.

The Southern Blot allows the visualization of one DNA fragment from a whole genome DNA extract.

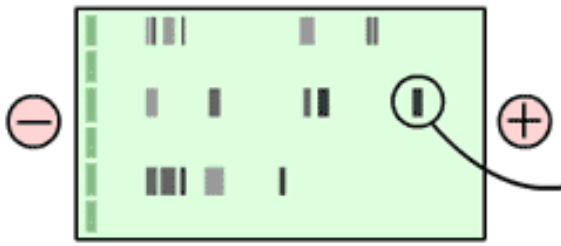
Electrophoresis

- We can separate DNA and RNA molecules by size using agarose gel electrophoresis

DNA (or RNA) samples loaded into wells

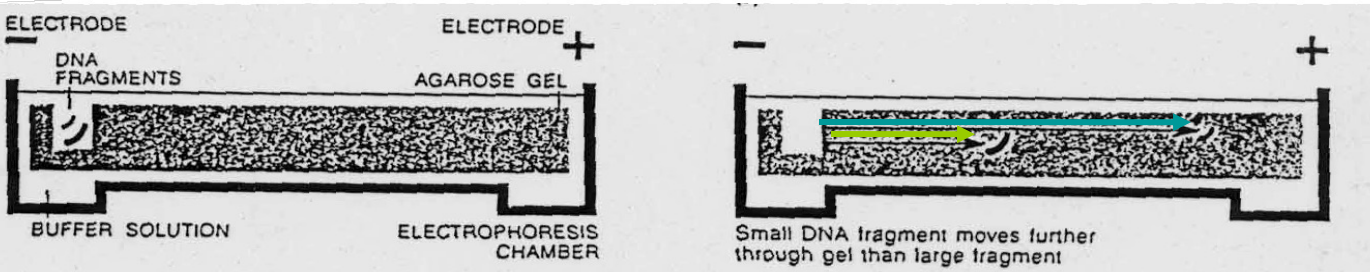


2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.

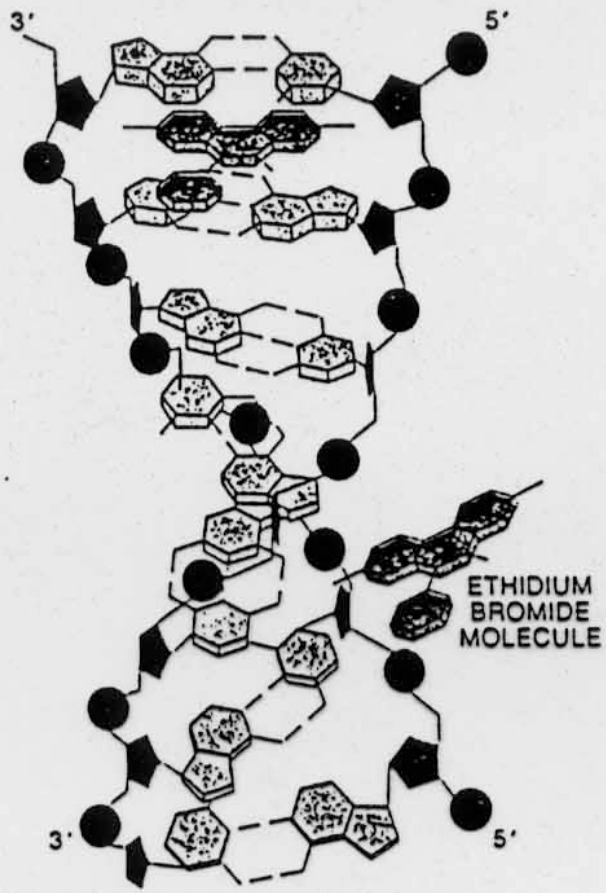


3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.

4. Smaller DNA segments move faster and farther than larger DNA segments.

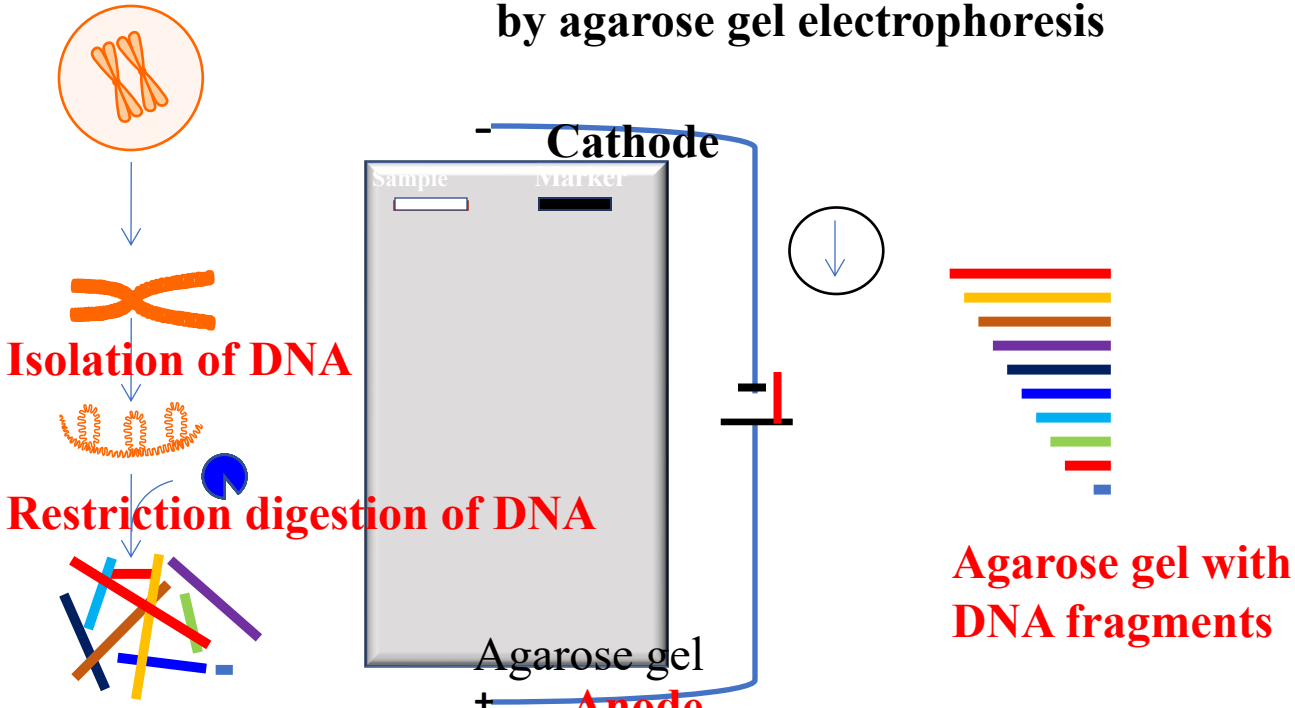


Agarose Gel Electrophoresis of DNA Fragments
(Art concept developed by Lisa Shoemaker.)



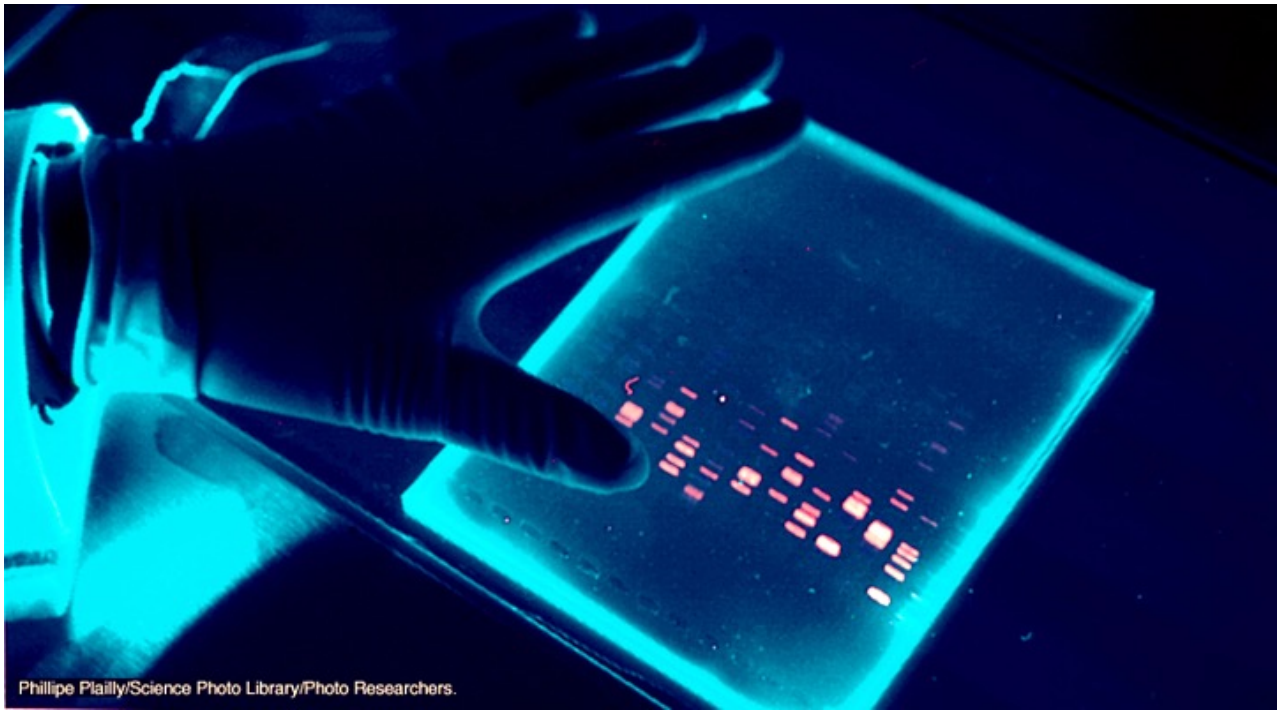
Intercalation of Ethidium Bromide into DNA Helix

Resolving DNA fragments according to their molecular size by agarose gel electrophoresis

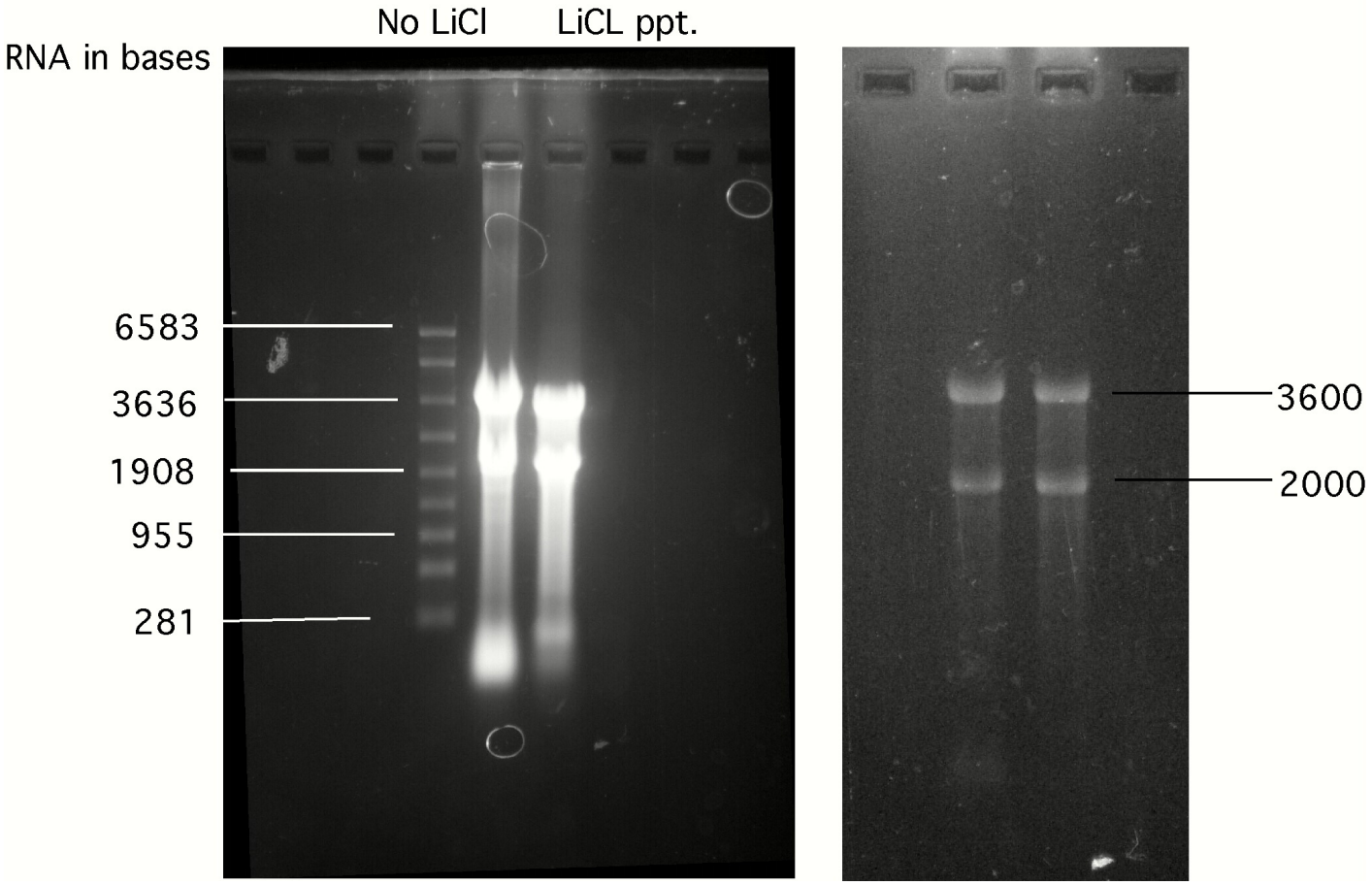


Restriction fragments of DNA

Ethidium bromide is fluorescent in UV light



RNA Gel



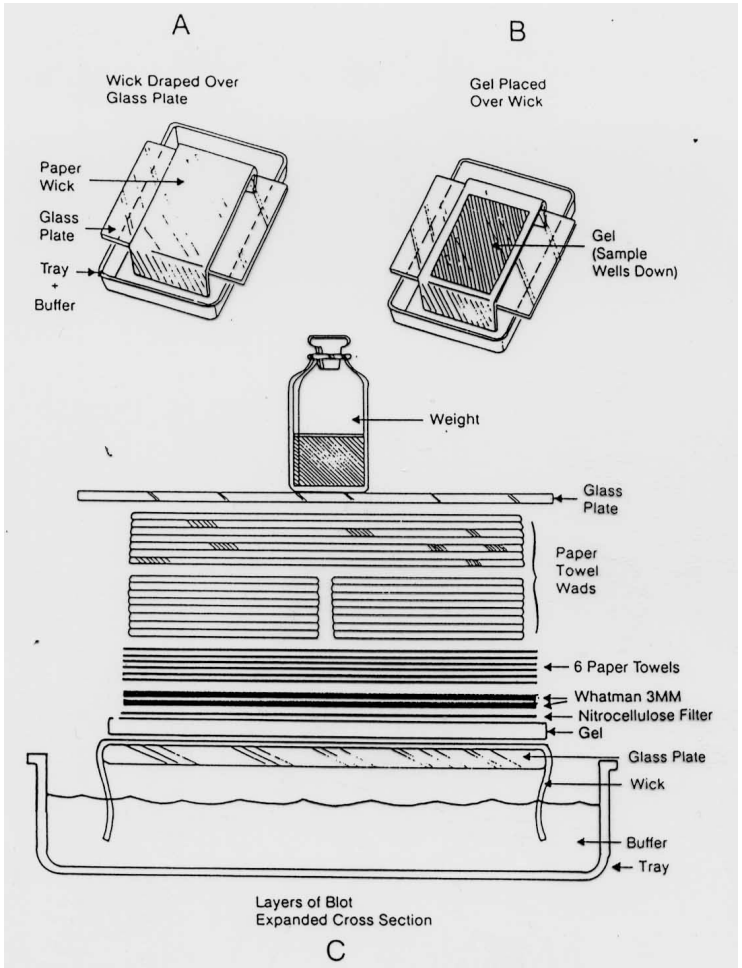


Figure 5.3
Preparation of a Southern or Northern blot. (A) Position of wick over glass plate. (B) Gel is placed on wick. (C) Schematic diagram of final layered organization of materials.

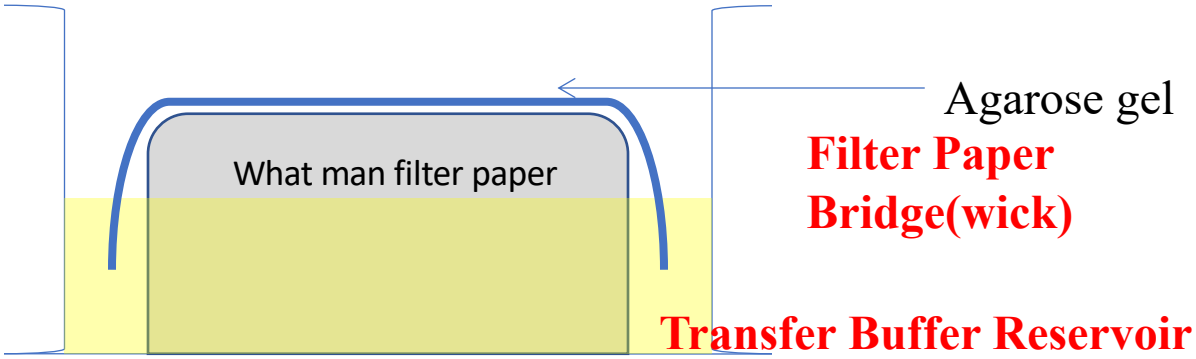
Denaturing solution
(NaOH)

Neutralizing solution
(Tris-Cl pH 7.4)

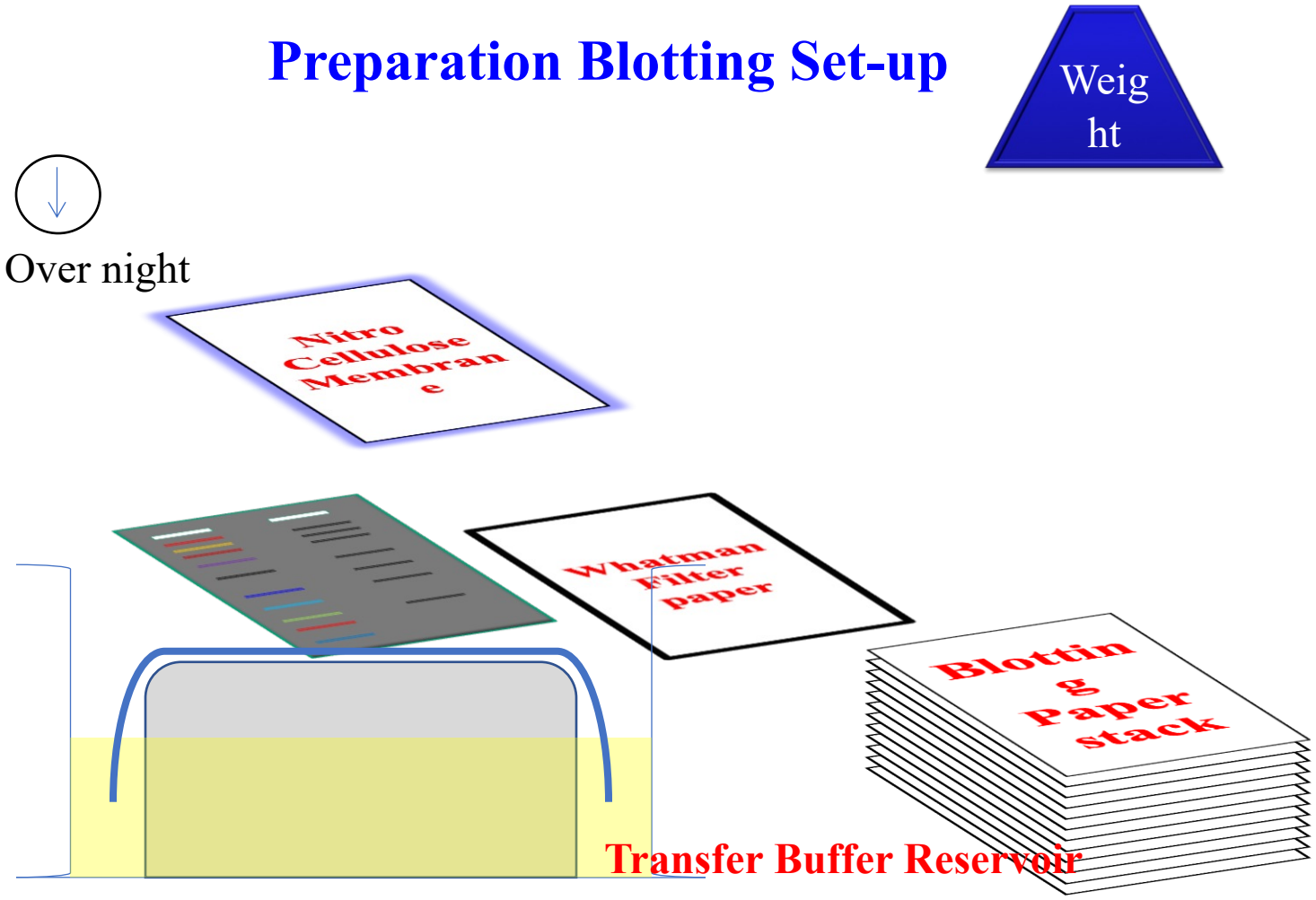
Single Strand DNA

Denaturing double strand DNA into Single Strand DNA by NaOH solution

Southern Blot Transfer Unit

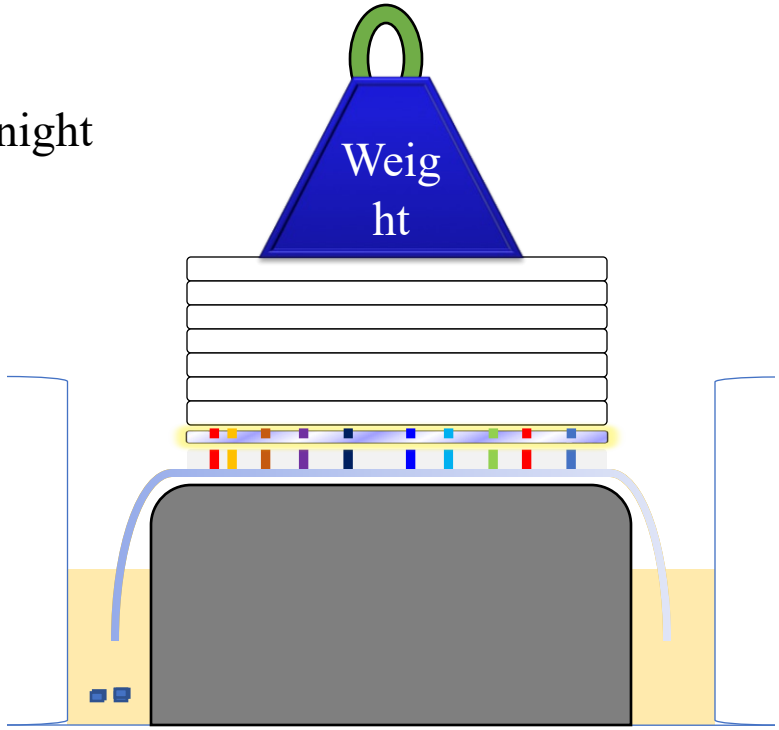


Preparation Blotting Set-up

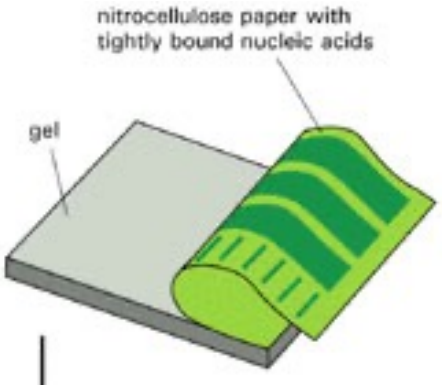


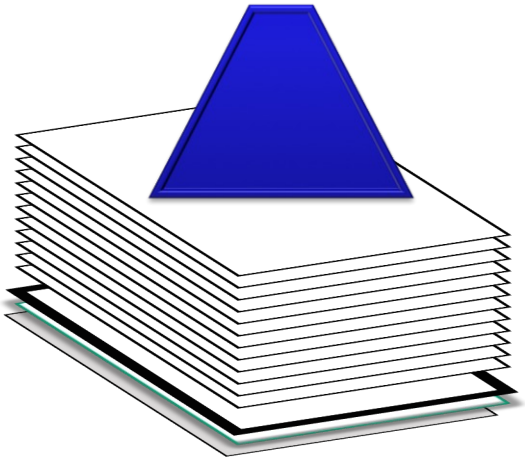
Southern Blot Transfer Process

Over night

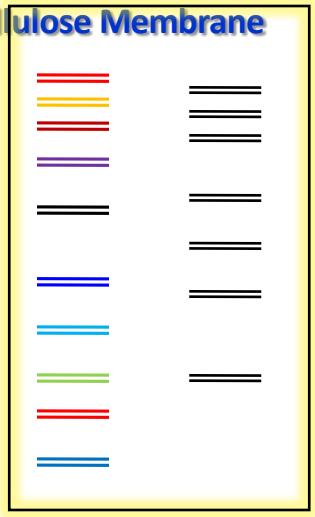


Paper towel sucks the buffer from Reservoir through Whatmann paper, Gel, nitrocellulose membrane, and then blotting paper towel





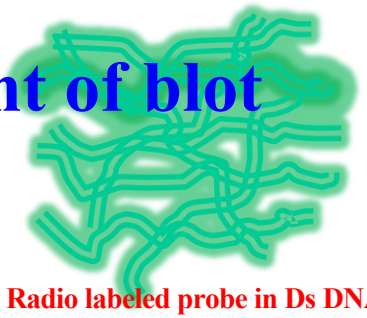
Nitrocellulose Membrane



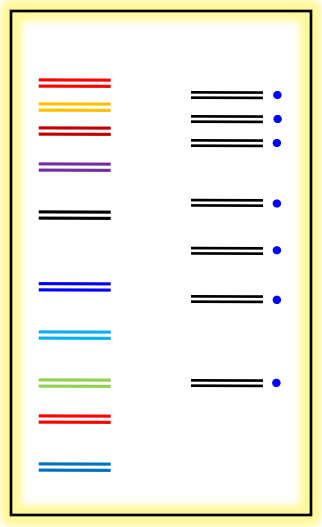
Agarose gel

Hybridization and Development of blot

Hybridzation solution



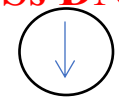
Radio labeled probe in Ds DNA



Blot with bound and unbound probes

Denatured Ss DNA is hybridized with Probe

A



Over night

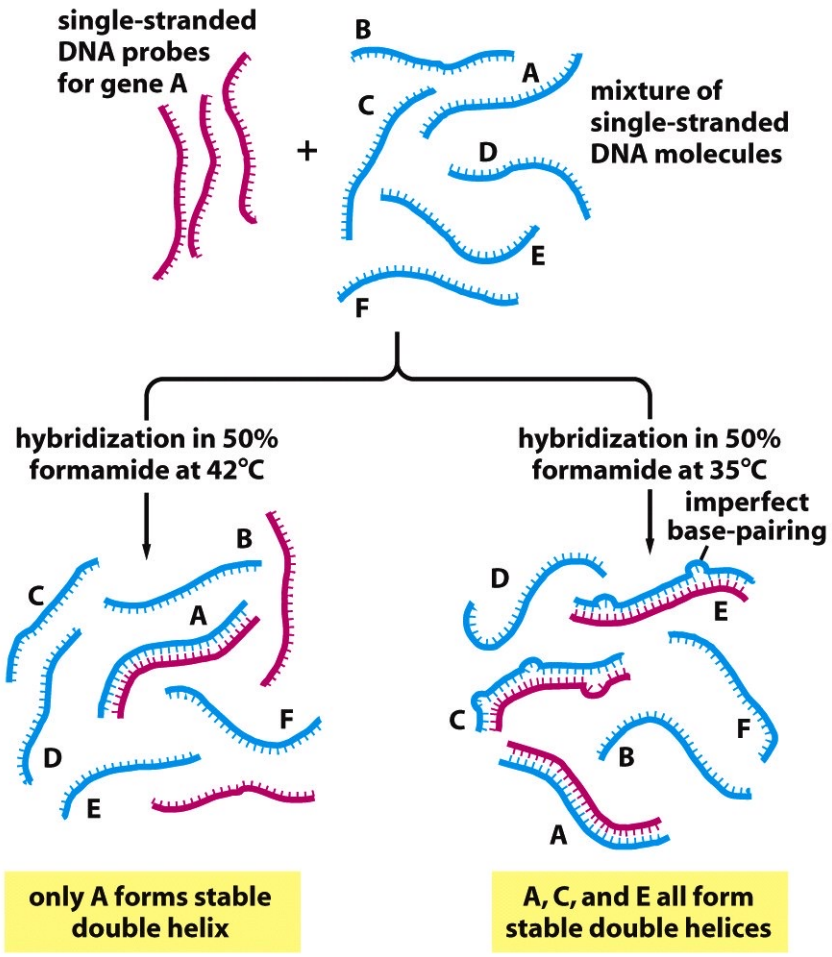
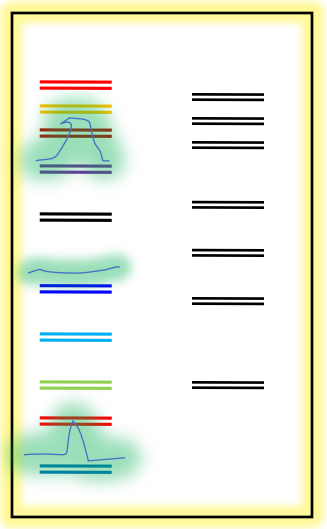


Figure 8-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Removing Non-specific bindng

45°C

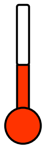
65°C



Specific DNA bound with probe

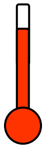
Washing @ 45°C to remove non-specific DNA and probes

Remove non specifically bound probe by washing at higher temperature or low salt solution



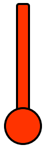
45^{o}C is enough to denature this

```
TGACGCATGCCCCCC CACTGCTAGCTGCACGAACCGGAATGCTGTGCATGCTT
| | | | | | | | |     |   |       | | | | | | | | | | |       | |       | | | | |
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```



60^{o}C is need to denature this

```
TGACGCATGCACCCACTGCACTAGCTGCTCGTTGGCCAATCGACTCGATGCAA
| | | | | | | | |     | | | | | | | | | | | | |  | | | | | | | | | | | | | |   | | | | | |
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```

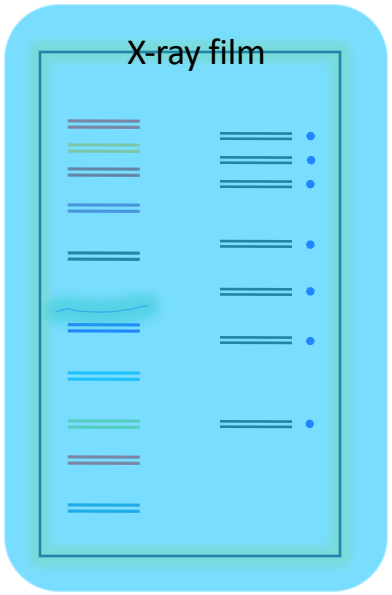


100^{o}C is need to denature this

```
TGACGCATGCAGGCACTGCACTAGCTGCACGTTGGCCAATCGACTGCATGCAA
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```

Development of Autoradiogram for blot

2 hr to overnight exposure



Exposure of blot to X-ray film for 2hr to overnight

Super imposition of blot and X-ray film