AGAROSE GEL ELECTROPHORESIS

Aim:

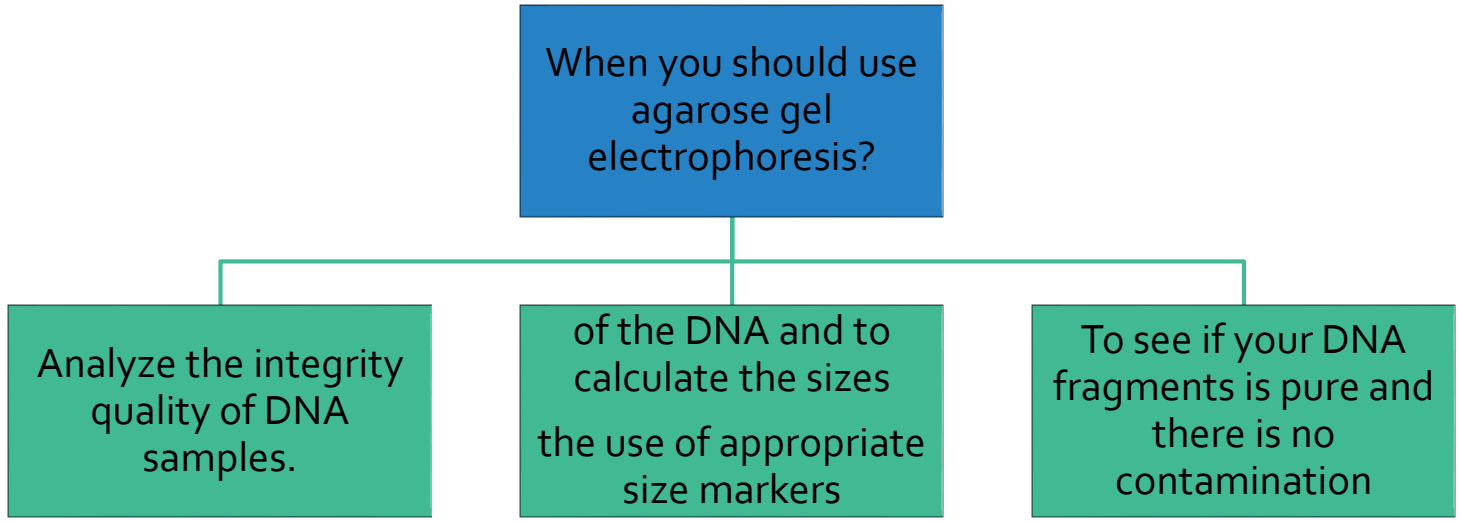
To calculate the molecular size of DNA fragment by comparing the separated band with known standard molecular weight marker.

To quantify DNA fragment by comparing the separated band with known quantity of DNA.

To obtain the purity of DNA preparation.

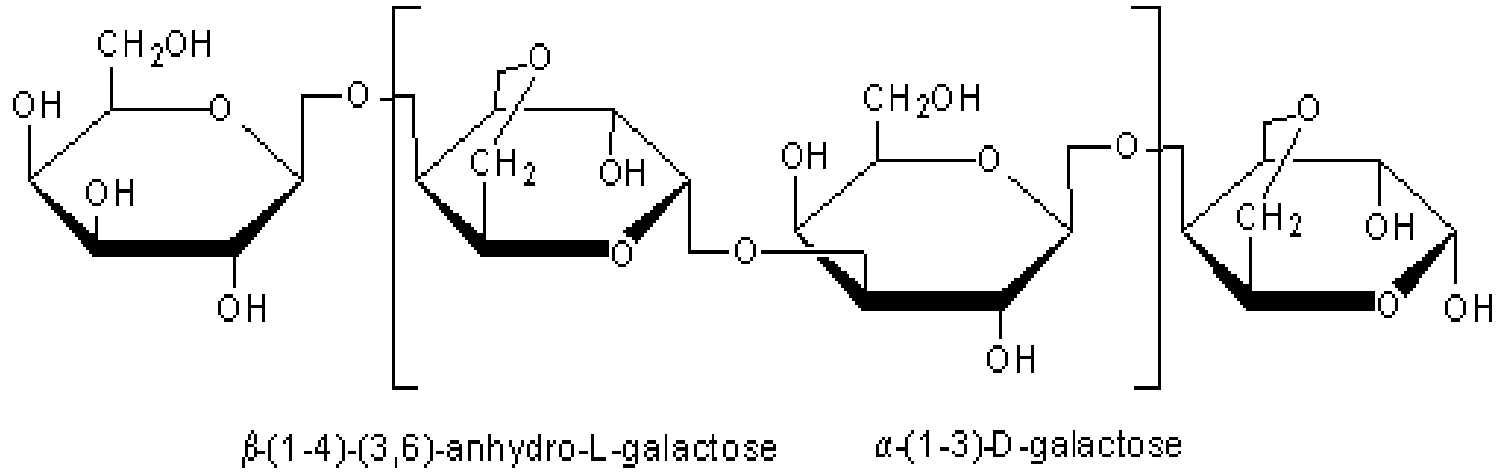
Agarose gel electrophoresis

- Is a method of gel (made of agarose) electrophoresis used to separate and analyze DNA or RNA molecules **by size**



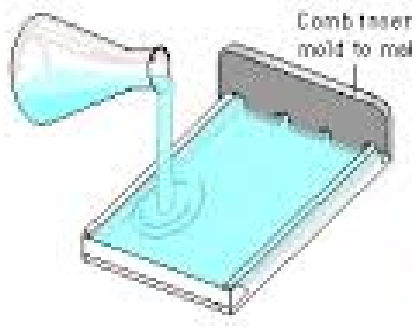
Agarose

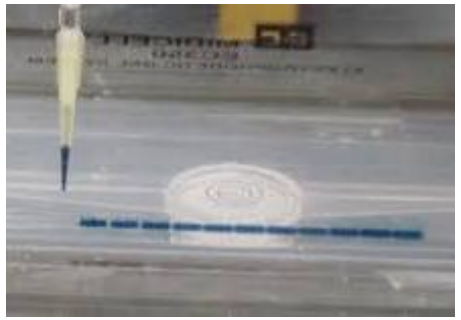
- Agarose is a linear polysaccharide made up of the basic repeat unit agarobiose, which comprises alternating units of galactose and 3,6-anhydrogalactose

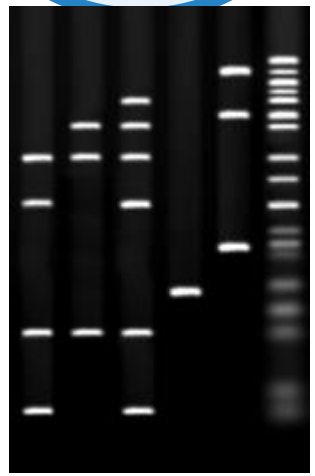


Agarose gel electrophoresis

Gel preparation → Load the running buffer → Loading the sample → Running the gel → Visualize the sample





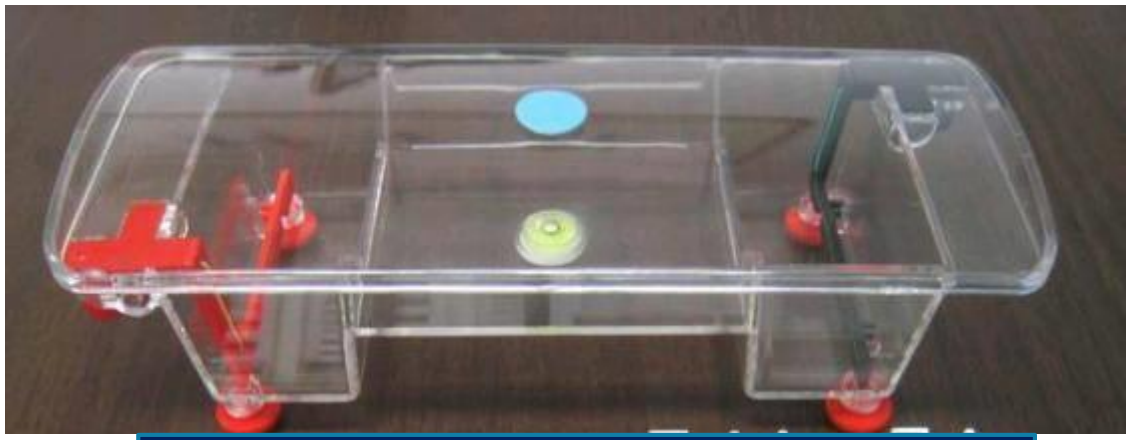


Components:

Comb

Gel casting trays

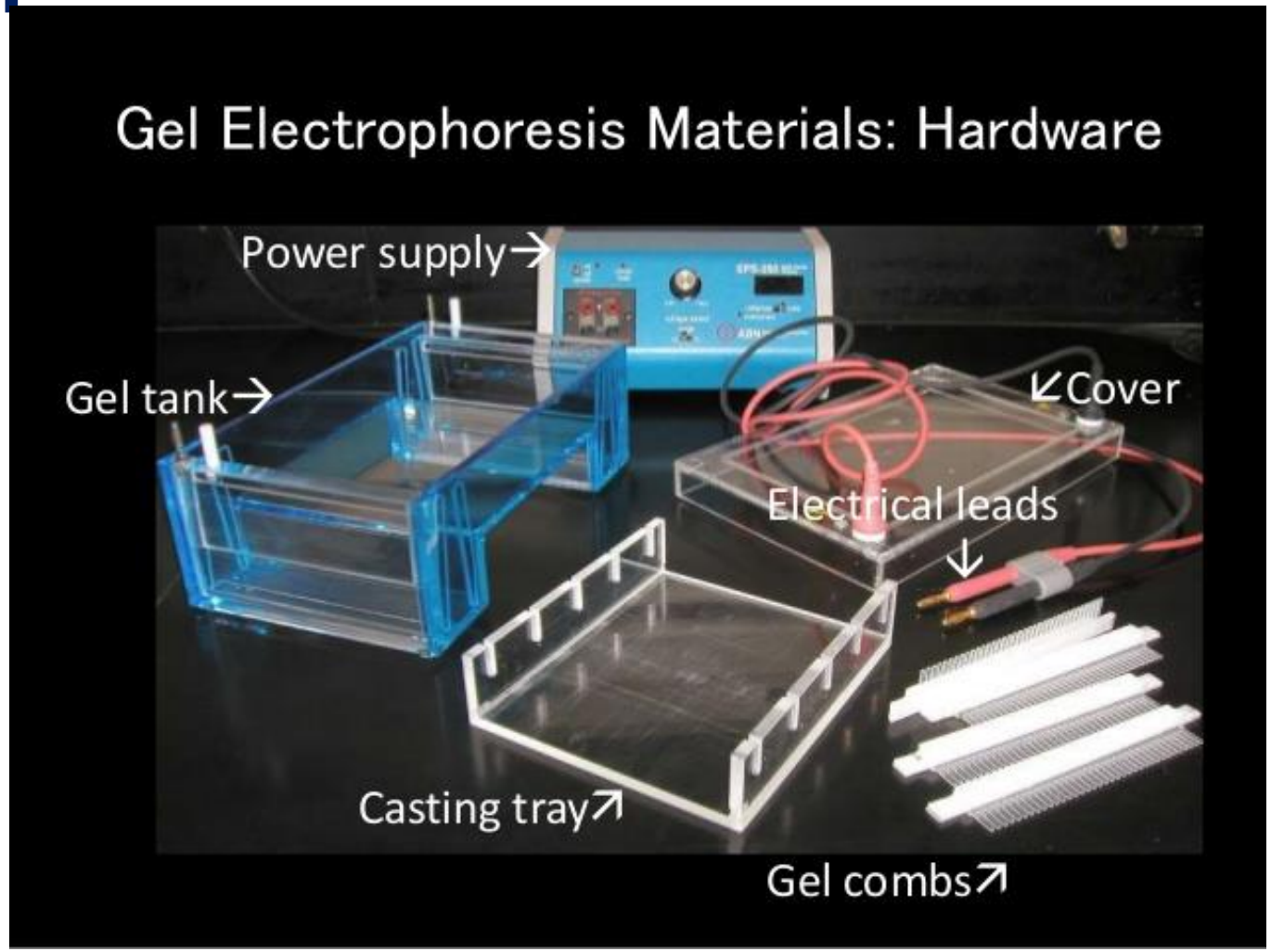
Rubber



electrophoresis chamber or gel tank

Power supply

Components:


Gel Electrophoresis Materials: Hardware
Power supply→
Gel tank→
↙Cover
Electrical leads
↓
Casting tray↗
Gel combs↗

1-Preparing the gel

Tris base

Acetic acid

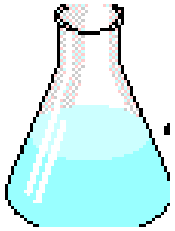
EDTA

OR

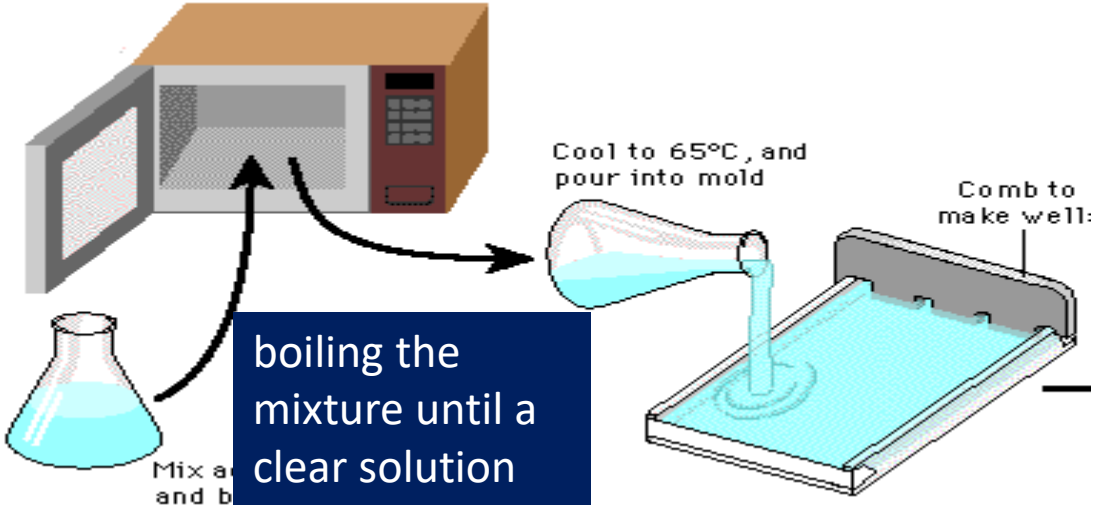
Tris base

Boric acid

EDTA

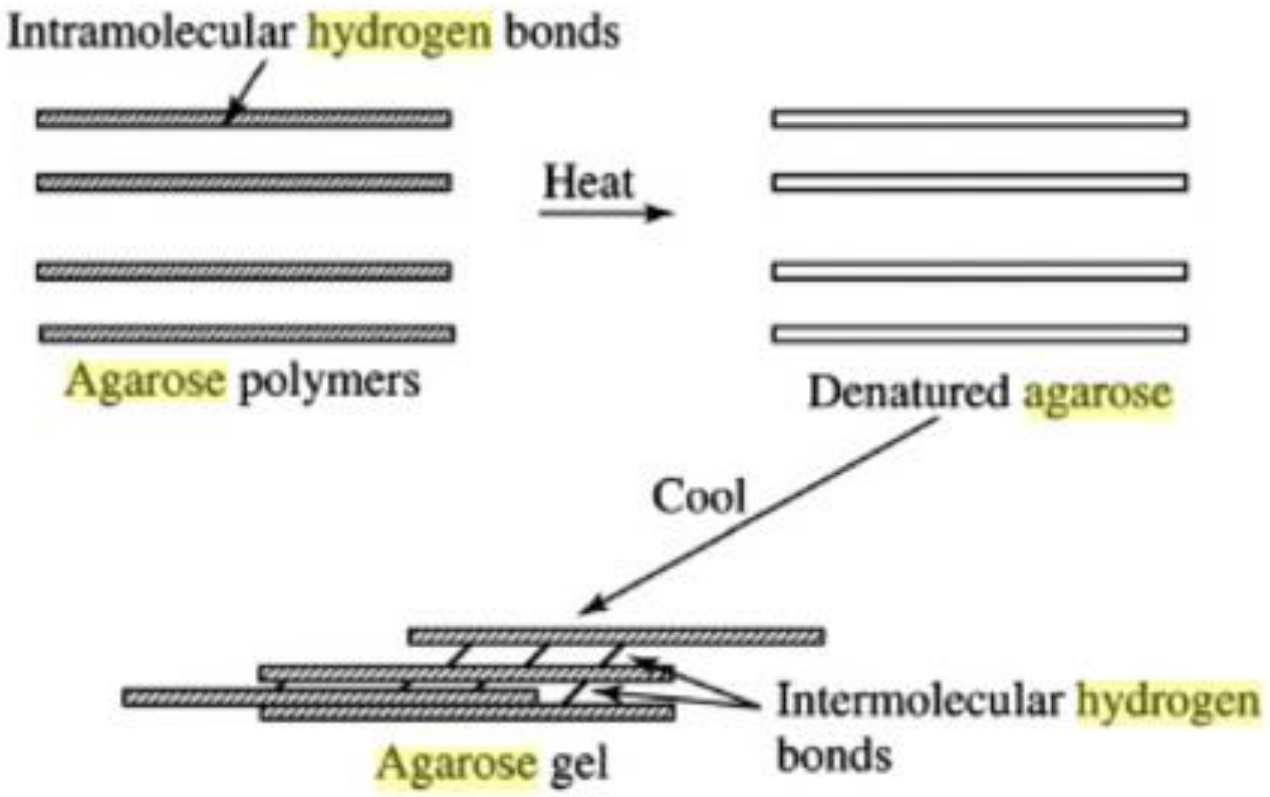


Add Ethidium bromide



boiling the mixture until a clear solution forms.

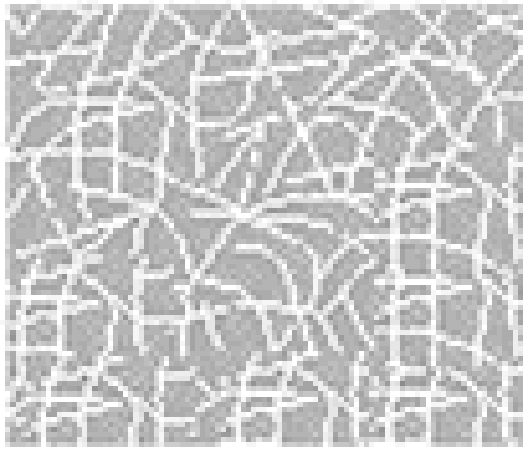
1-Preparation of gel (Behind the steps)

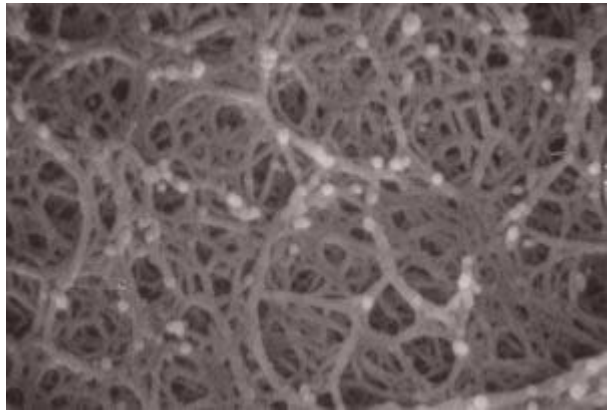


- Heating disrupts the intramolecular hydrogen bonding pattern of agarose

- The gelling properties are attributed to both inter- and intramolecular hydrogen bonding

1-Preparation of gel (Behind the steps)



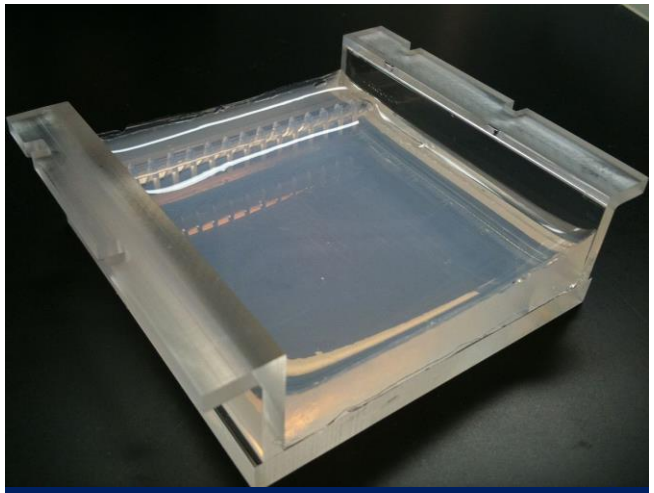


- The concentration of agarose in the gel determines the size of the pores.
- [high concentration of the gel → small pore size]

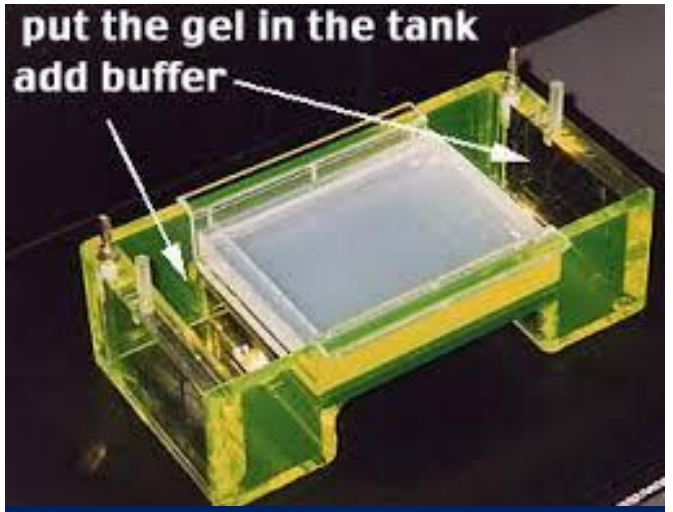
Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

2-Loading the sample into the well and running the gel



Remove the comb



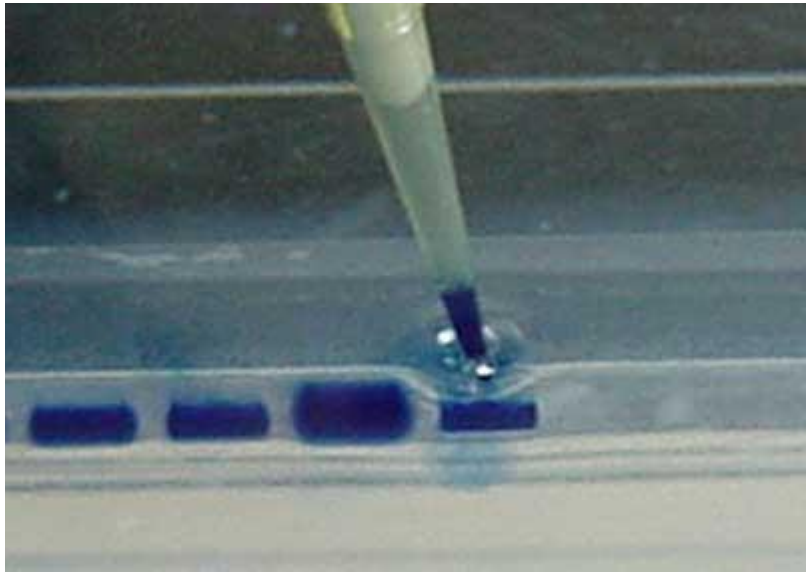
covered the gel with buffer

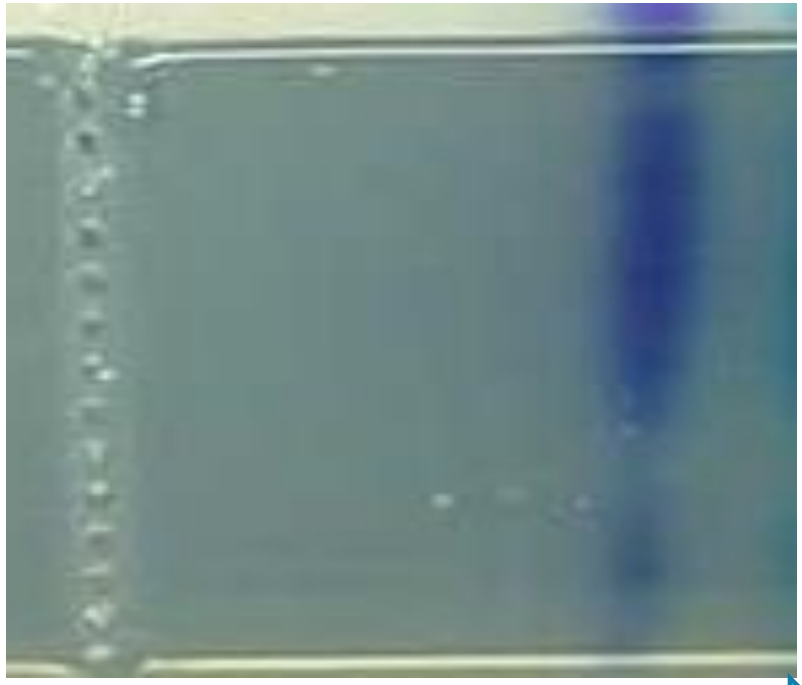
You Must Mix your sample with Loading Dye that contain:

Glycerol
Tracking Dye (Orange Dye)
7 microliter from the sample to 4 microliter from loading dye

You Can not Load DNA sample directly, WHY?

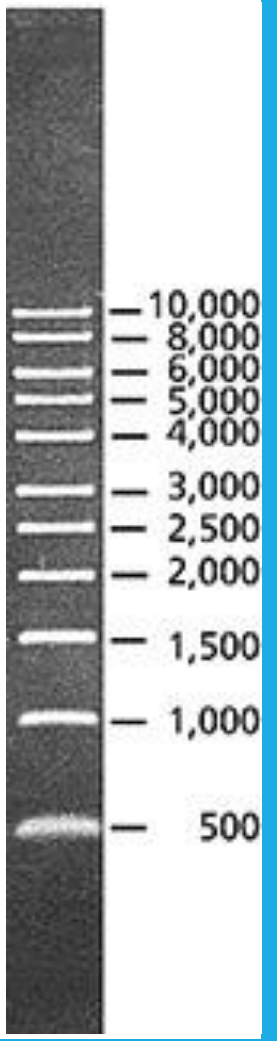
Loading Dye:





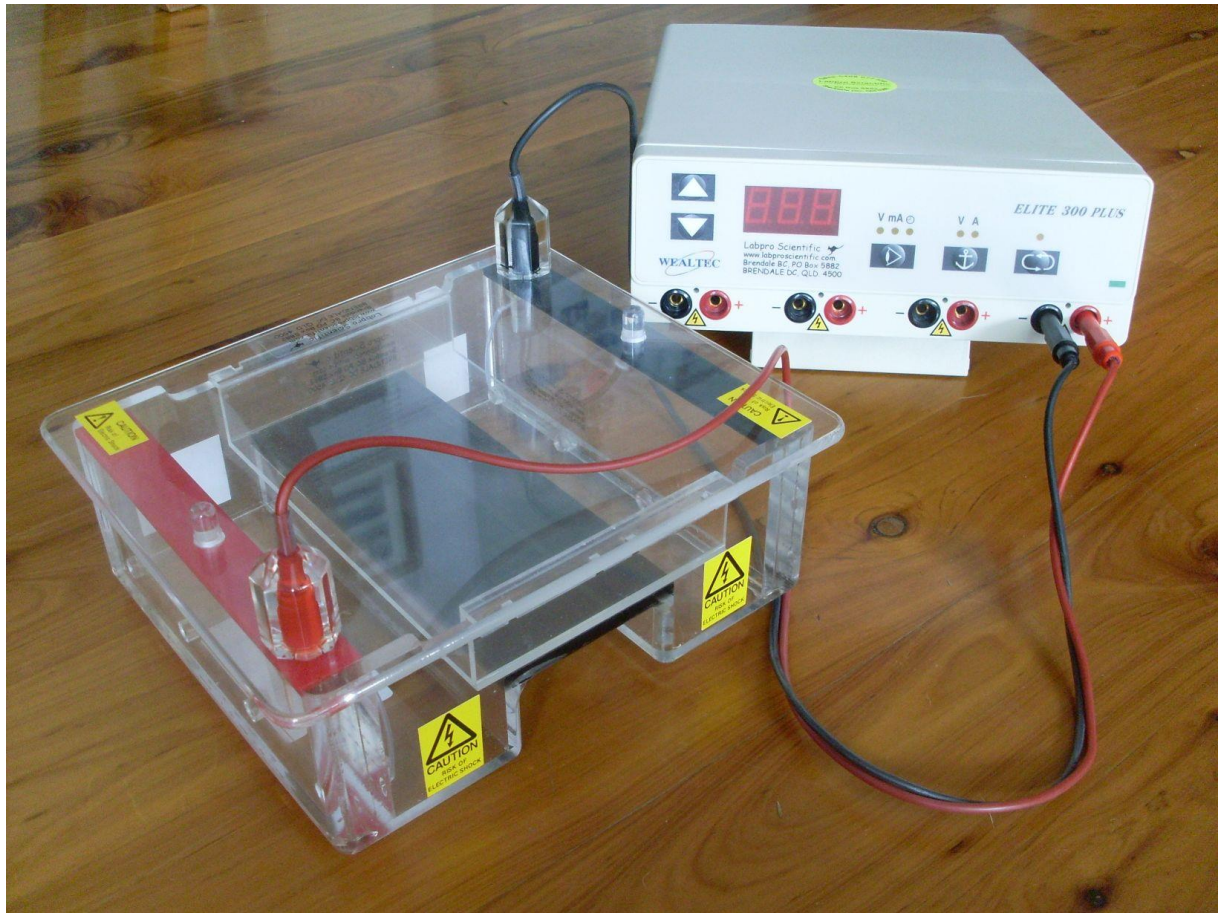
DNA Marker (Ladder)

- DNA and RNA size markers contain a mixture of DNA (or RNA) fragments of known length, making them suitable for estimating the fragment length of concurrently run samples.

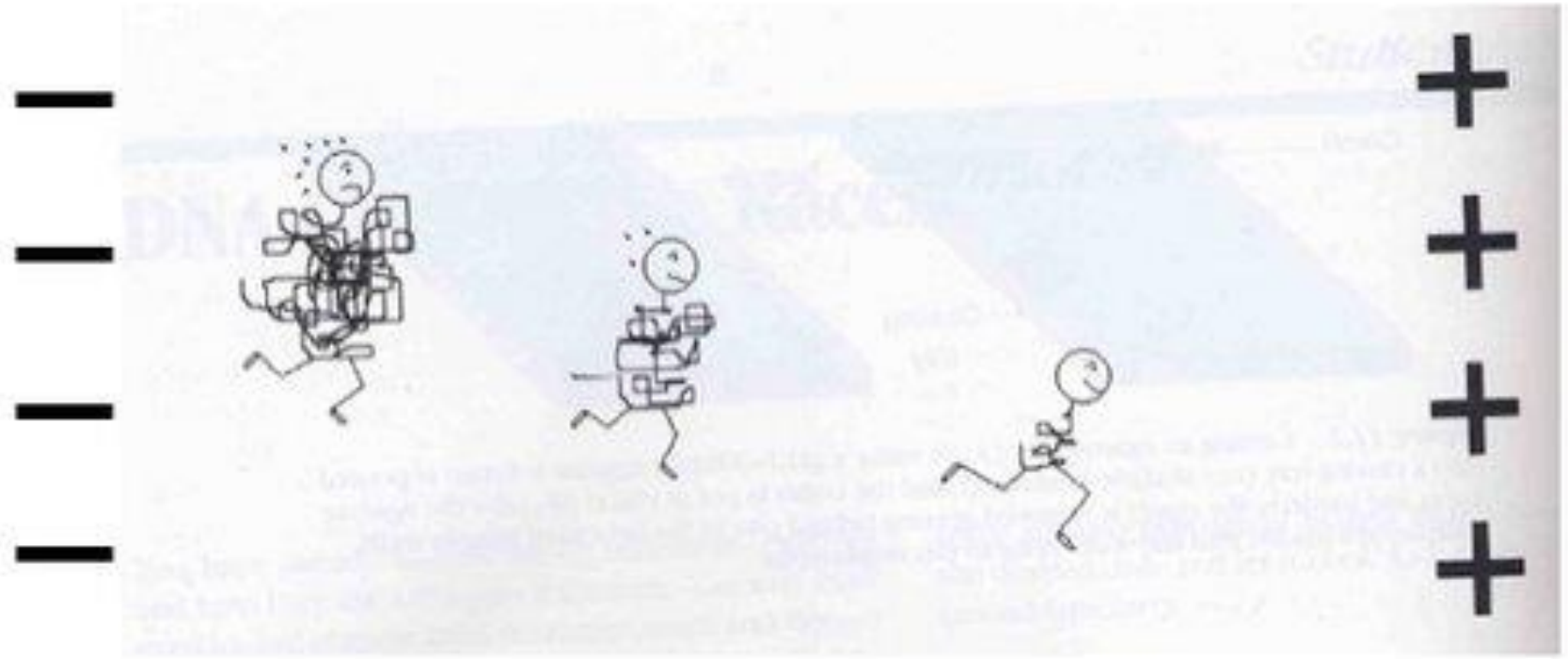


3-Running the Gel

- Put the cover of the container (Insure that you have put it in the right way)
- *Black is negative, red is positive.*
- For fragments 1-12 Kb use a voltage of 3-6 V/cm, for >12Kb use 1-2 V/cm.
- The higher the voltage → the more quickly the gel runs



3-Running the Gel



4-Gel staining and visualization

1. The DNA in the gel needs to be stained and visualized

When exposed to **ultraviolet** light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA.

[Ethidium bromide is a cyclic planar molecule that binds between the stacked base-pairs of DNA.].

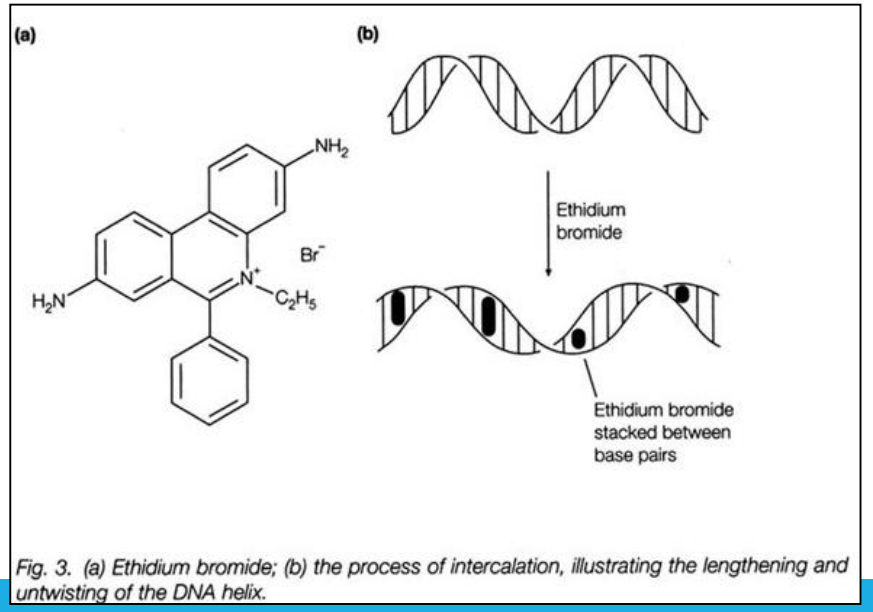
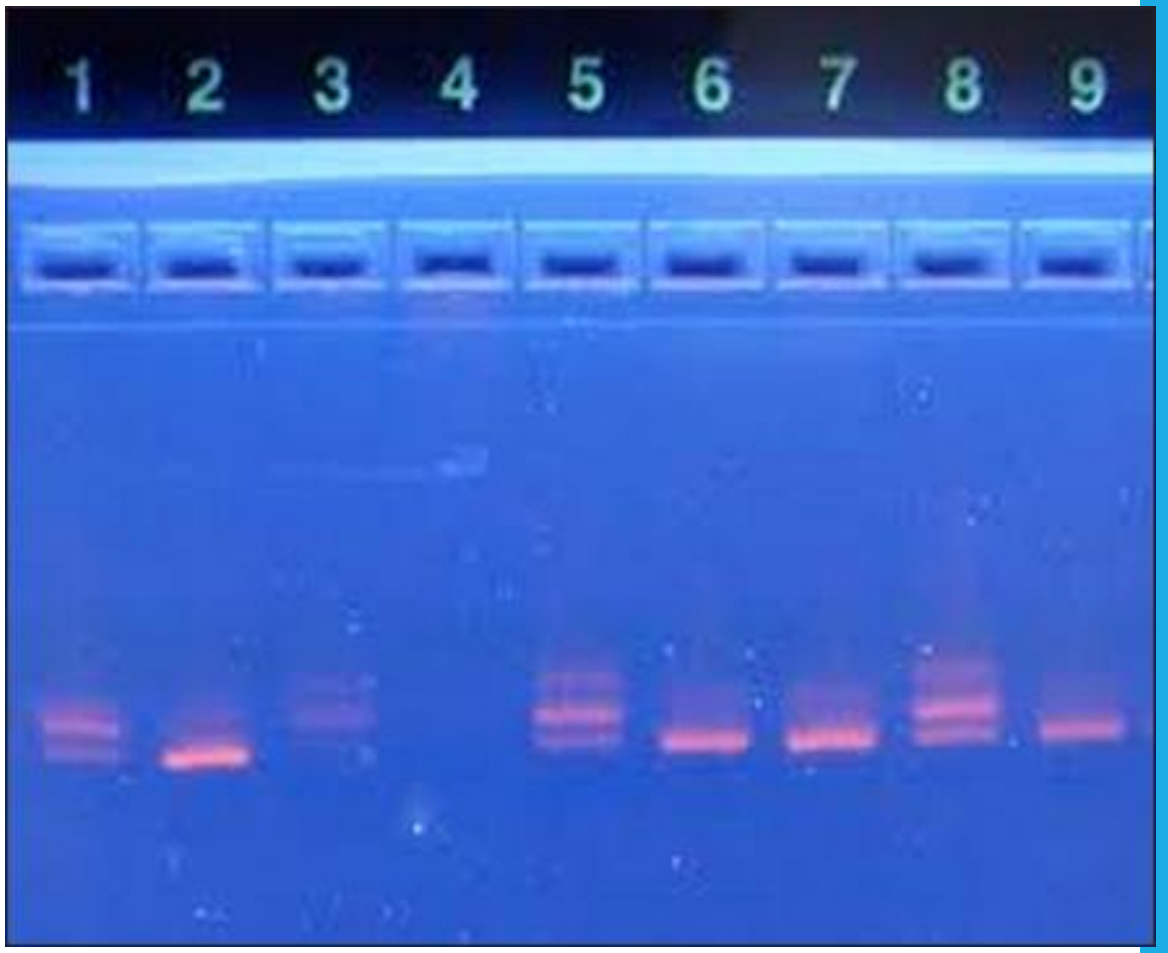
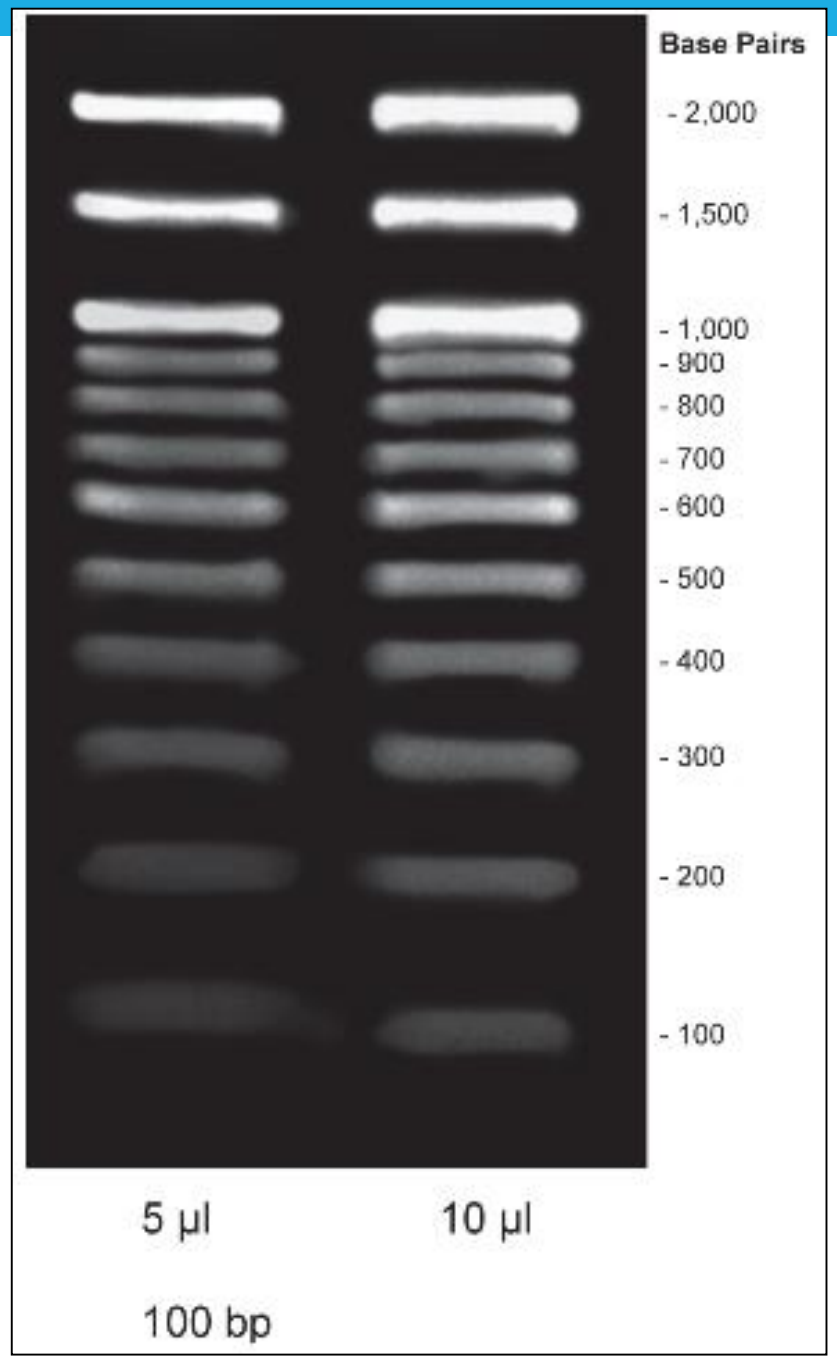


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.



"EtBr"orange light after binding to DNA.



Determine the size of the DNA fragment:

-Since agarose gels separate DNA according to size, the size of a DNA fragment may be determined from its electrophoretic mobility by running a number of standard DNA markers of known sizes on the same gel.

- **Ladder can come in different ranges of fragments!!You must choose your ladder carefully!!!!!**

Figure: The 100 bp DNA Ladder is suitable for sizing double-stranded DNA fragments from 100-2000 bp.

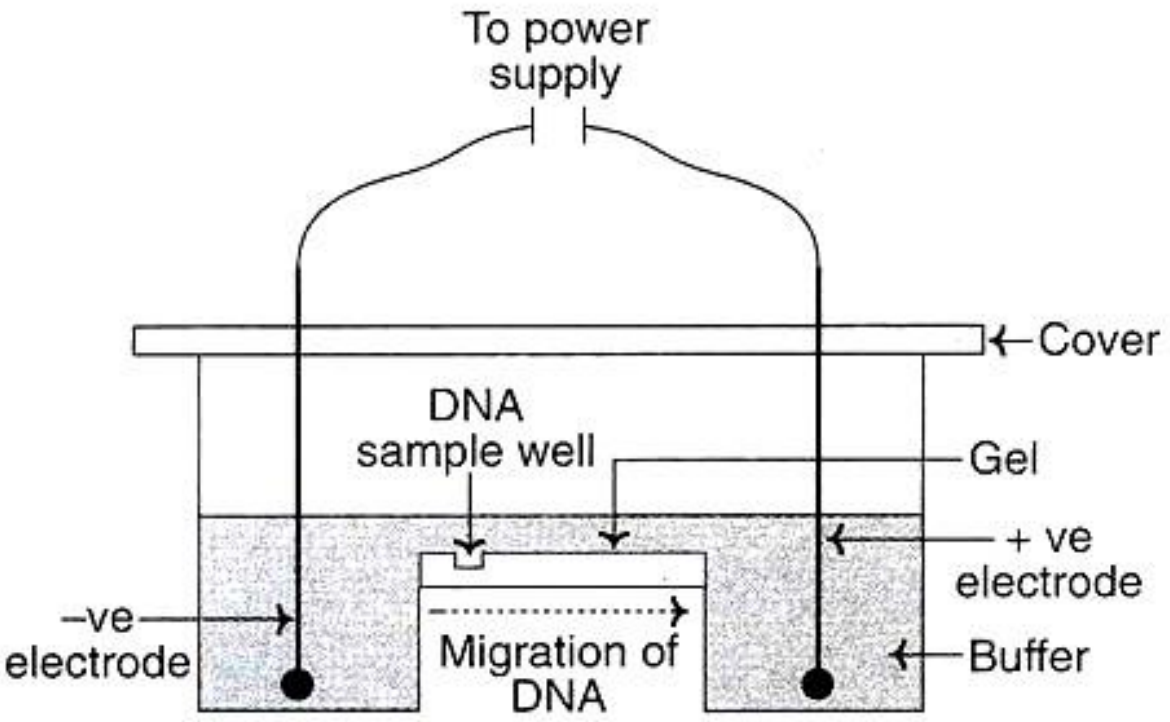


Fig. 7.1 : *A diagrammatic representation of agarose gel electrophoresis system.*