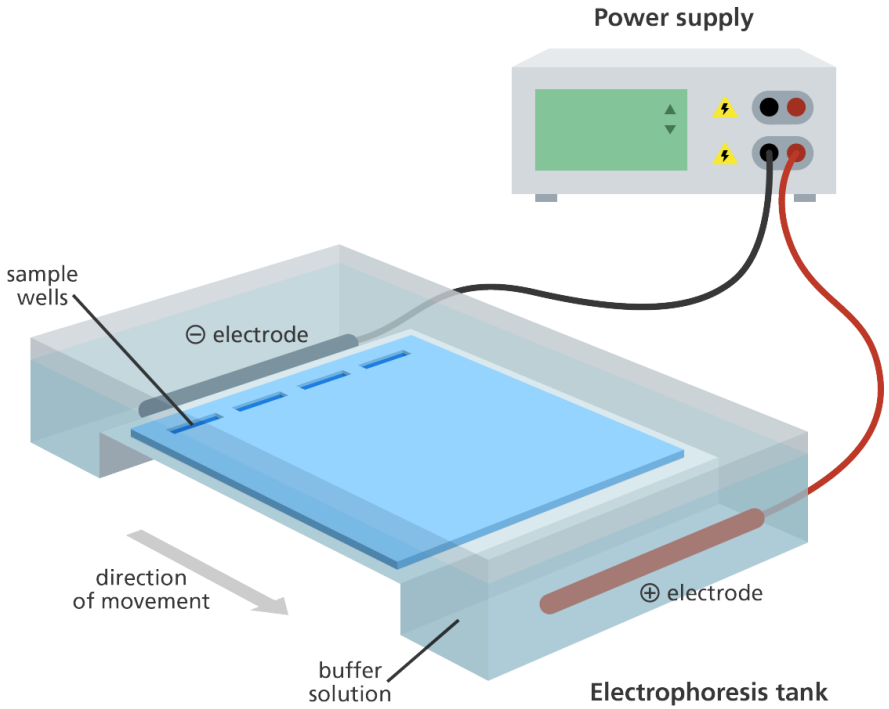
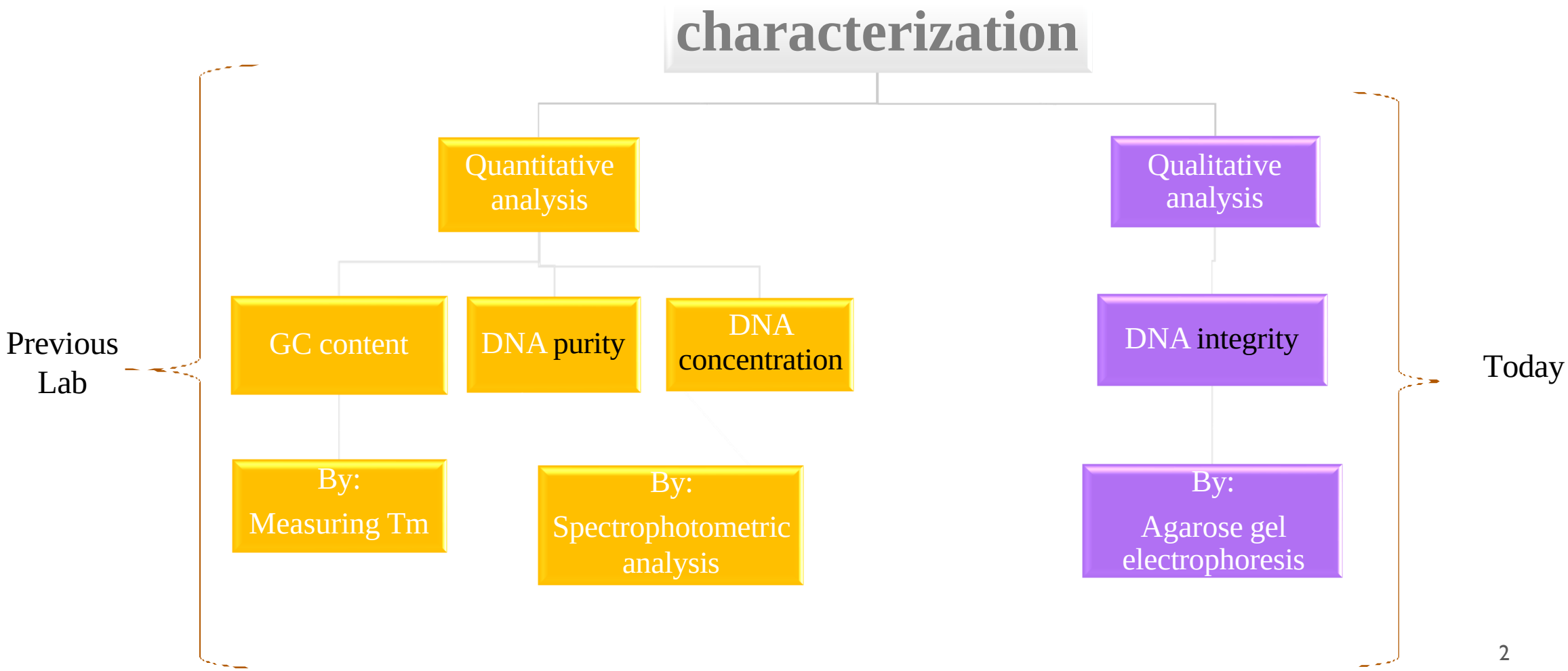
Agarose Gel Electrophoresis (AGE)



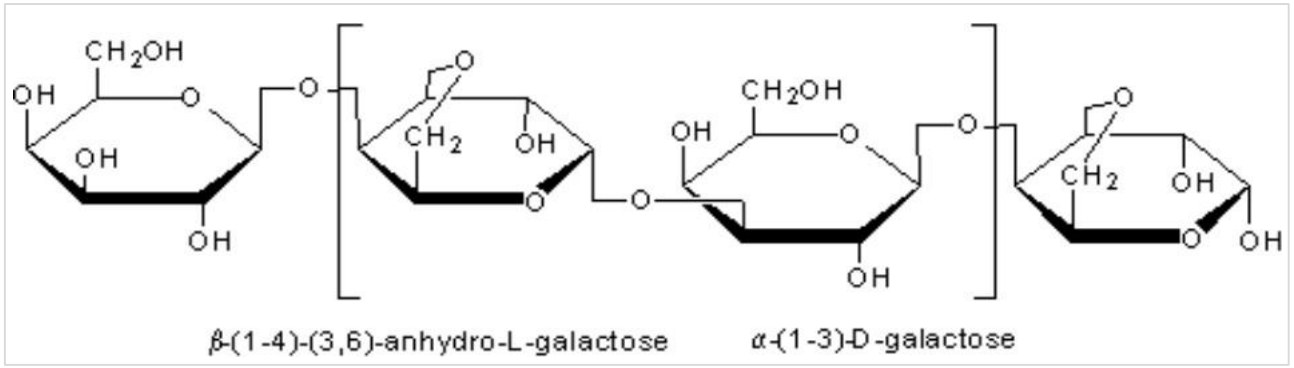
After DNA isolation:

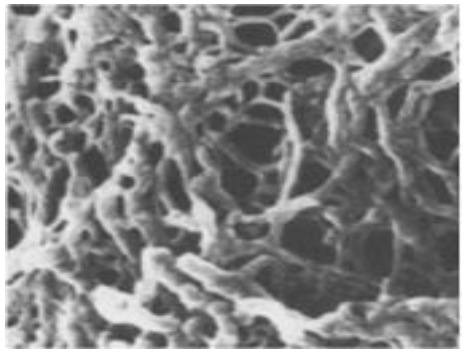


Agarose gel electrophoresis:

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

Agarose: is a liner polymer composed of alternative residues of D-galactose and 3,6-anhydro-L-galactopyranose joined by α (1→3) and β (1→4) glycosidic linkages.





Polymerized agarose

Electrophoresis: is the movement of charged particles under the influence of electric field.

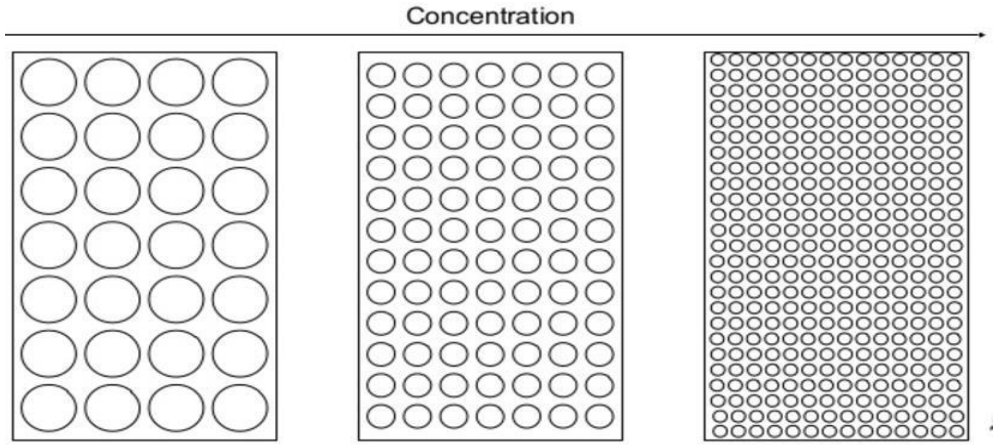
Agarose gel electrophoresis: is method for separation nucleic acids by size.

Agarose gel electrophoresis:

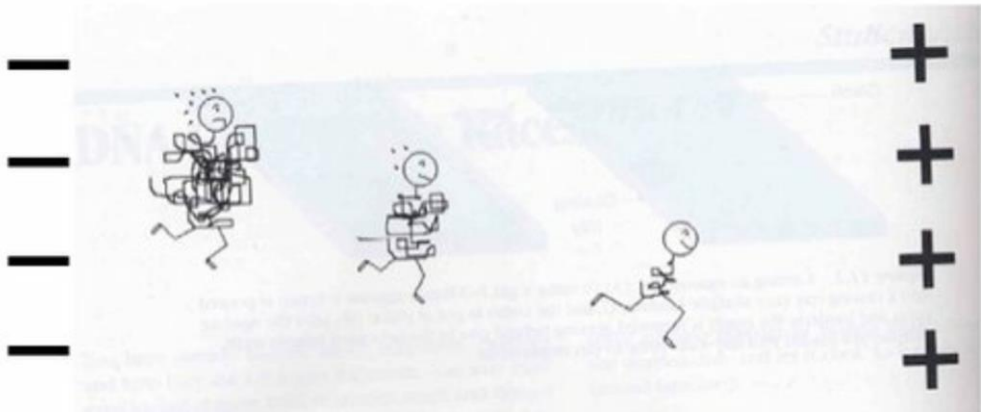
- **The electrophoretic migration rate of nucleic acids depends on:**
  - Size of DNA molecules.
  - Concentration of agarose gel.
  - Voltage applied.
  - Conformation of DNA.
  - Buffer used for electrophoresis.

How to control the pores size?

The pore size in the gel is controlled by the initial concentration of agarose.



The largest molecules will have the most difficulty passing through the gel pores.



When you should use agarose gel electrophoresis ?

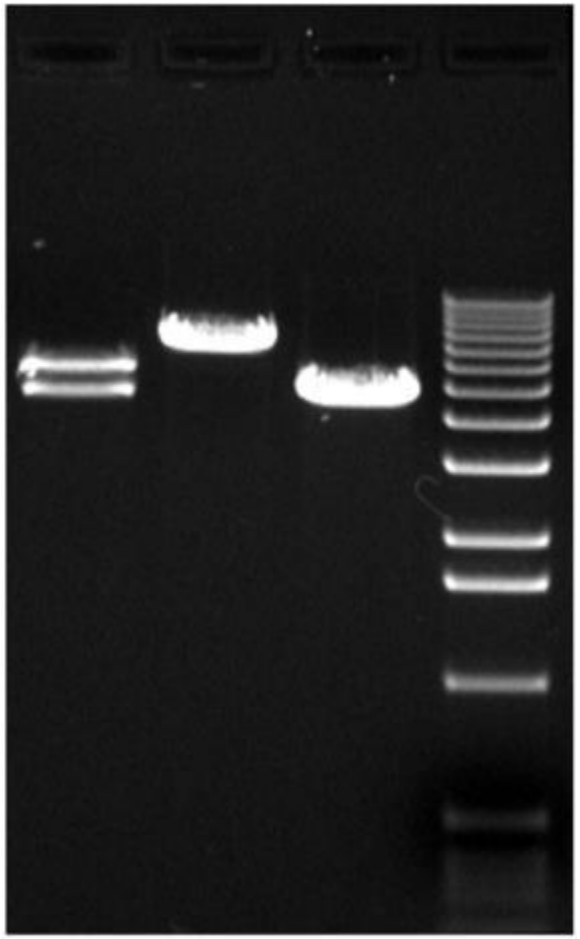
Analyse the integrity of DNA samples.

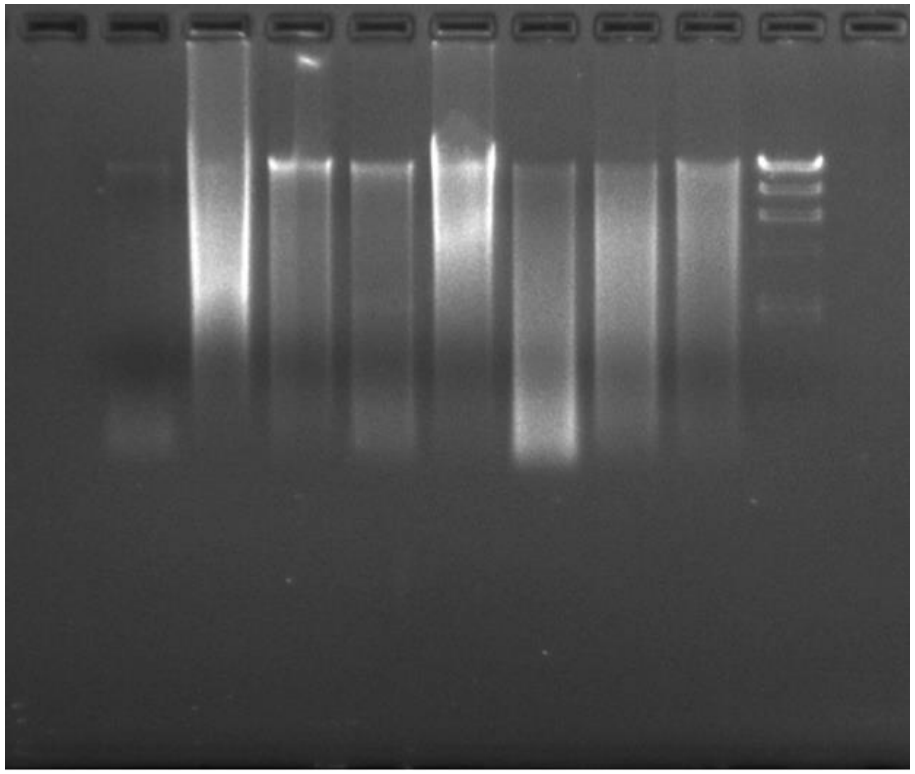
Calculate the size of DNA ➔ **by the use of appropriate size markers.**

To see if your DNA fragments is pure and there is no contamination (?).

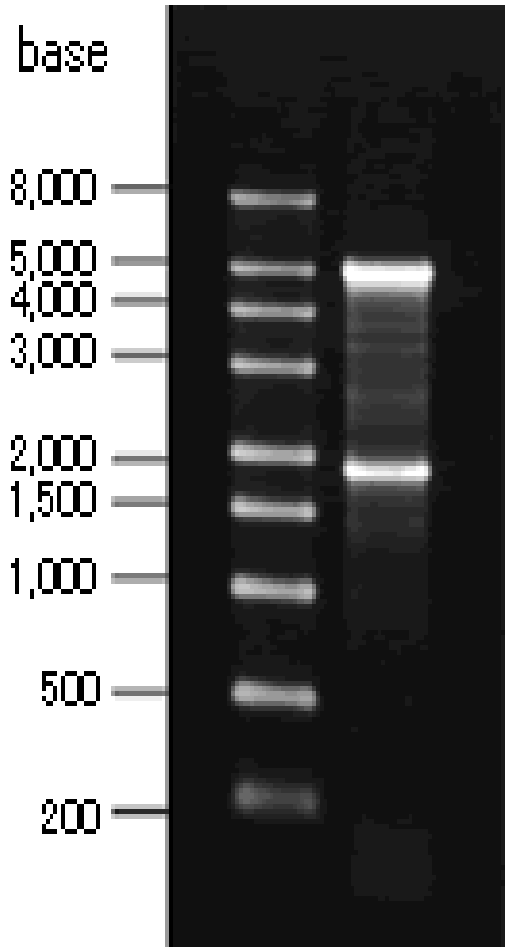
Purification of nucleic acids fragments mixture

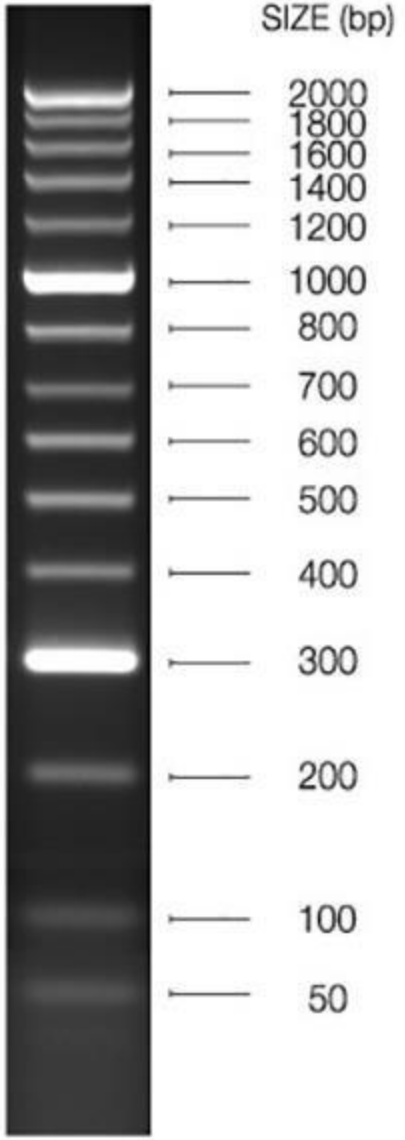
Analyse the integrity of DNA samples.





Calculate the size of DNA.





Practical Part

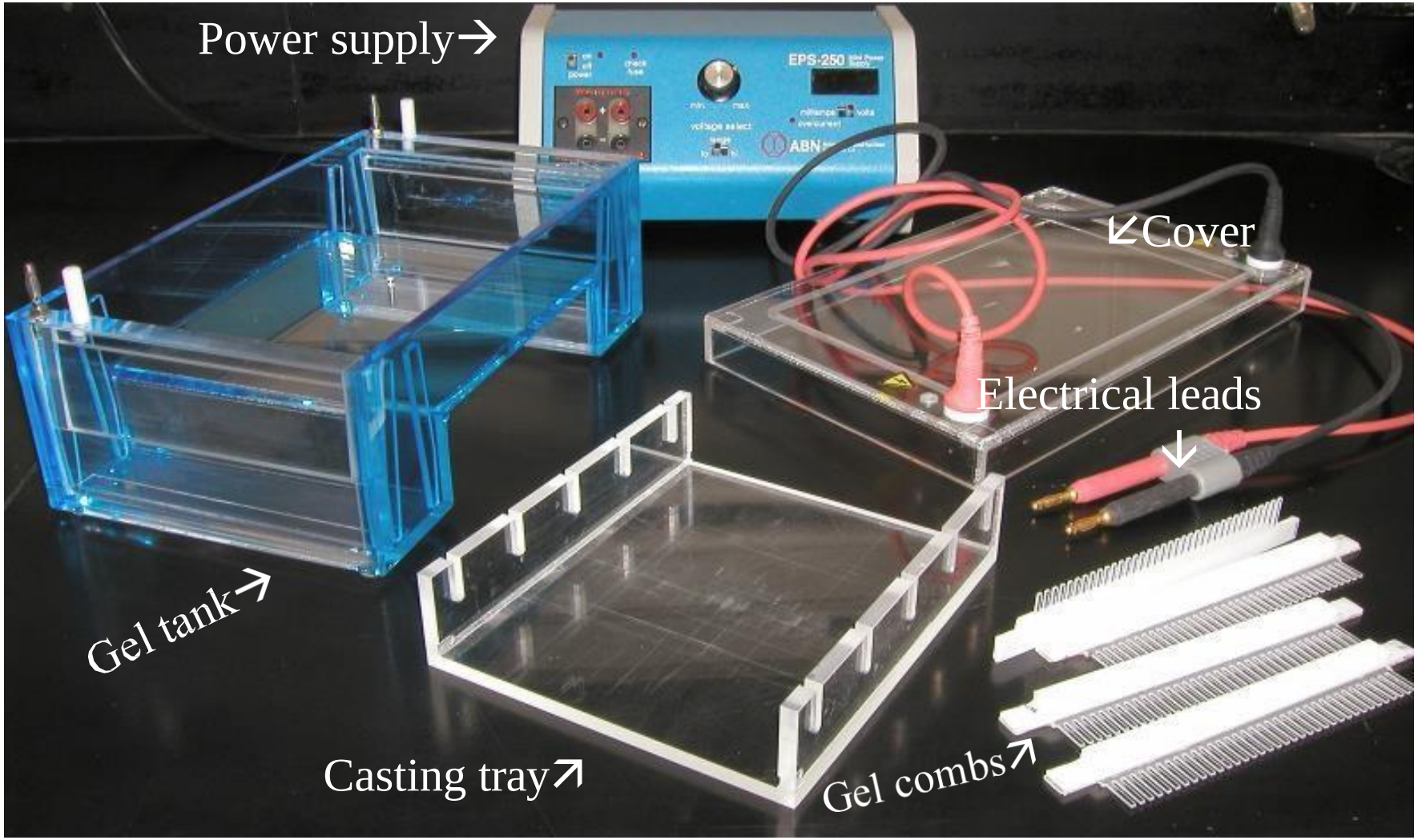
Aim:

- Examination of extracted DNA by agarose gel electrophoresis.
- To separate and calculate the molecular size of DNA fragment by comparing the separated bands with known standard molecular weight marker.

Principle:

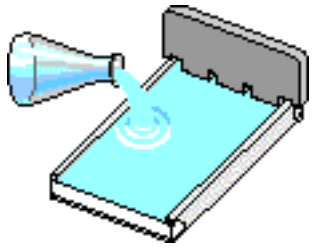
- Nucleic acids are separated by applying an electric field, so these **negatively charged molecules** will move through an agarose matrix towards the anode, and the biomolecules are separated by size in the agarose gel matrix, **where the distance travelled by a DNA molecule is inversely correlated with its size.**

Electrophoresis glassware:

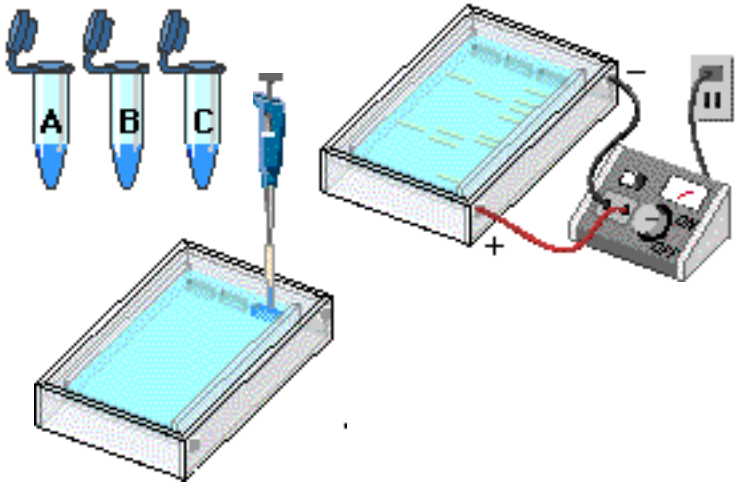


Performing Agarose gel electrophoresis:

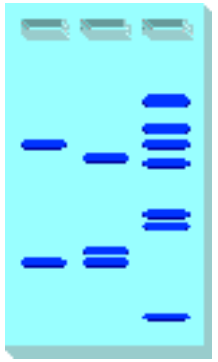
(1) Gel preparation



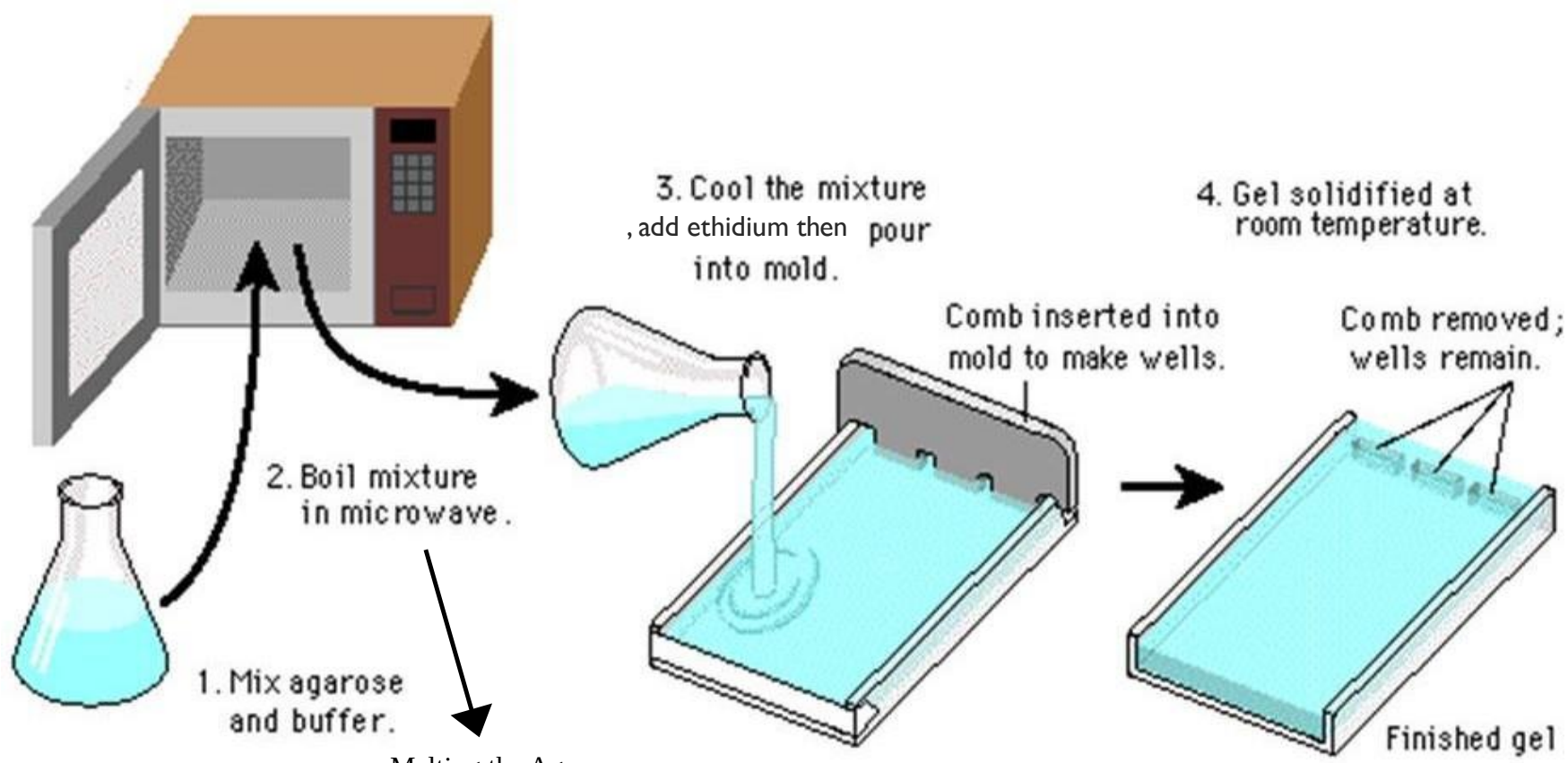
(2) Load the sample and start the run



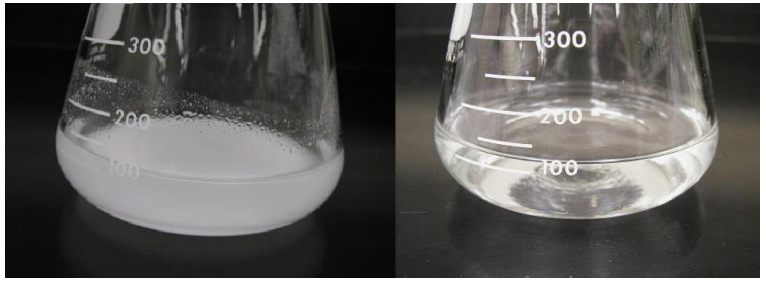
(3) Visualizing the sample



(1) Agarose Gel Preparation

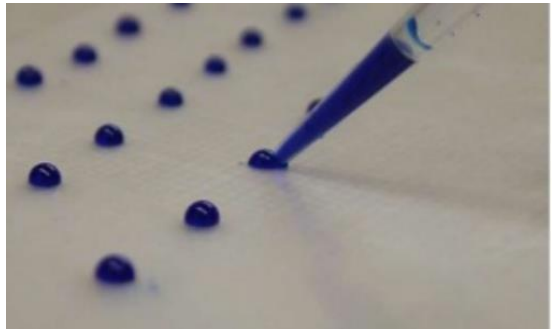


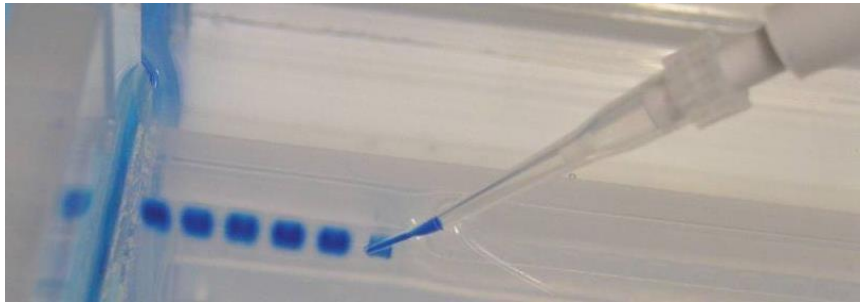
Melting the Agarose

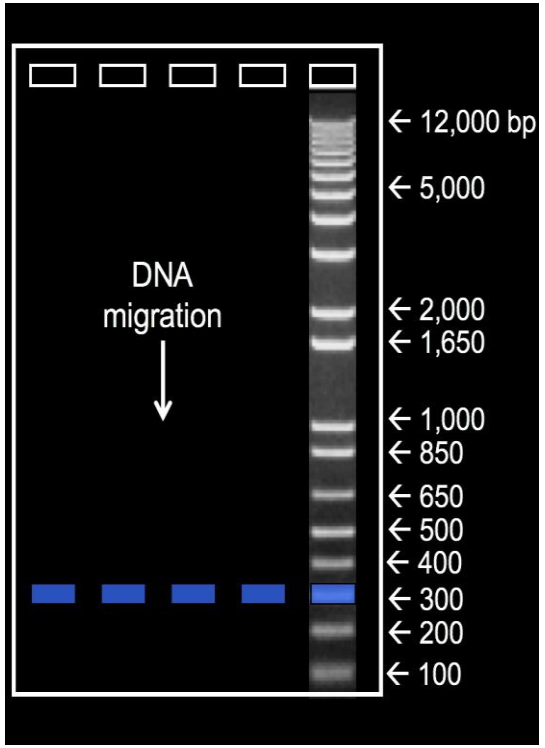


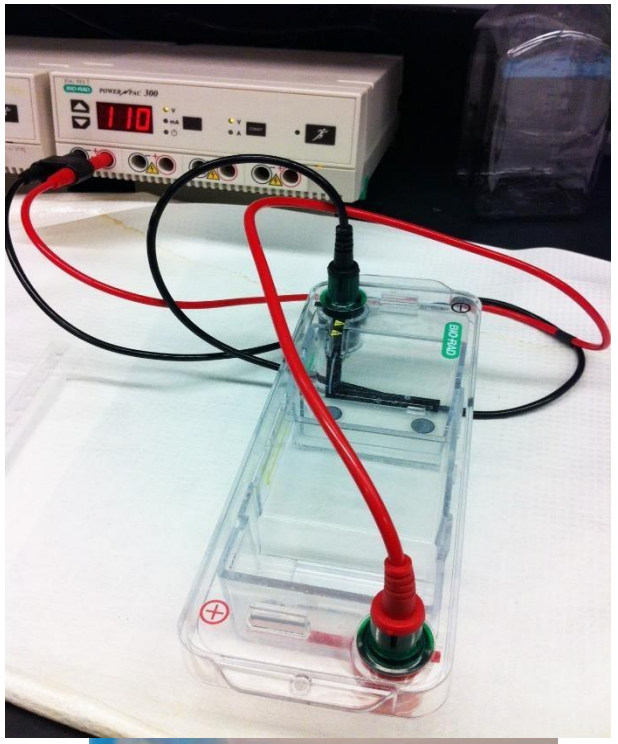
(2) Load the sample and start the run

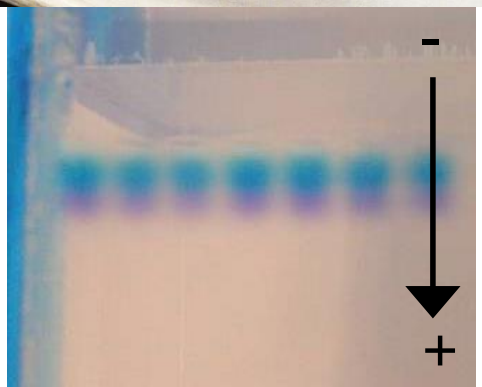
1) Mix the DNA samples with the loading dye **… why?**
2) Load the sample into the well using pipette tip.
3) Load the DNA marker (Ladder).
4) Run the gel and track the sample.





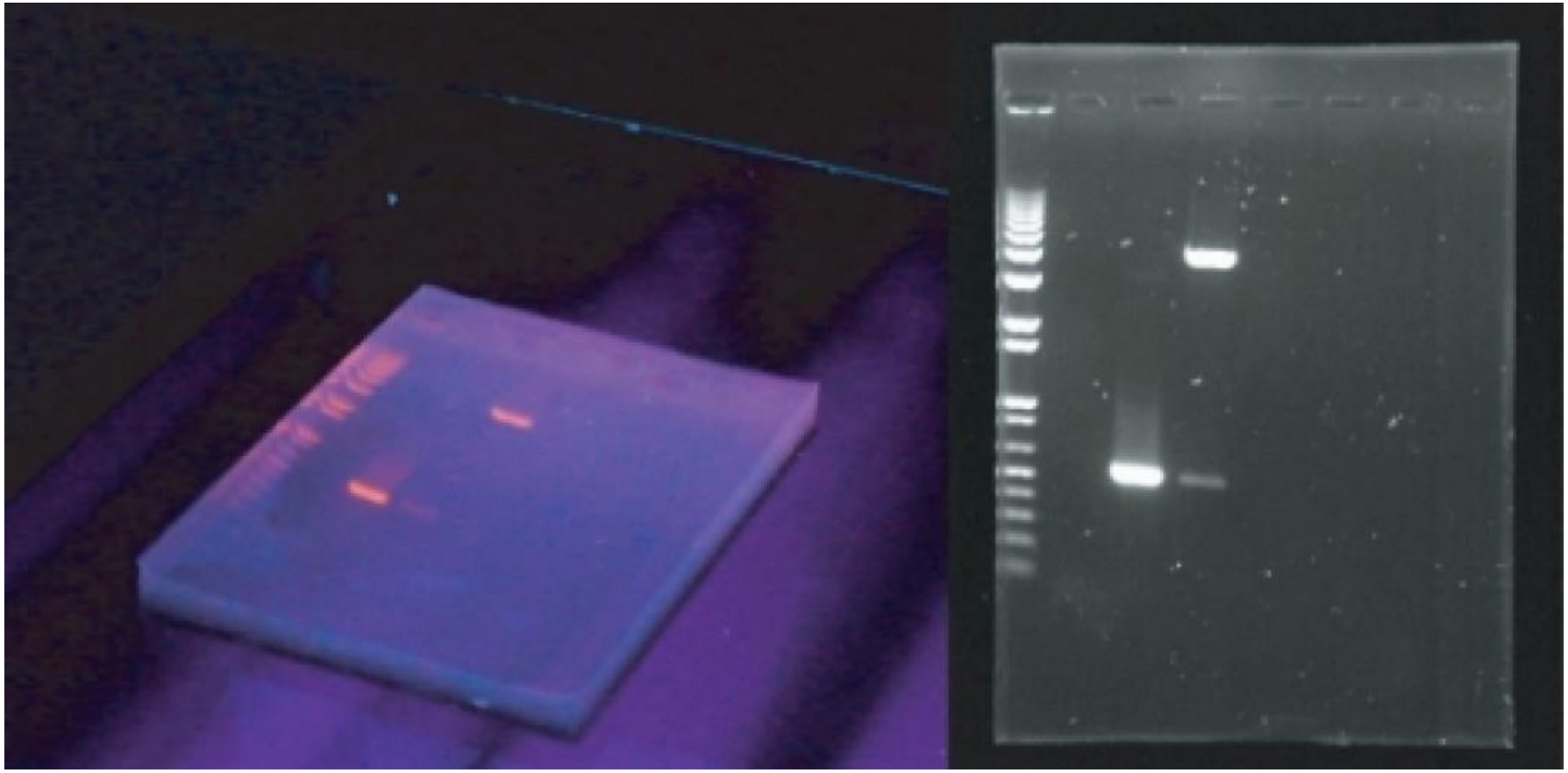






(3) Visualizing the sample

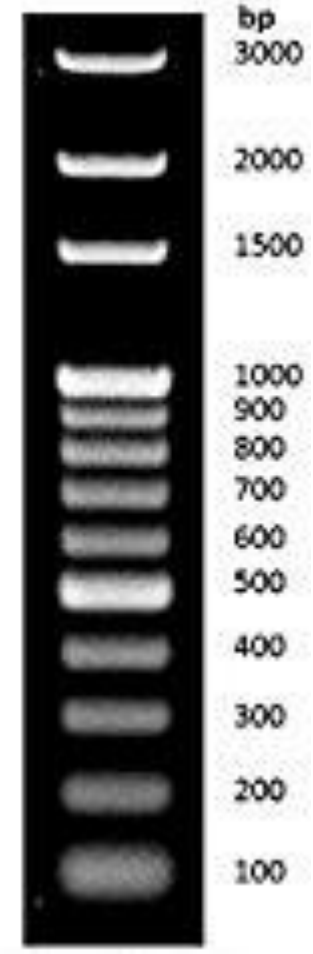
Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a gel.



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.

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



Results:

- Picture of the gel.

Home Work:

- When Run total RNA on agarose gel three bands will be detected, what dose each band represent ?