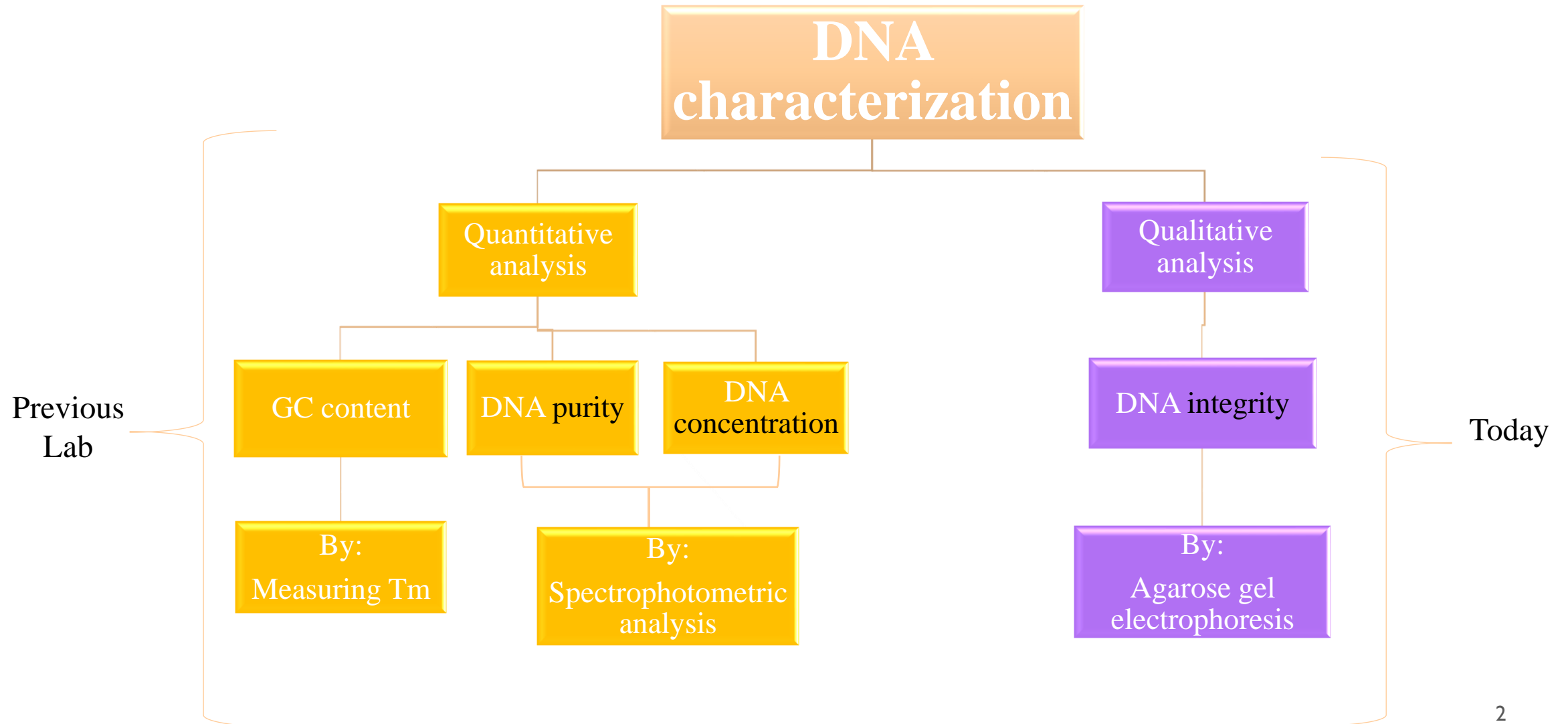




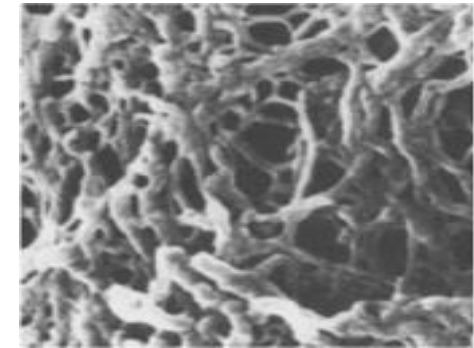
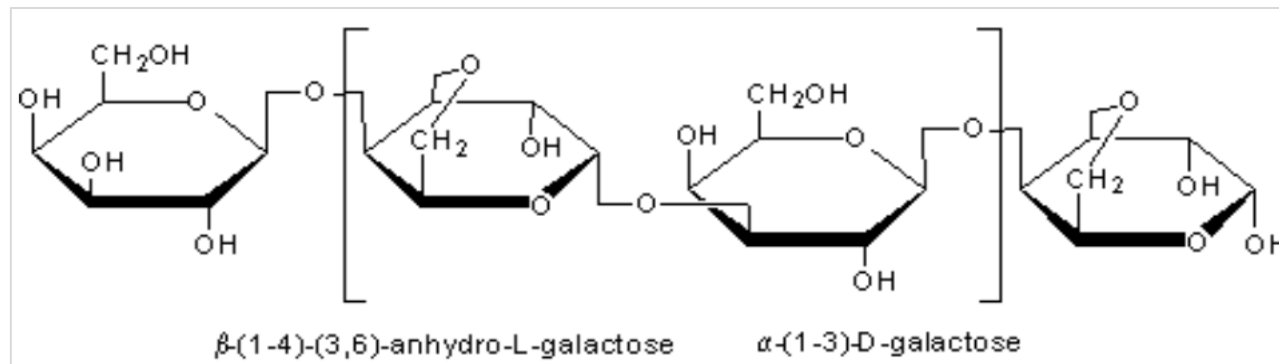
Agarose Gel Electrophoresis (AGE)

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 linear polymer composed of alternative residues of D-galactose and 3,6-anhydro-L-galactopyranose joined by α (1 \rightarrow 3) and β (1 \rightarrow 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.

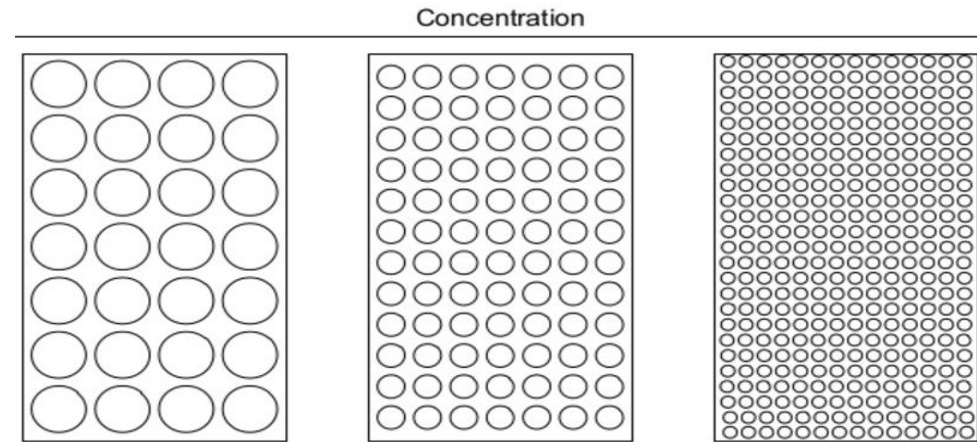


Agarose gel electrophoresis:

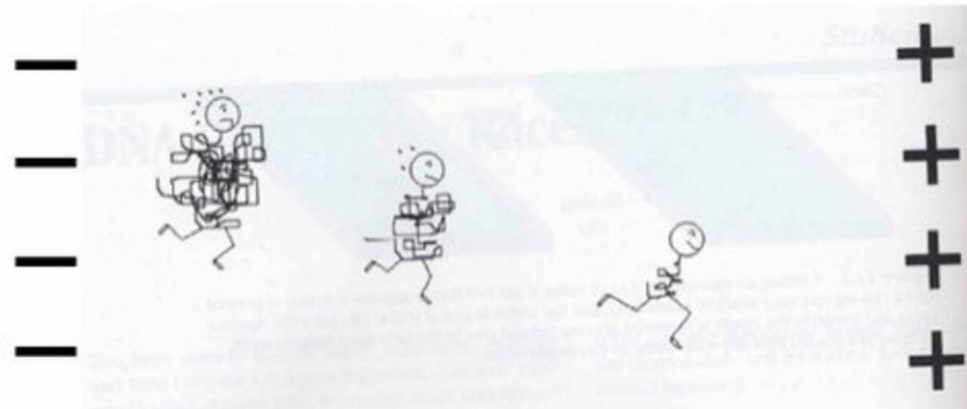
- **The electrophoretic migration rate of nucleic acids depends on:**
 - Size of DNA molecules.
 - Concentration of agarose gel.
 - Voltage applied.
 - Conformation of DNA.
 - Buffer used for electrophoresis.

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.

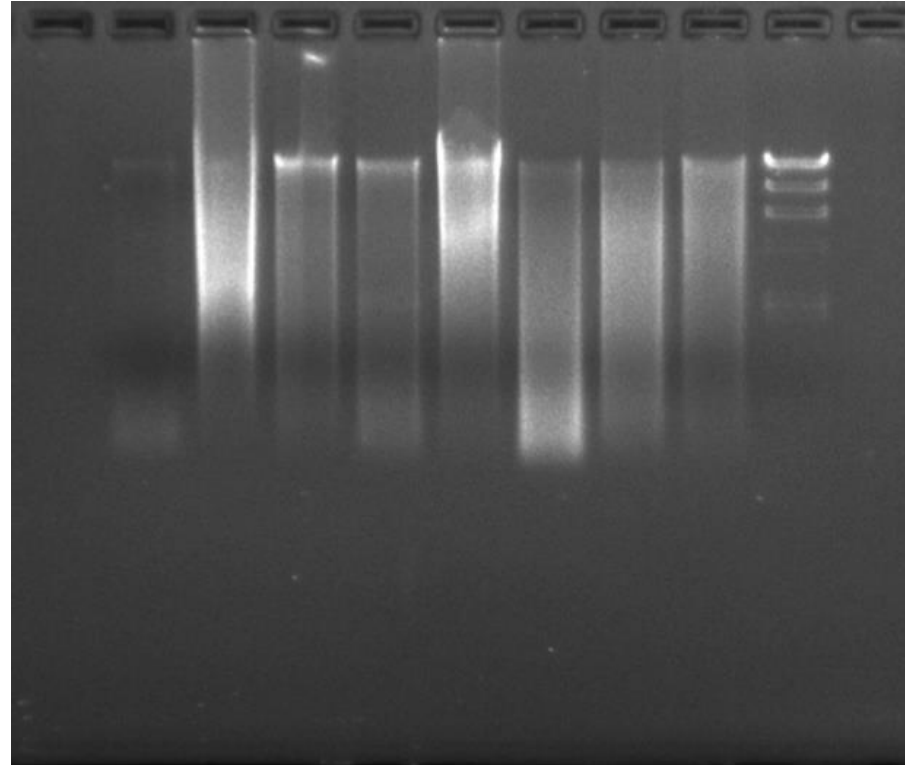
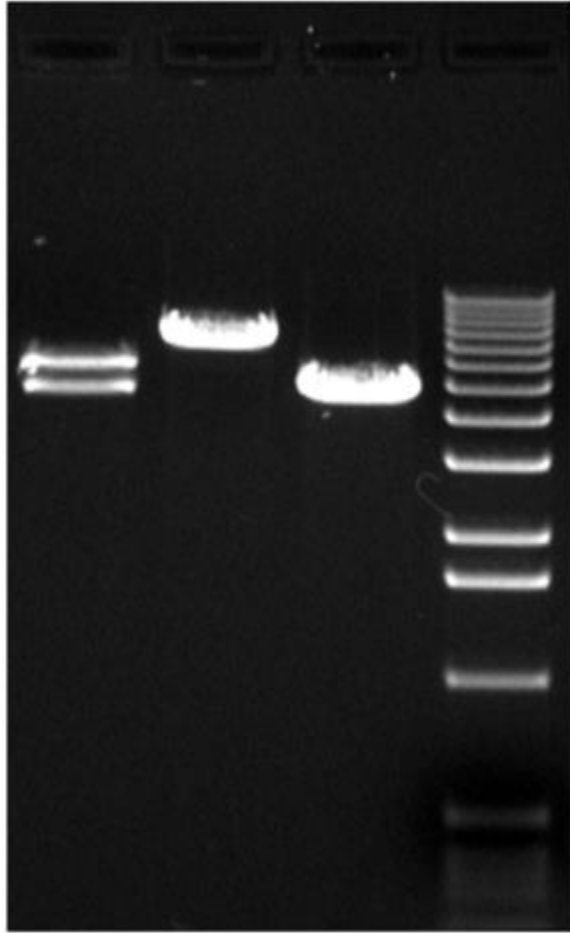




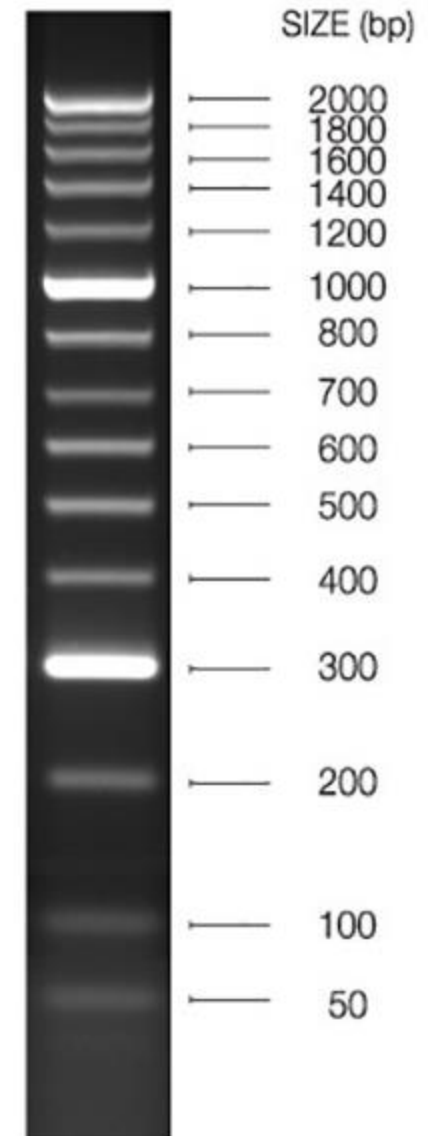
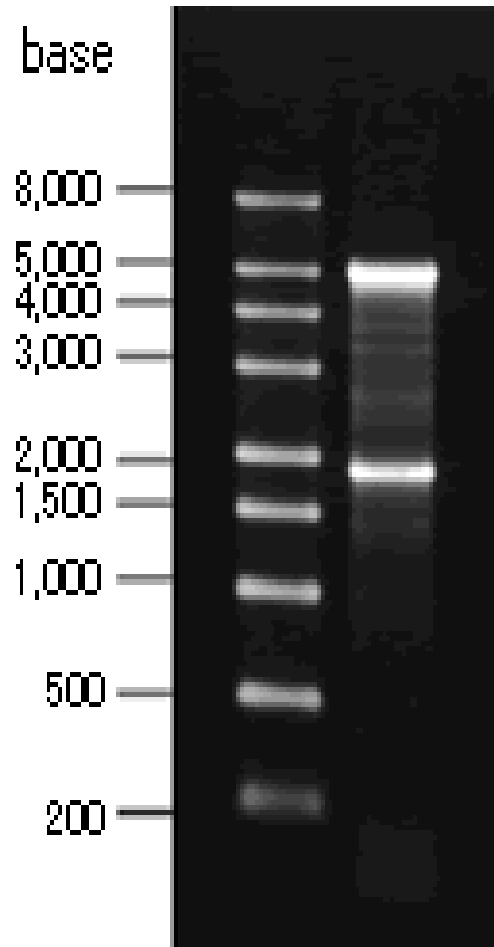
When you should use agarose gel electrophoresis ?

- 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



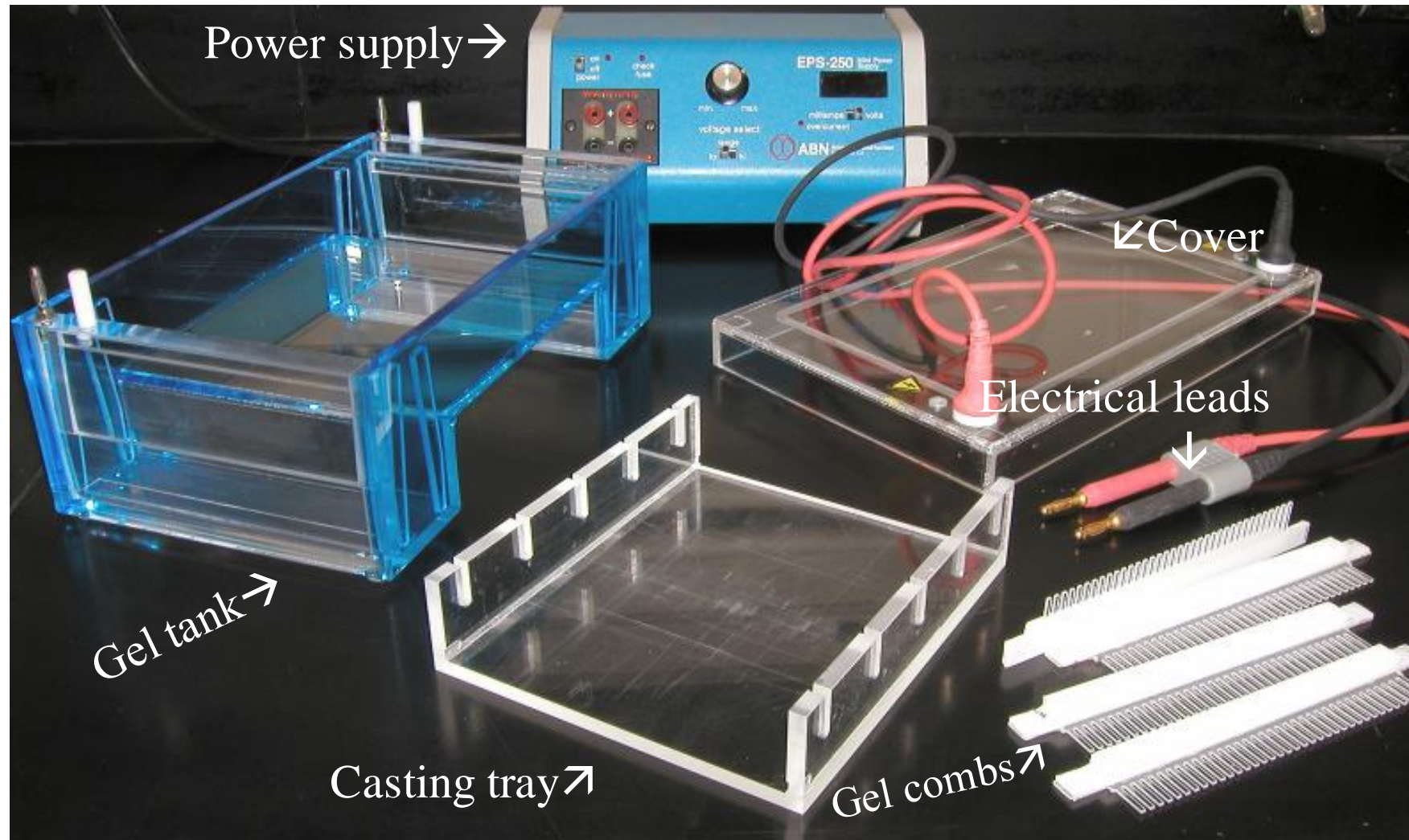
Aim:

- 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:

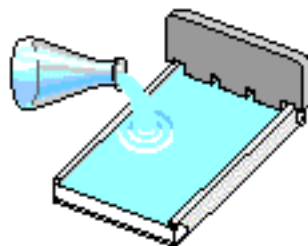
- Nucleic acids are separated by applying an electric field, so these **negatively charged molecules** will move through an agarose matrix towards the anode, and the biomolecules are separated by **size** in the agarose gel matrix, where the distance travelled by a DNA molecule is inversely correlated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

Electrophoresis glassware:

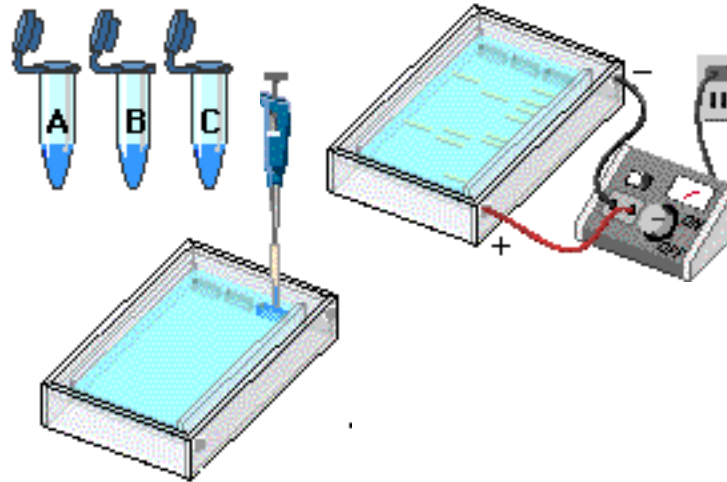


Performing Agarose gel electrophoresis:

(1) Gel preparation



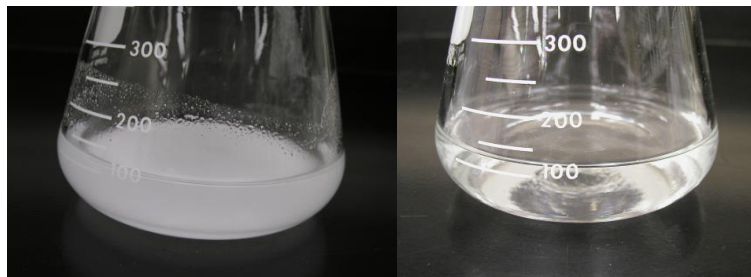
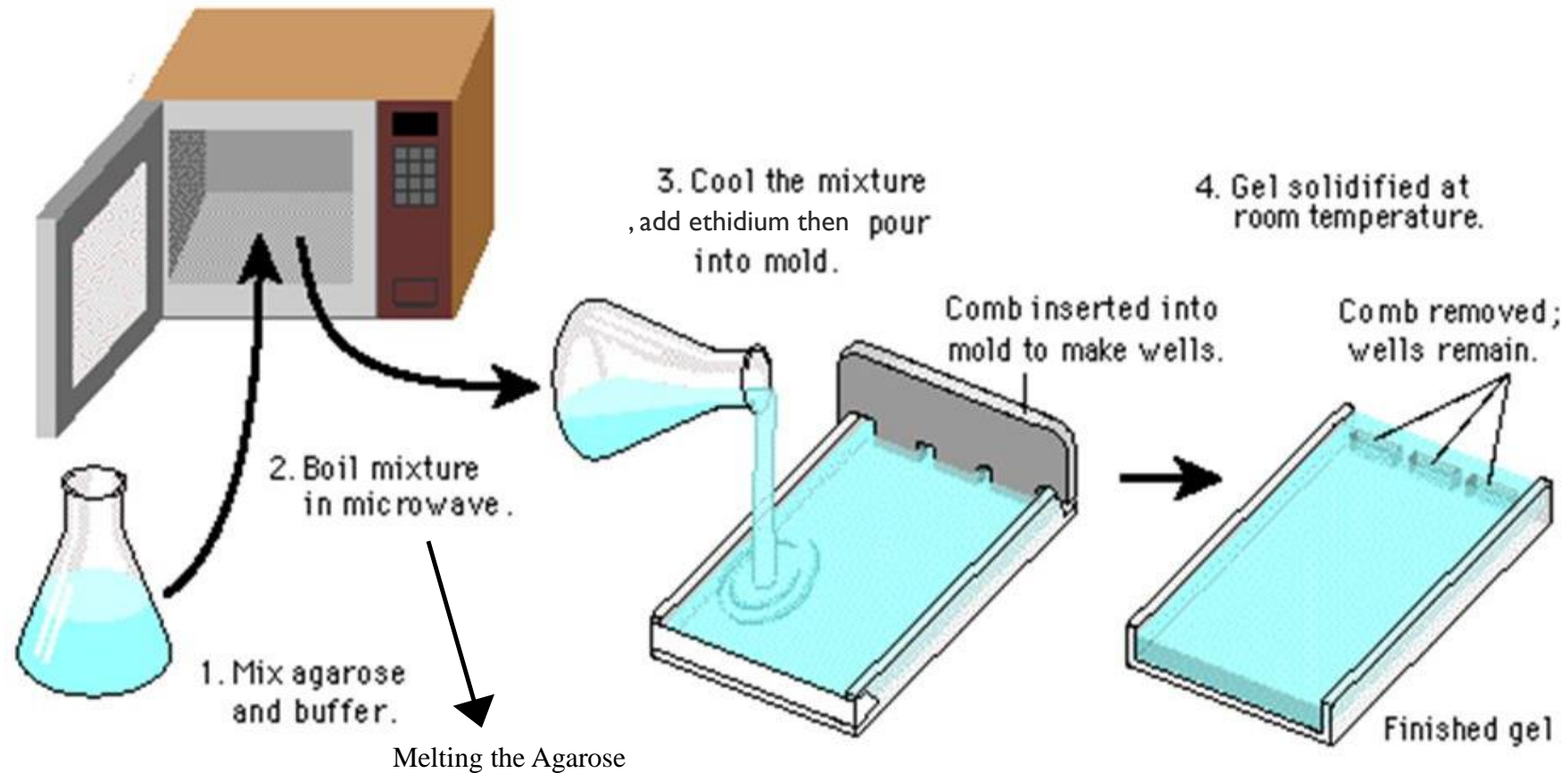
(2) Load the sample and start the run



(3) Visualizing the sample

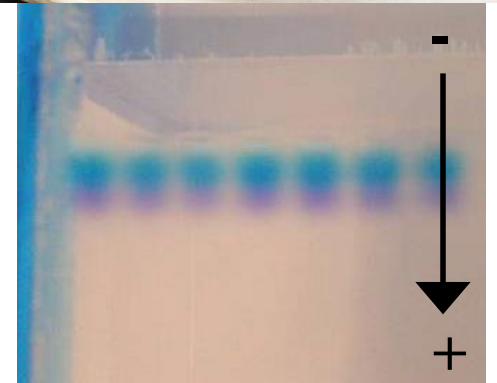
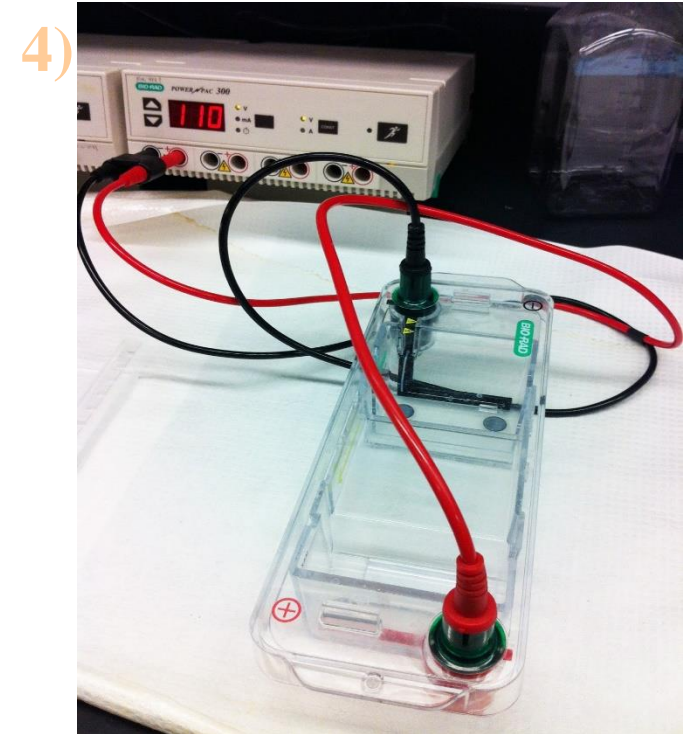
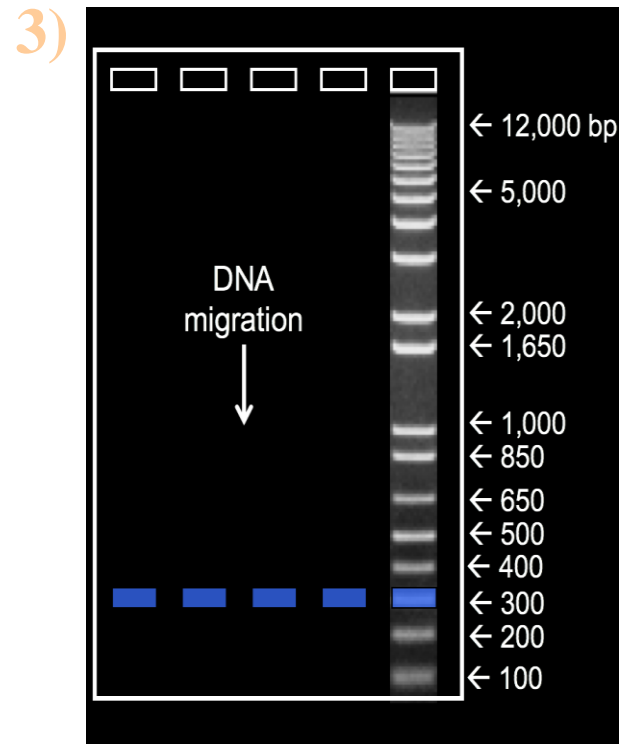
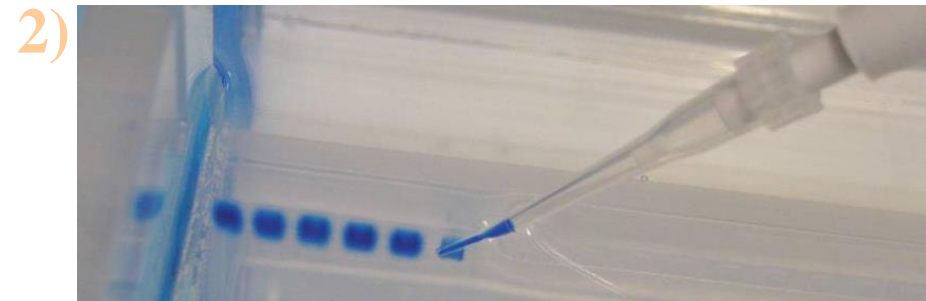
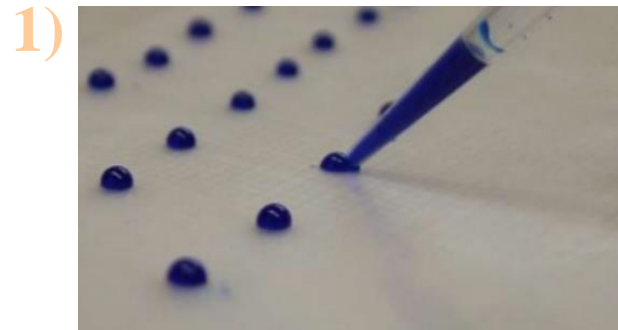


(1) Agarose Gel Preparation



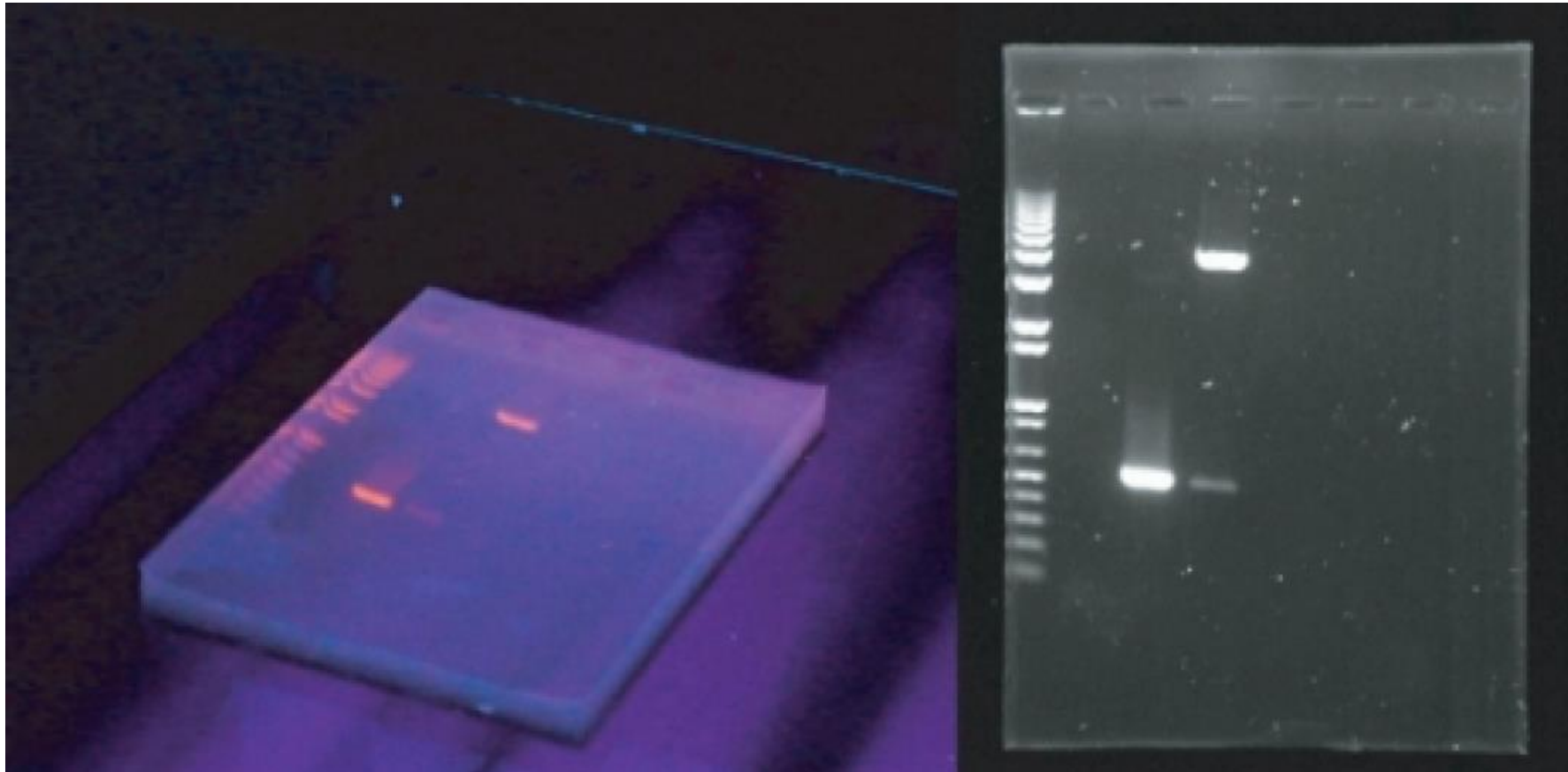
(2) Load the sample and start the run

- 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.



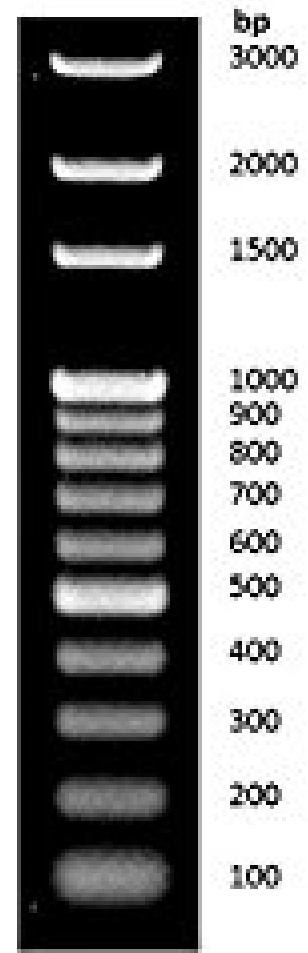
(3) Visualizing the sample

- ❖ 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.