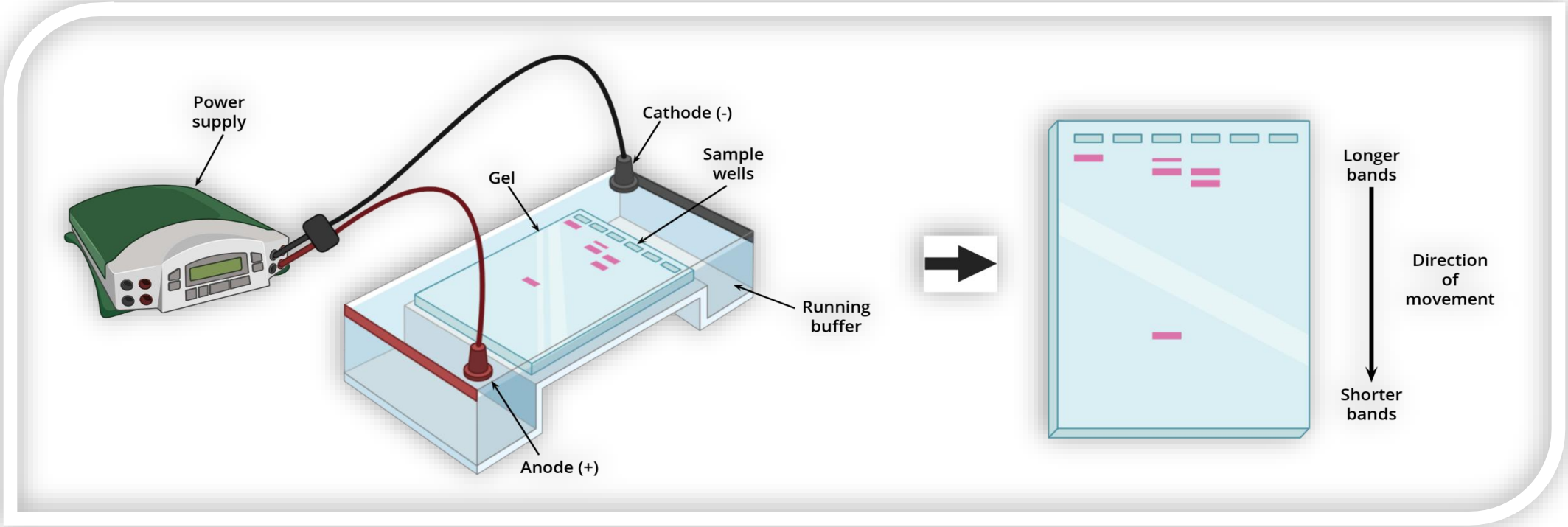
DNA electrophoresis



By:

Dr/ Aya El-Hanafy

Associate Prof. Of Biochemistry & Molecular Biology

Learning outcomes

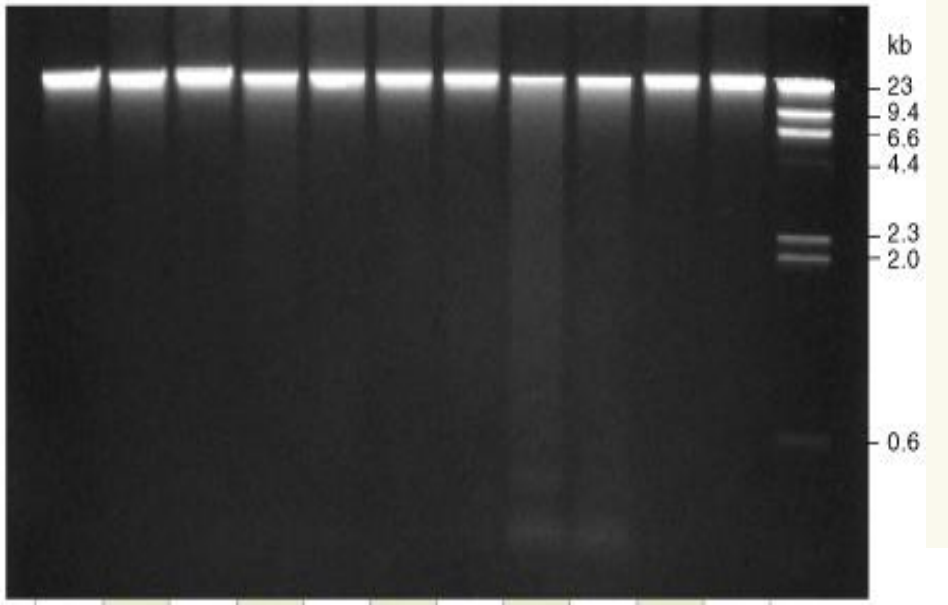
❑ **At the end of this session, students should be able to:**

- Understand **agarose gel electrophoresis** technique.
- Demonstrate **DNA separated by agarose gel electrophoresis**.

Assessment of quality and quantity of extracted DNA

- The product of DNA extracted will be used in subsequent experiments.
  Poor quality DNA will not perform well in PCR.

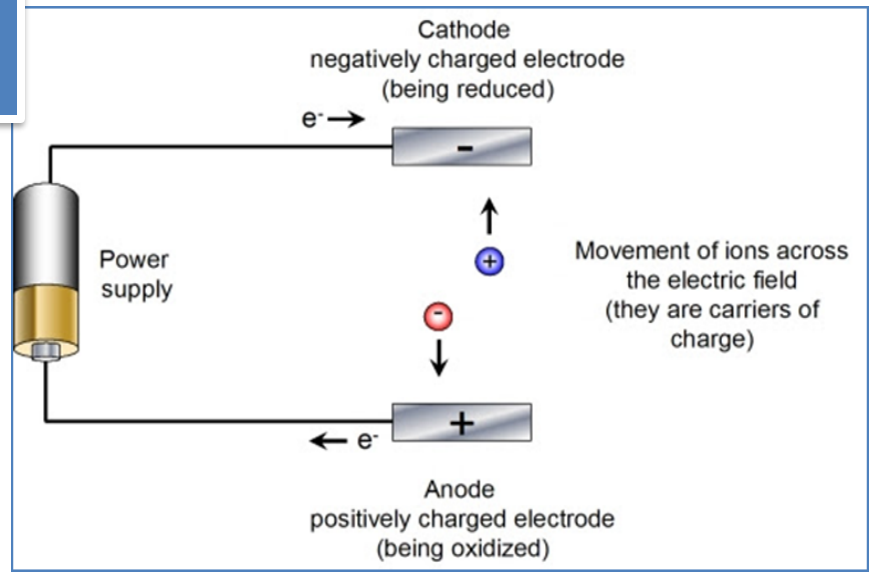
- For assessing quality & quantity of extracted DNA:
  1. **Agarose gel electrophoresis**
  2. **UV spectrophotometry (Nanodrop)**

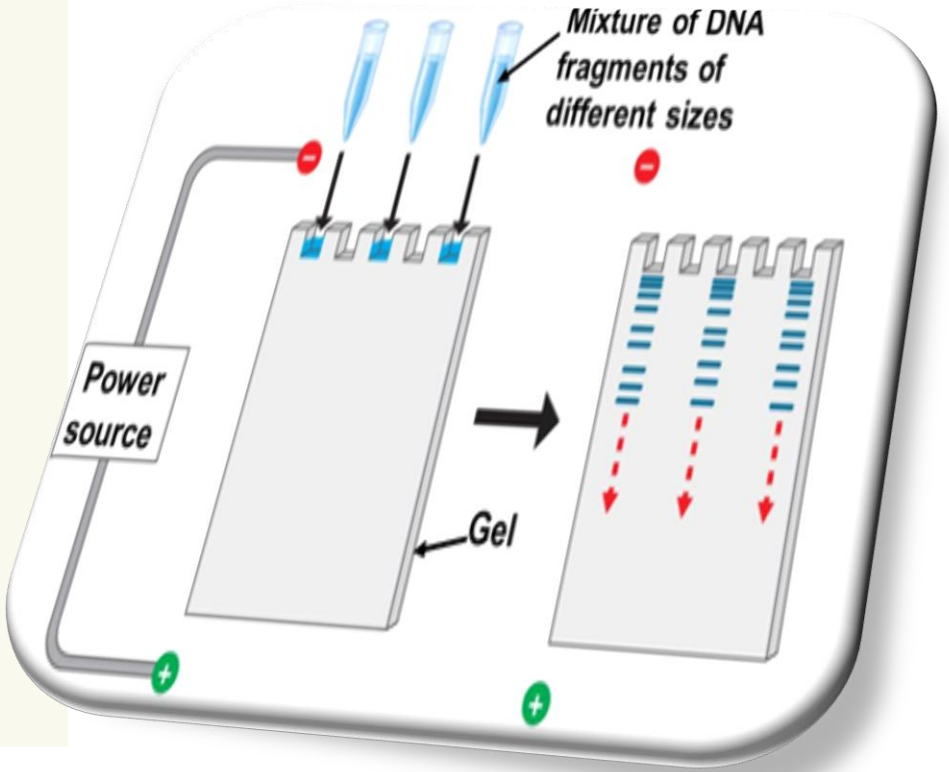


Electrophoresis

Definition

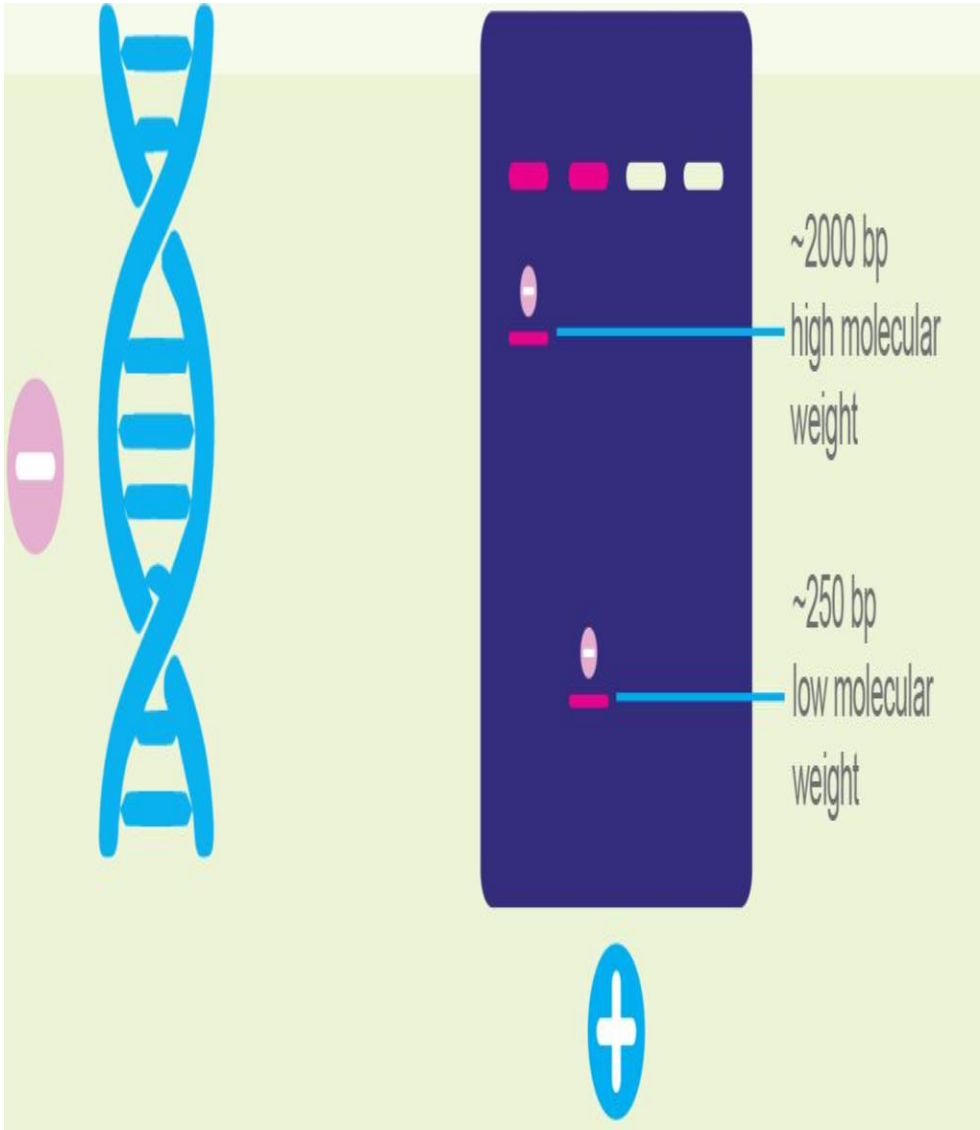
- It is the movement of charged particles under the influence of an electric Field
  -Cations move towards cathode
  -Anions move towards anode
- Used to identify and separate biomolecules e.g **nucleic acids or proteins** based on **size and charges**





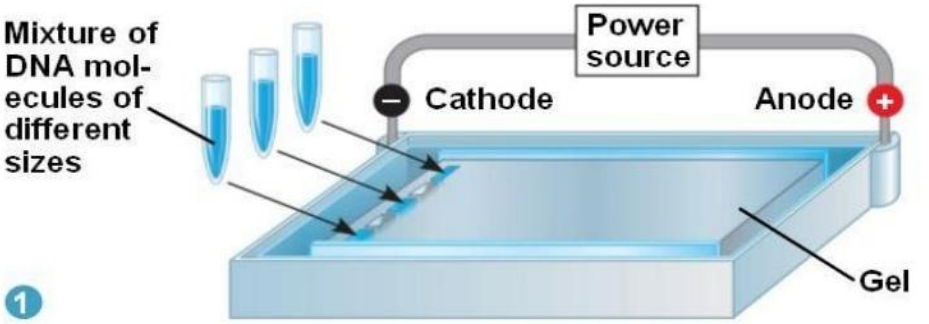
Principle:

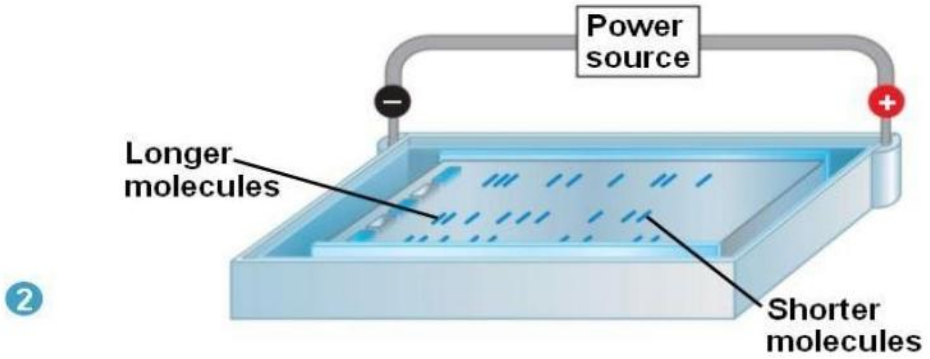
- The **+ve charged particles (cations)** move to **cathode (-)** and -ve charged particles (anions) move to **anode(+).**
- In an electric field, DNA (contains -ve charged phosphate groups) will migrate toward the **+ve anode.**

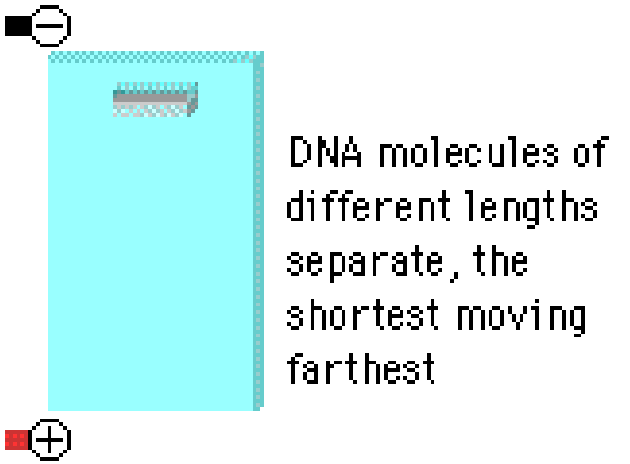


Principle: (cont.)

- The **fixed -ve DNA charge** makes the separation of the DNA chains to occur on the basis of the **molecular size.**
- **Shorter molecules** migrate more **rapidly** through the pores of a supporting media (gel) than do longer molecules.

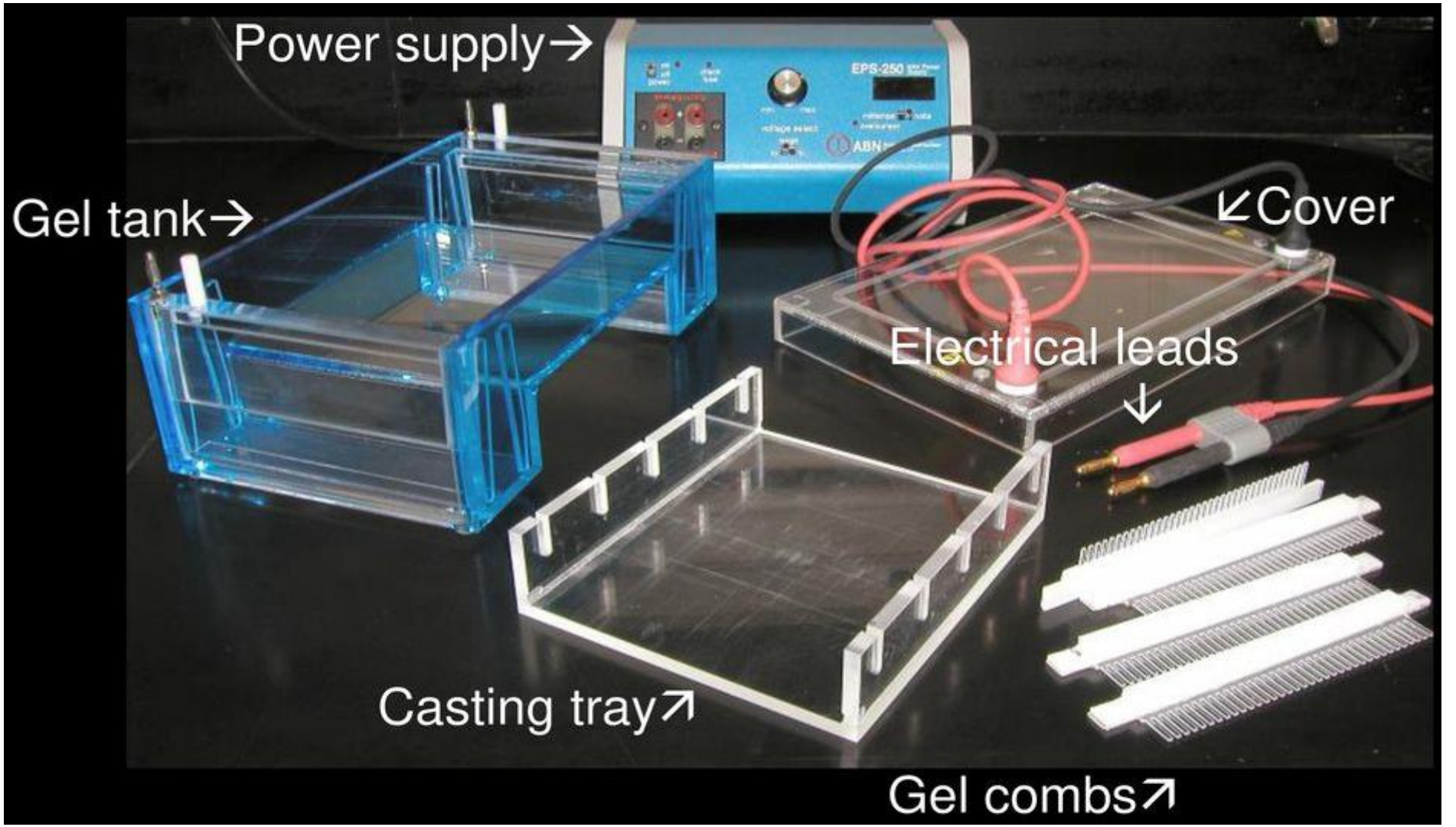






Electrophoresis Apparatus

Electrophoresis Apparatus



Types of Gel Electrophoresis

- **According to the supporting media used**

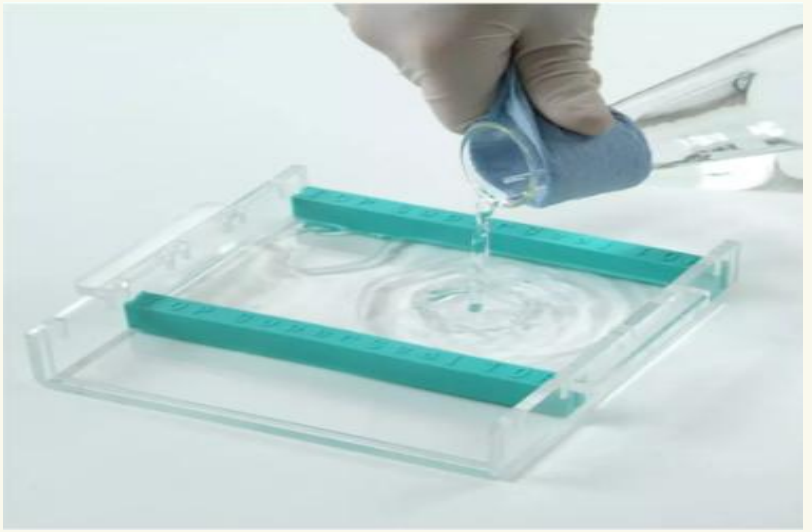
(the matrix at which separation takes place) :

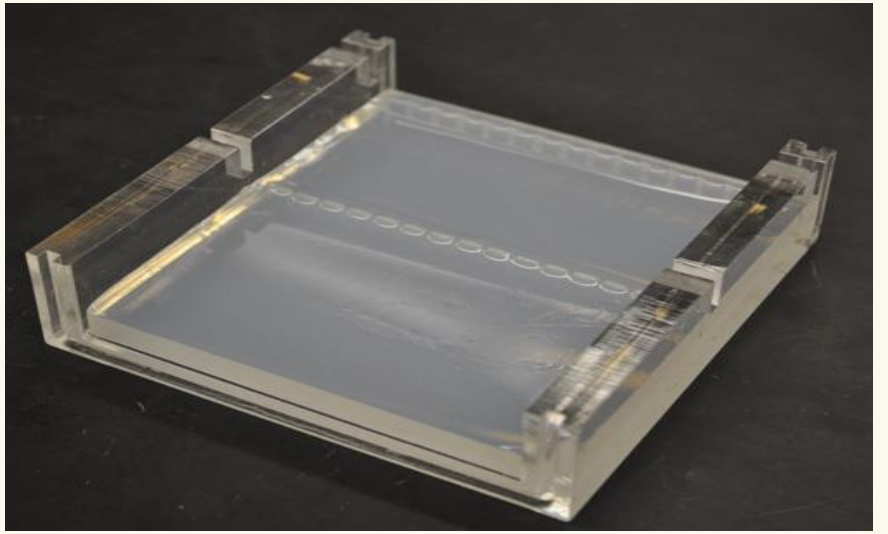
1)Agarose gel electrophoresis.

2)Polyacrylamide gel electrophoresis (PAGE).

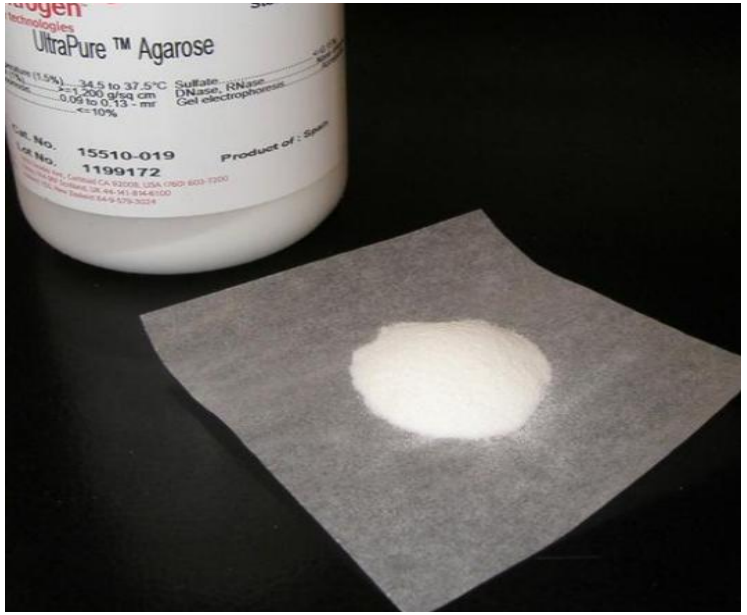
Agarose Gel Electrophoresis

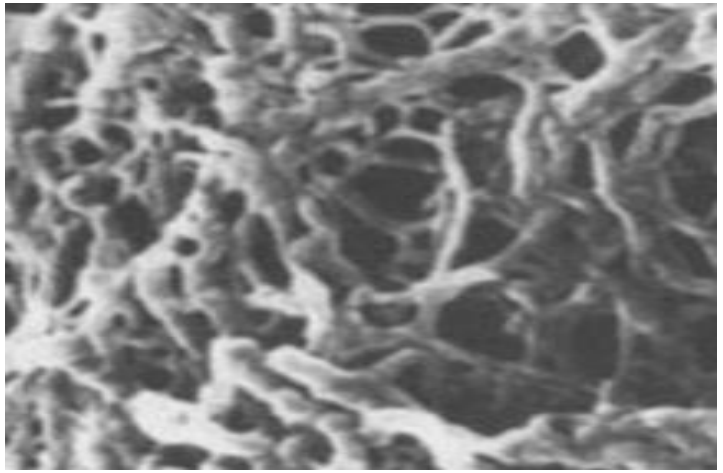
- **Agarose is hetero-polysaccharide that forms viscous liquid when hot that solidify to a gel on cooling.**
- **The gel is prepared in the buffer , spread over a casting tray and allowed to cool.**





Agarose gel used in Electrophoresis



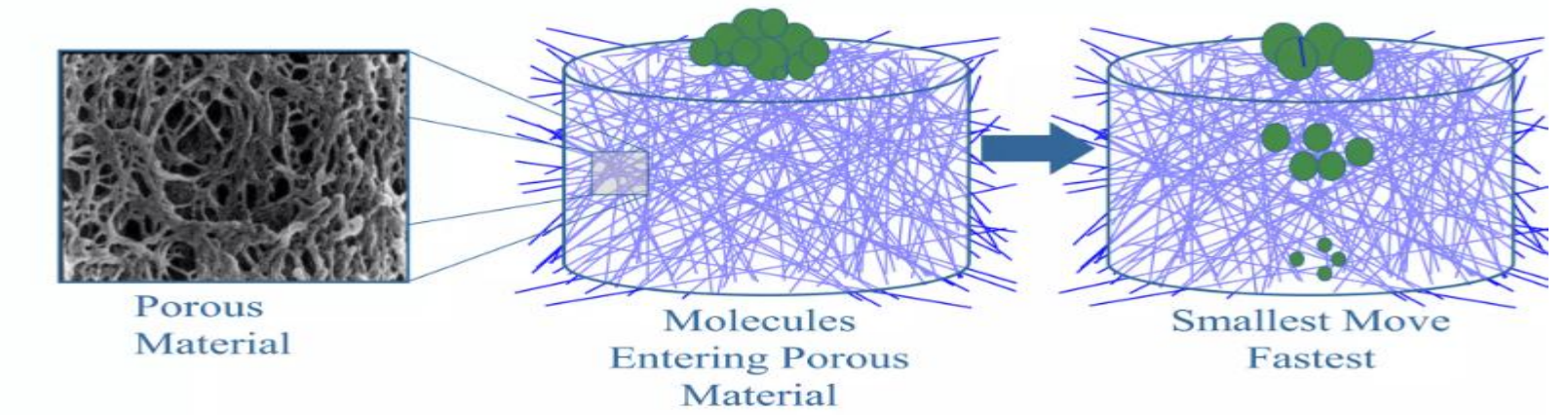


- Agarose is porous, allowing for the movement of DNA.
- The gel resembles a sponge with holes; DNA travels through "holes")

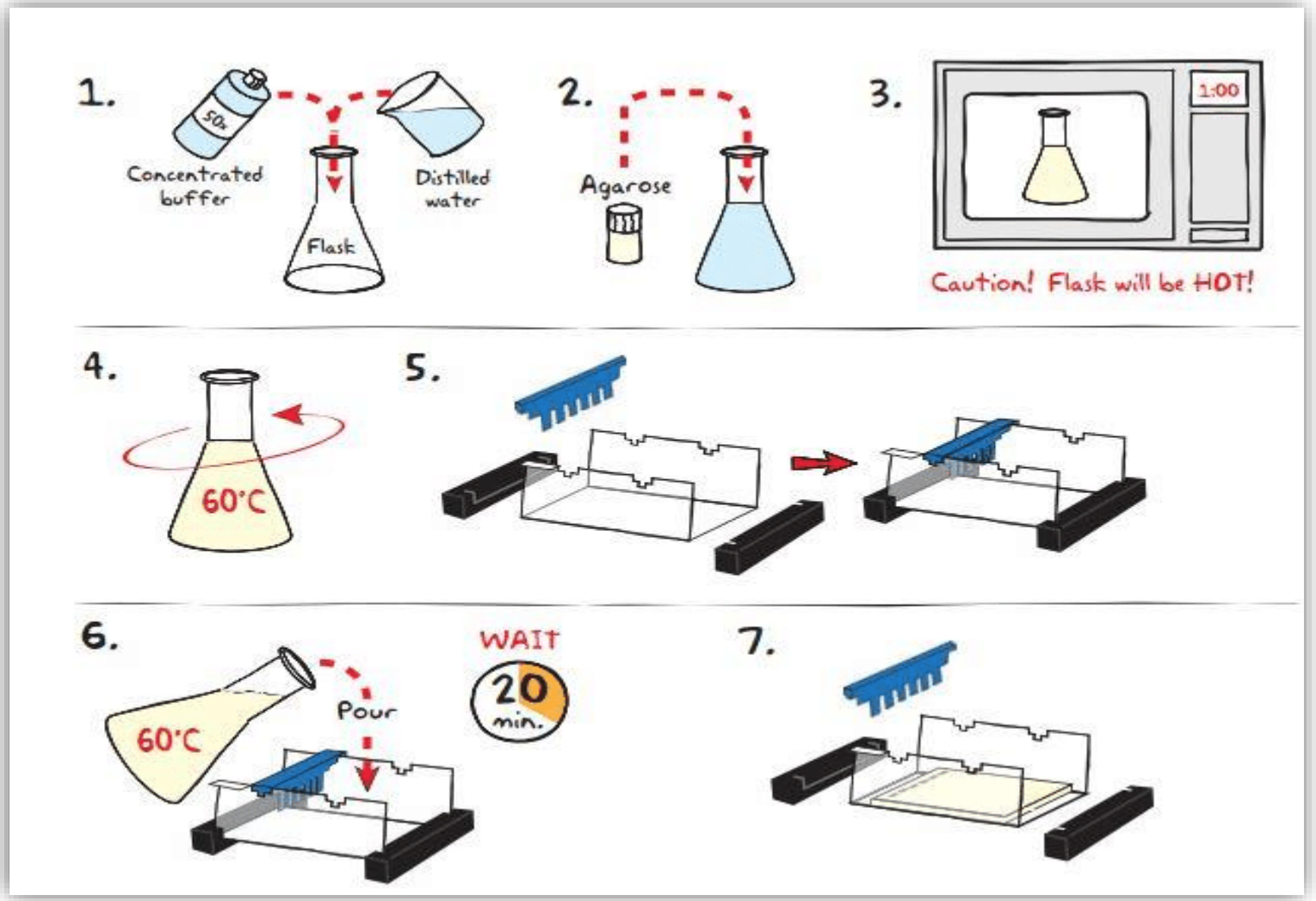
Agarose gel used in Electrophoresis

- **Low conc. = larger pores → better resolution of larger DNA fragments**
- **High conc. = smaller pores → better resolution of smaller DNA fragments**

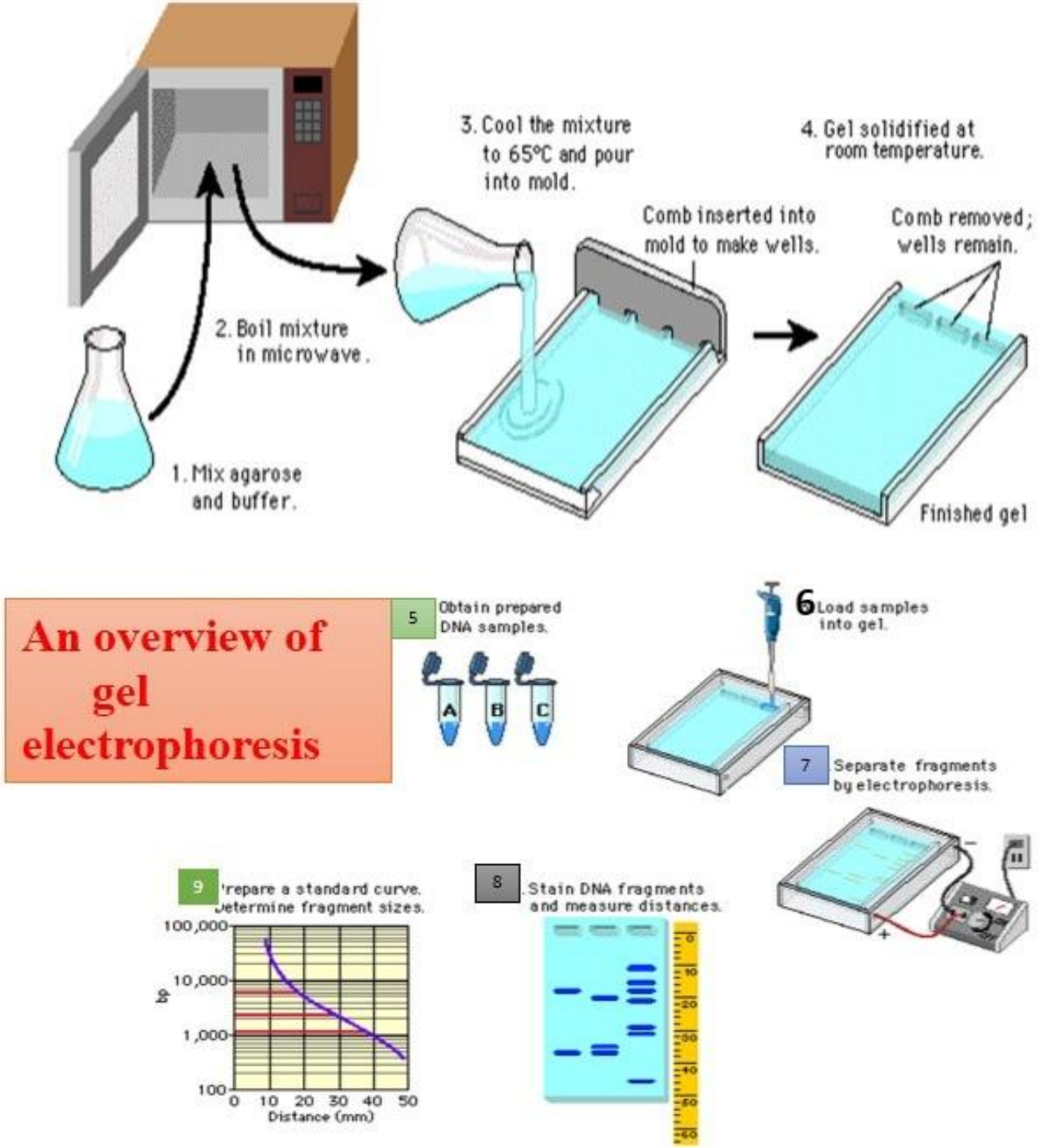
❑ **So smaller molecules move faster and migrate farther than larger ones because smaller molecules migrate more easily through the pores of the gel. This phenomenon is called sieving.**



Agarose Gel Electrophoresis: Steps



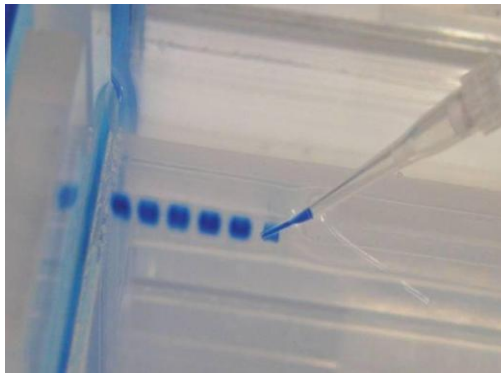
Agarose Gel Electrophoresis: Steps

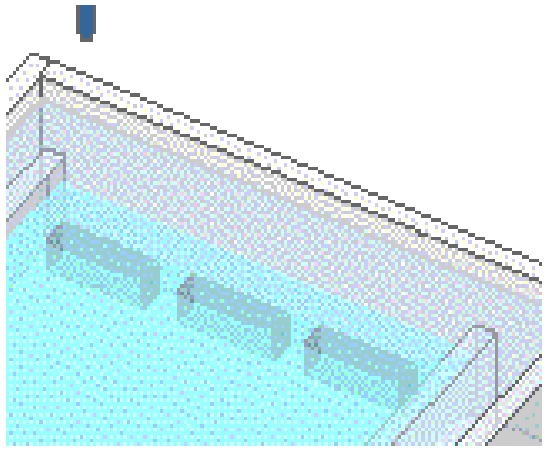


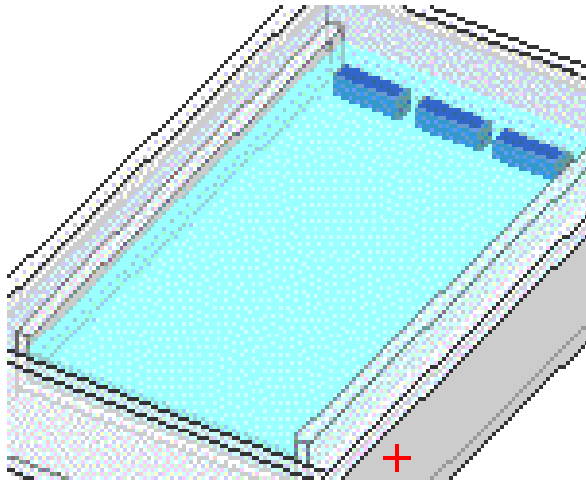
Agarose Gel Electrophoresis: Steps

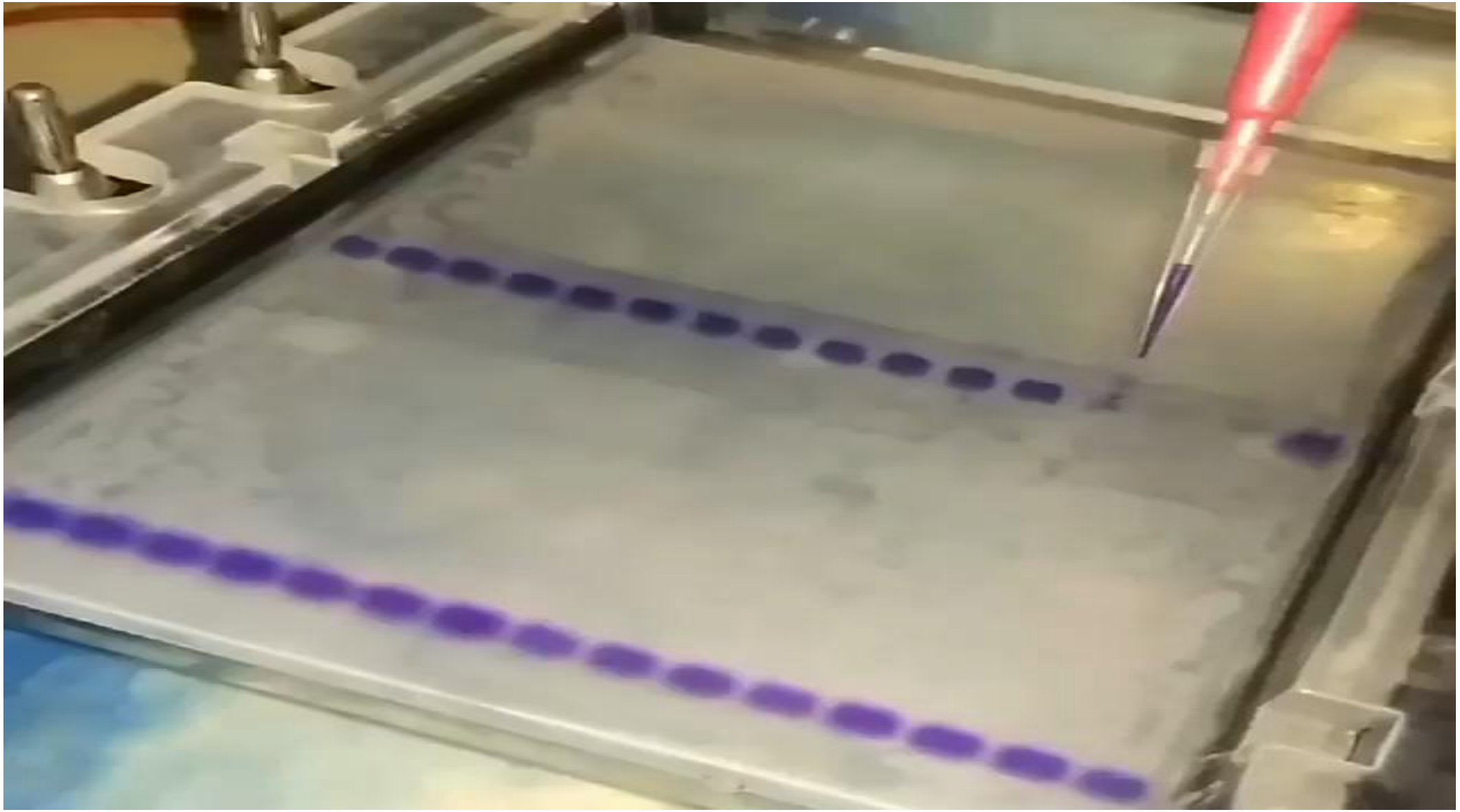
- A small sample (few µls) is applied into the gel wells.

- A colored <u>loading dye</u> (Bromophenol blue) is mixed to DNA sample to:
  - *Increase sample weight/density to help sample loading*
  - *Track the DNA migration (color)*





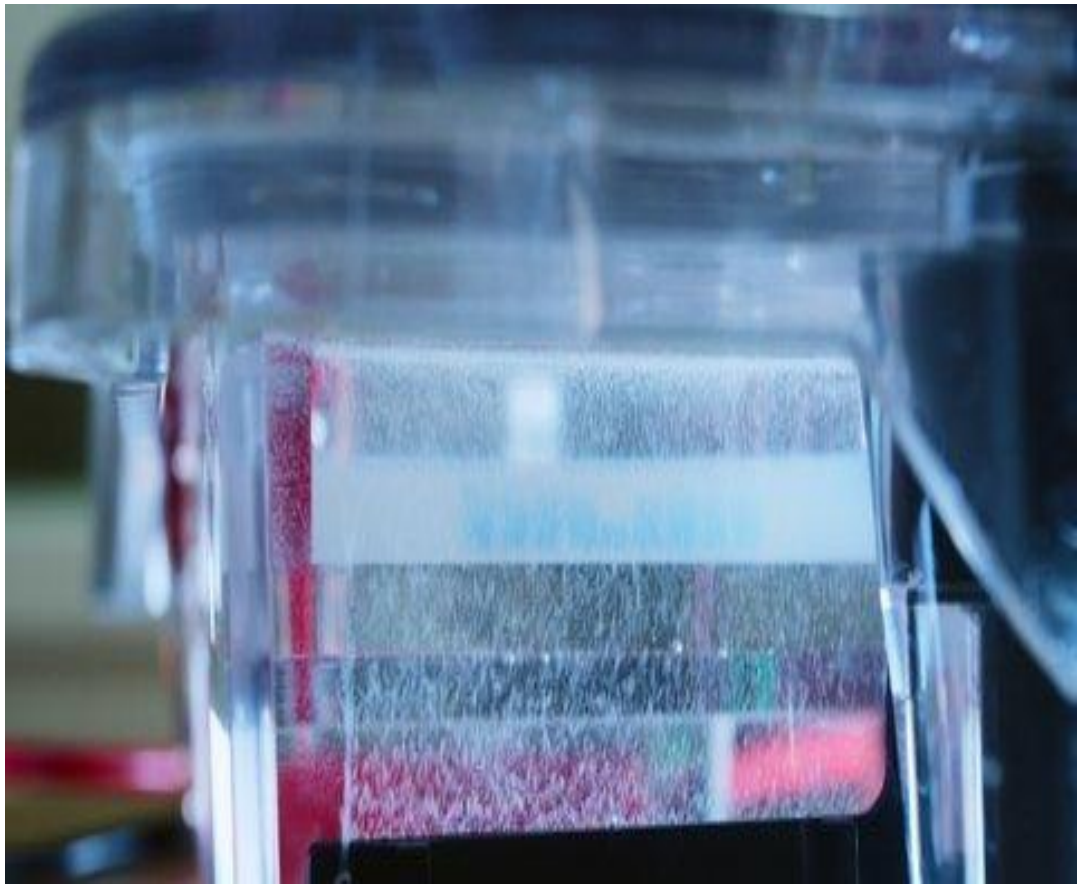


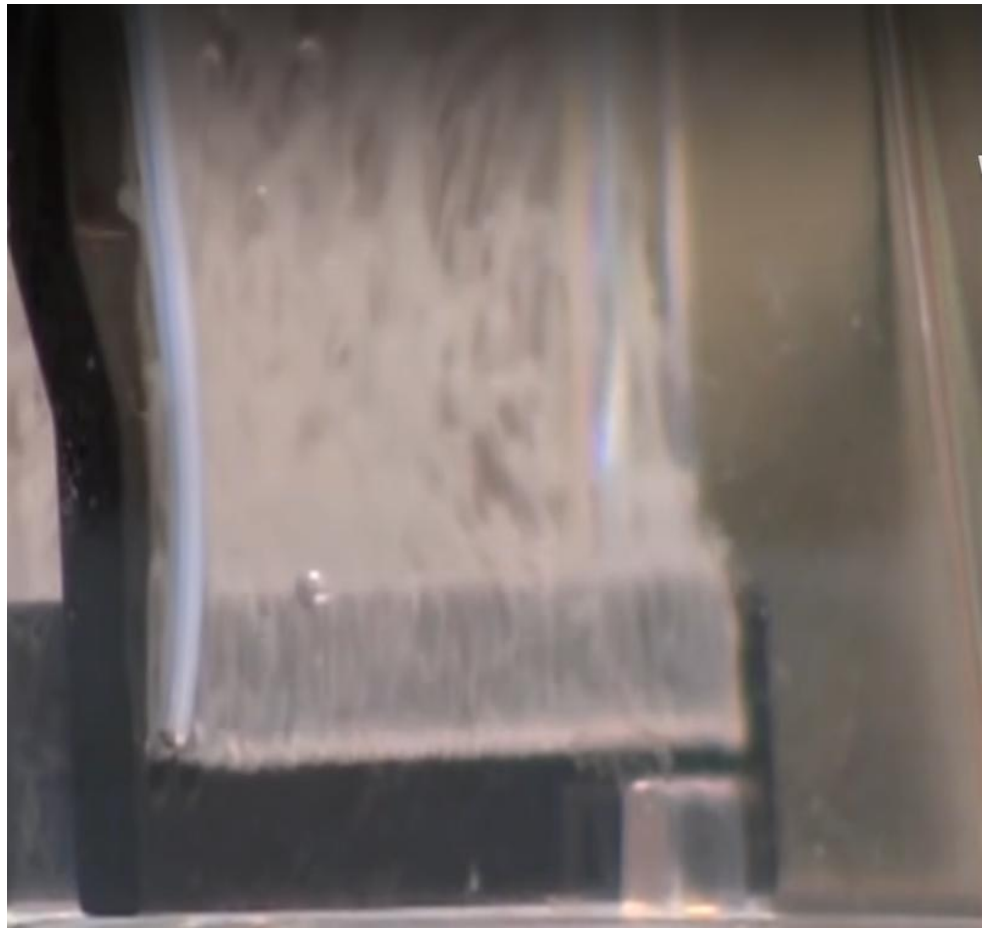


Running the gel (exposing it to an electric field)

- Black cable is connected to the negative pole, and red cable is connected to the positive.
- The DNA is negatively charged and will run towards the positive electrode
- Run the gel until the dye line is approximately 75-80% down of the gel.

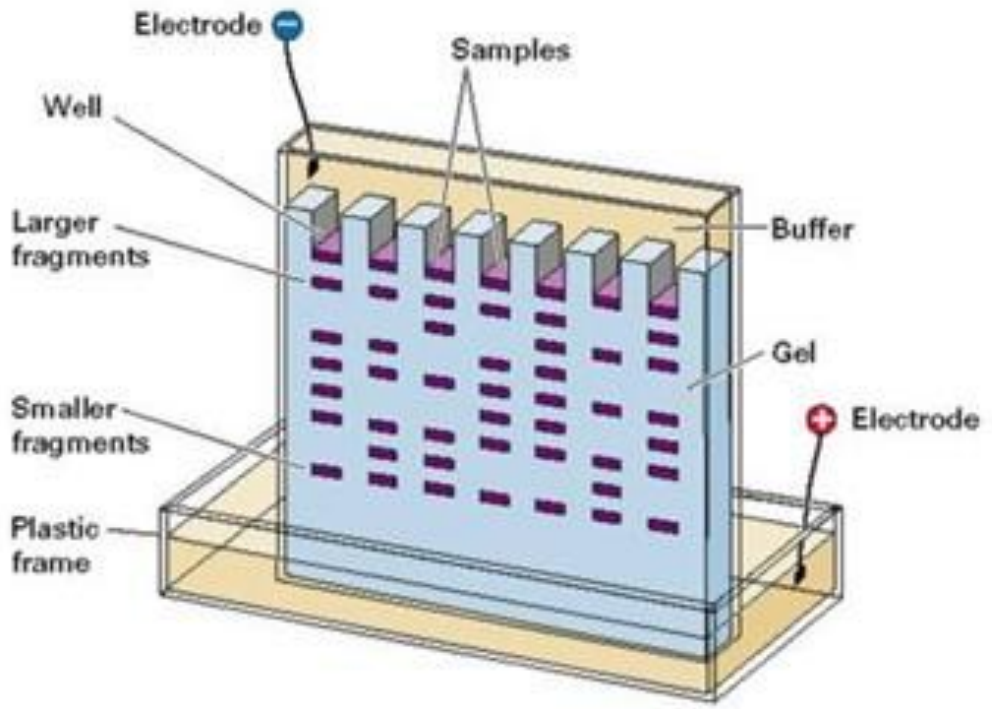
The presence of bubbles means that the electrodes are connected, plugged in, and that current is flowing.





Polyacrylamide gel electrophoresis (PAGE)

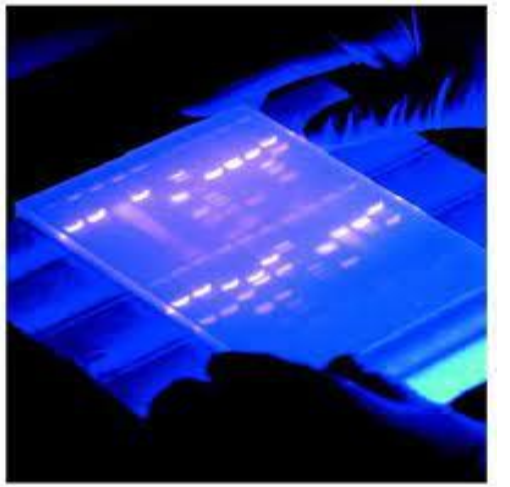
- **Very efficient separation.**
- **Polyacrylamide gel separate short DNA chains that differ in length by only one nucleotide while agarose gel separate chains of larger size.**

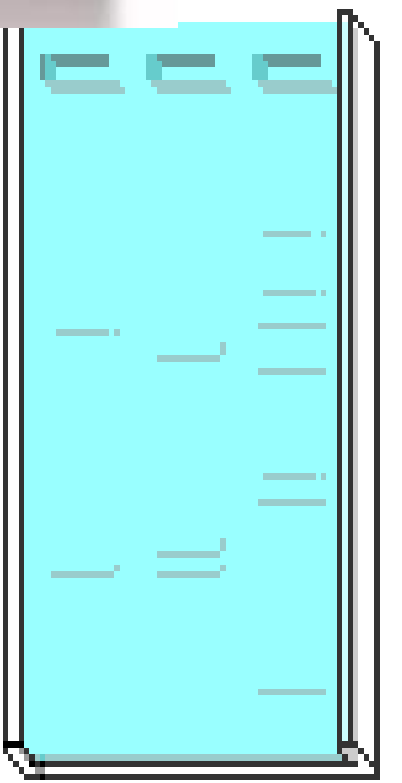


Visualization of DNA

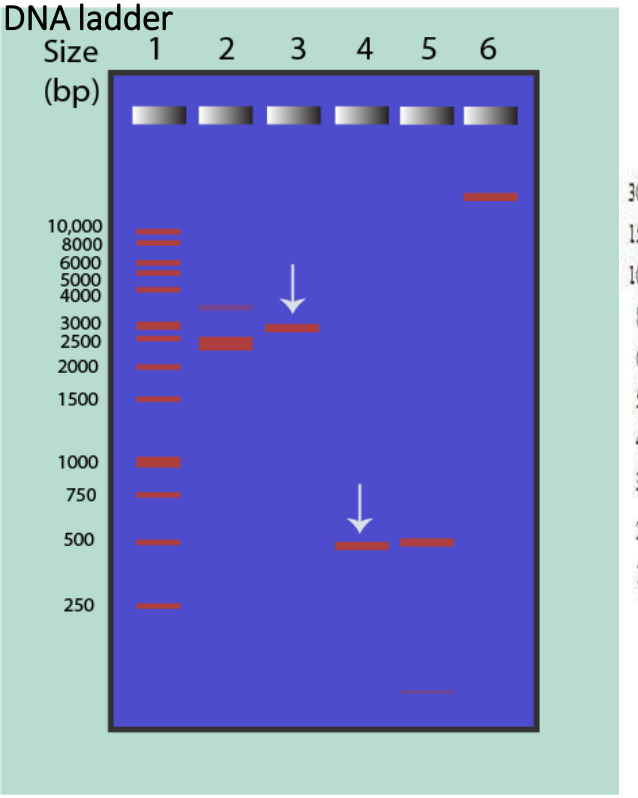
- DNA bands in a gel can be visualized by **Staining with dyes (e.g., ethidium bromide):** allows direct visualization of DNA bands **under ultraviolet light** (UV transilluminator)

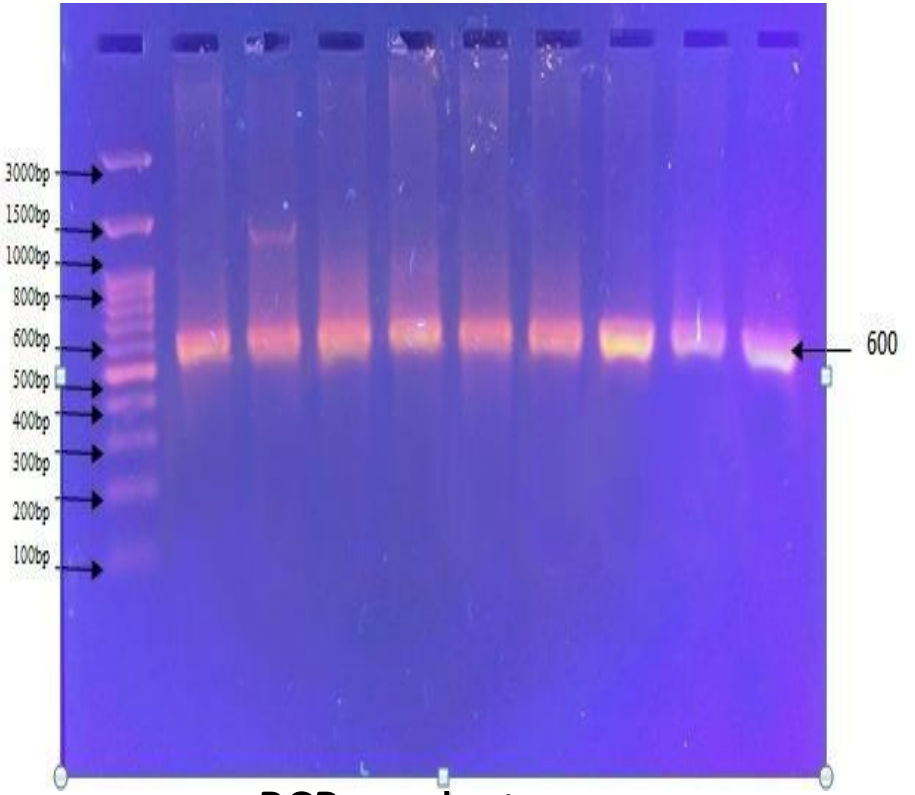
(Ethidium bromide intercalates between the nitrogenous bases of DNA and fluoresces under UV light).





Visualization of DNA





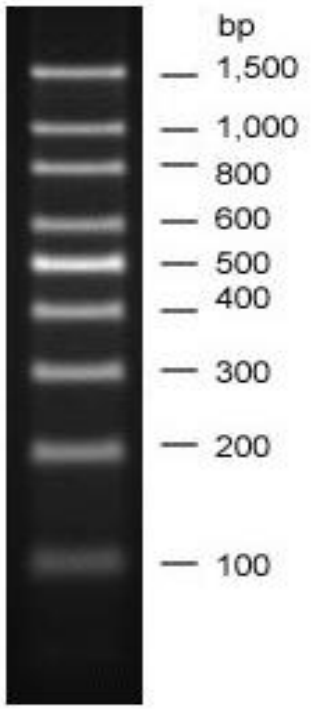
PCR product

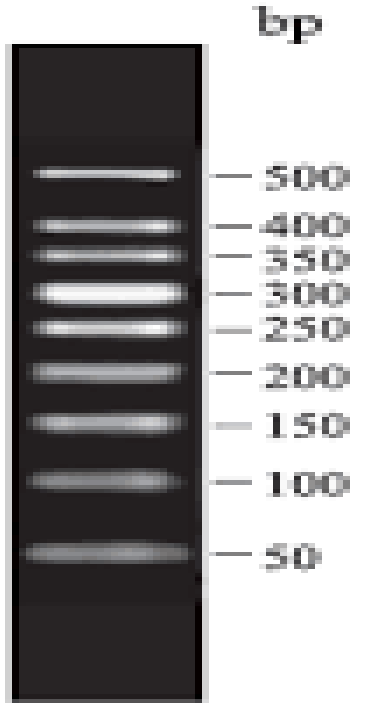
CAUTION! Ethidium bromide is a powerful *mutagen* and is moderately toxic. Gloves should be worn at all times

What is DNA ladder?

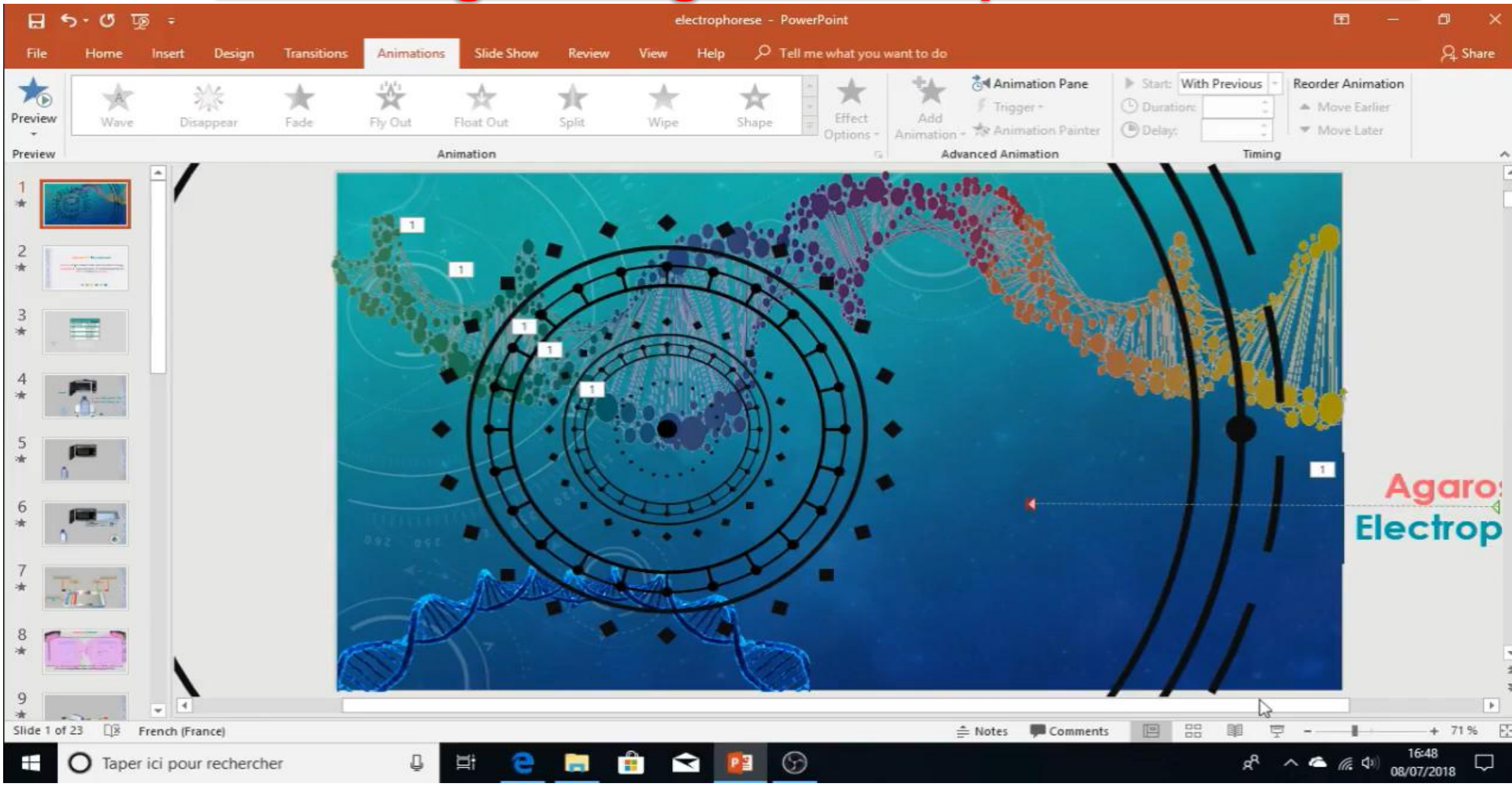
It is a solution of DNA molecules of **different lengths** used in agarose or acrylamide gel electrophoresis.

It is applied as a **reference** to estimate the size of unknown DNA molecules by comparing them to the closest fragment in the ladder.





Agarose gel Electrophoresis



https://youtu.be/saJIWFUGEbw?si=OVk7B4KUpvupKaSY

Agarose gel Electrophoresis (virtual lab)

- https://learn.genetics.utah.edu/content/labs/gel/

Further Readings

- BRS Biochemistry, Molecular Biology and Genetics, 5th edition, 2010.

https://www.youtube.com/watch?v=f-_8eT4wt5Q

- https://www.sciencedirect.com/topics/neuroscience/electrophoresis
- https://www.khanacademy.org/science/.../gel-electrophoresis-dna


THANK YOU