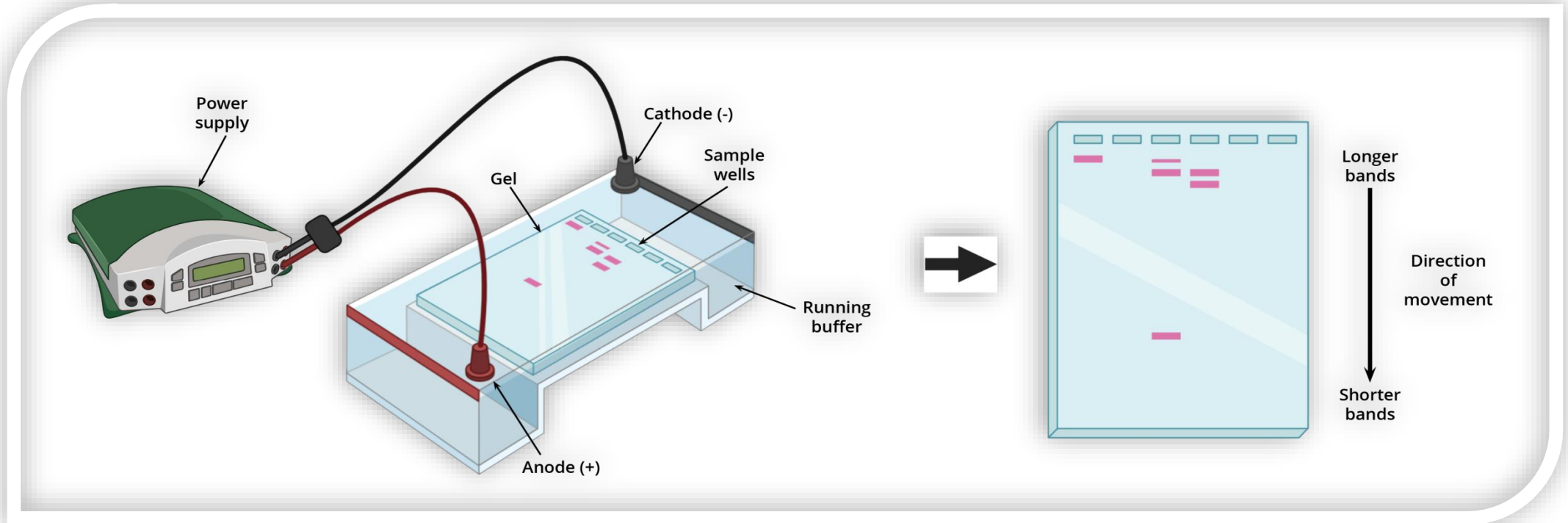


DNA electrophoresis



By:
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Associate Prof. Of Biochemistry & Molecular Biology

Learning outcomes

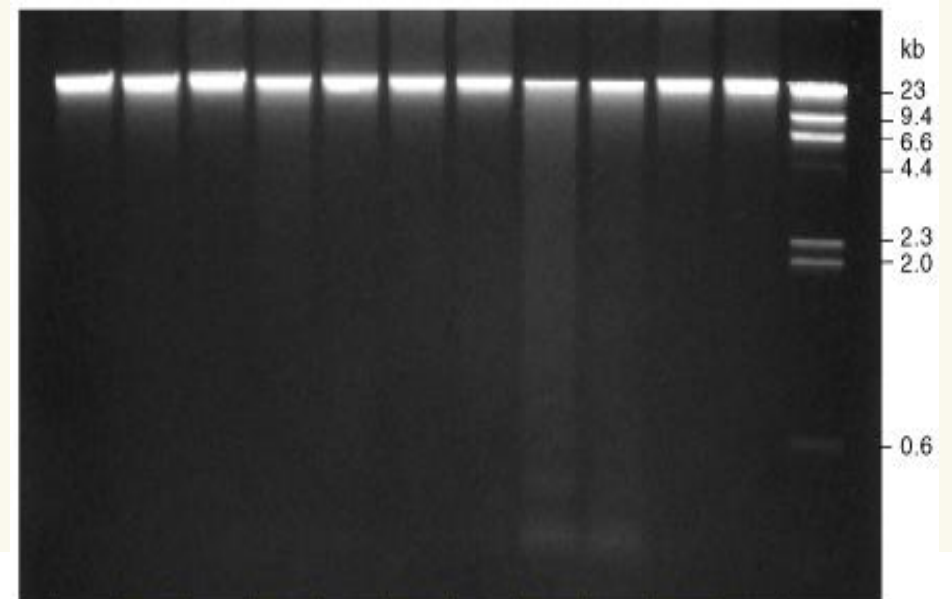
- ❑ **At the end of this session, students should be able to:**
 - Understand **agarose gel electrophoresis** technique.
 - Demonstrate **DNA separated by agarose gel electrophoresis**.

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:

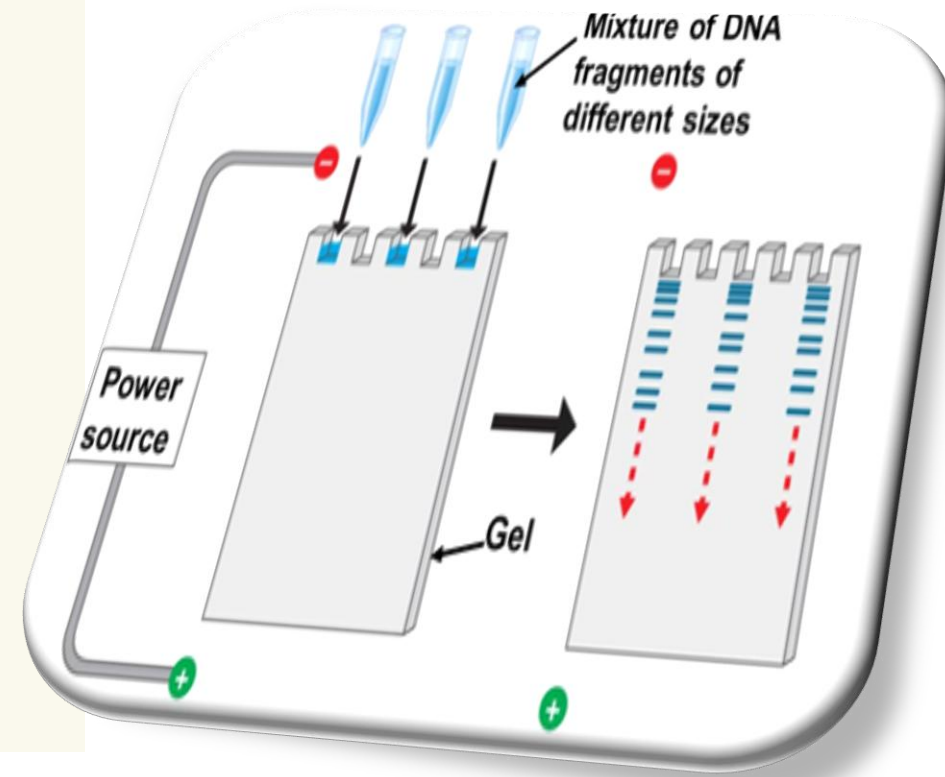
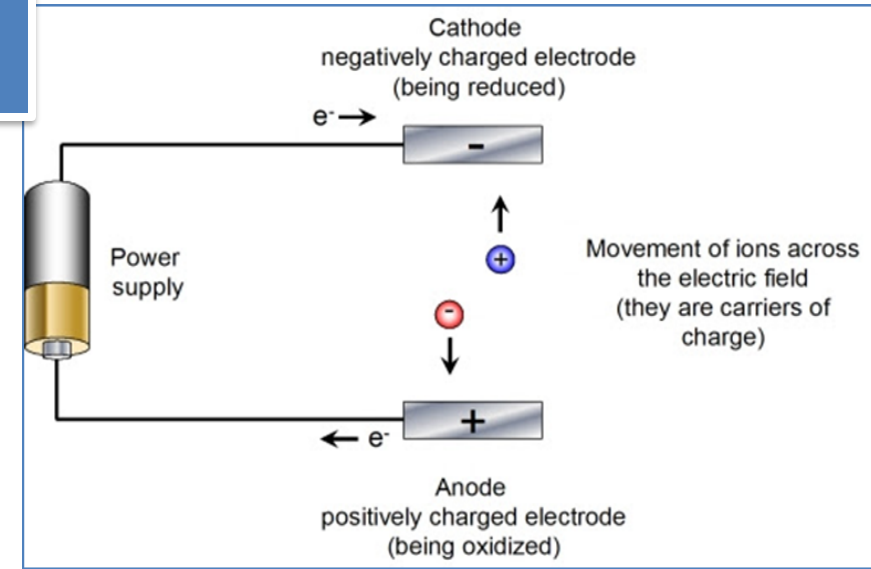
1. **Agarose gel electrophoresis**
2. **UV spectrophotometry (Nanodrop)**



Electrophoresis

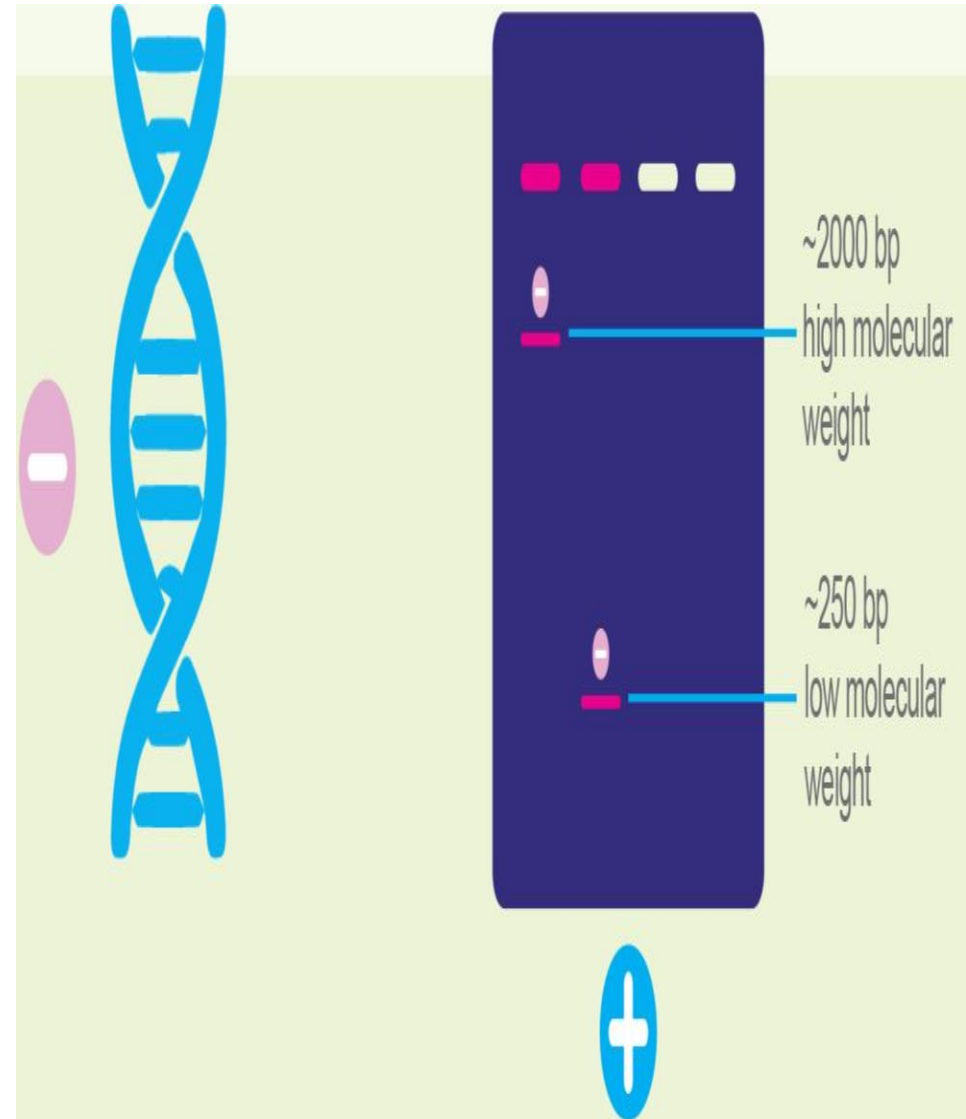
Definition

- 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**



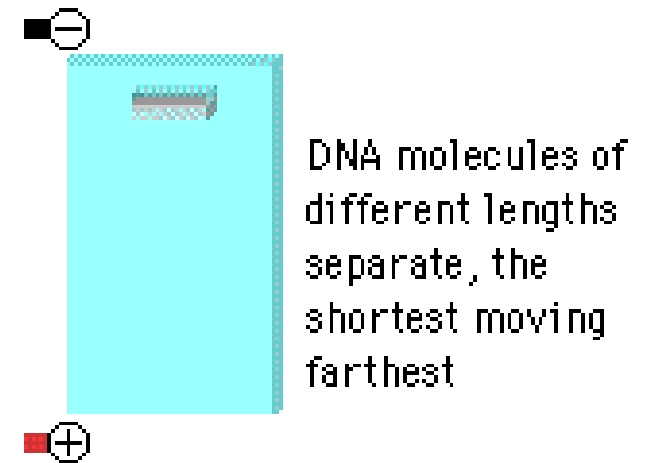
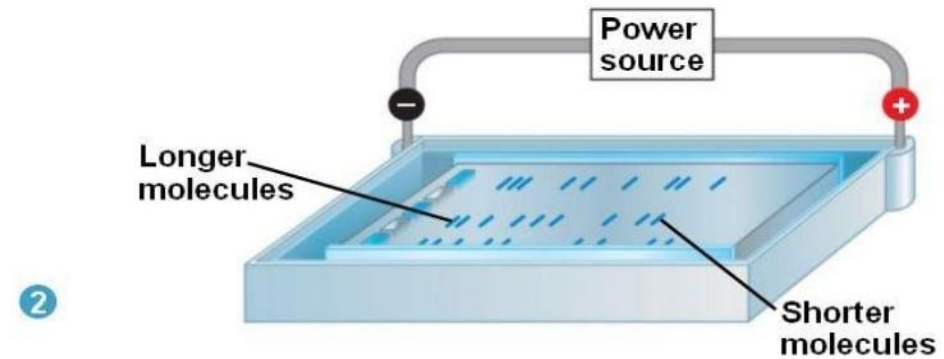
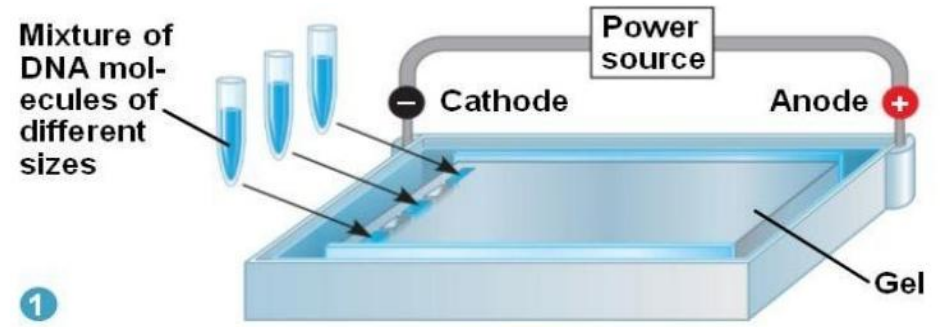
Principle:

- 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.)

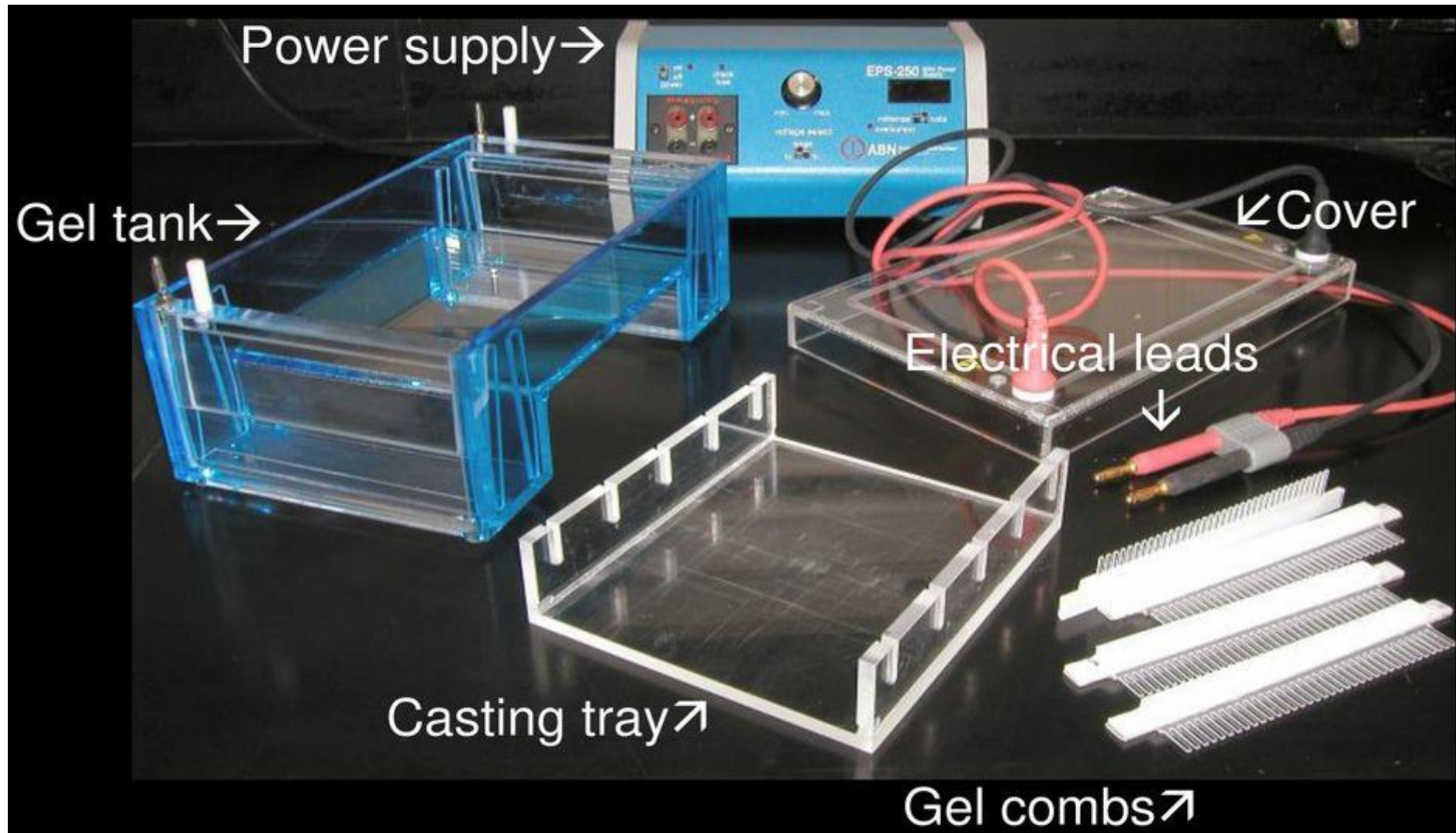
- The **fixed -ve DNA charge** makes the separation of the DNA chains to occur on the basis of the **molecular size**.
- **Shorter molecules** migrate more **rapidly** through the pores of a supporting media (gel) than do longer molecules.



Electrophoresis Apparatus



Electrophoresis Apparatus



Types of Gel Electrophoresis

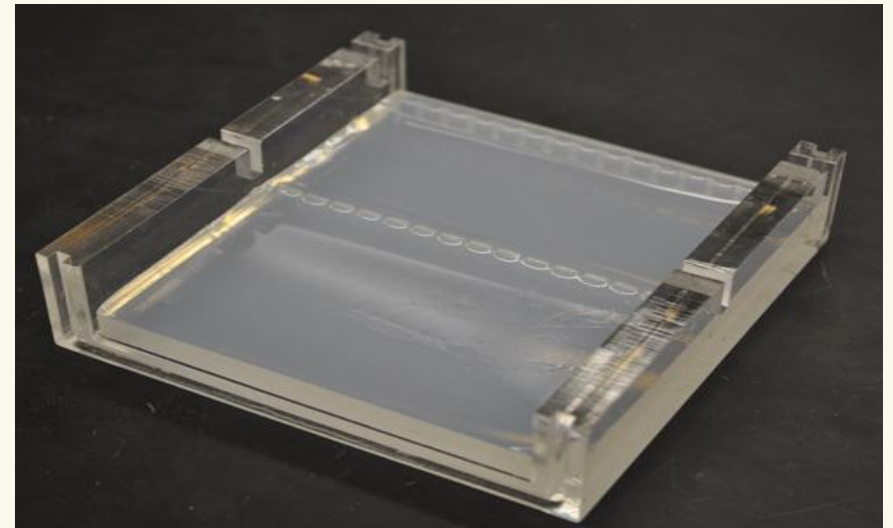
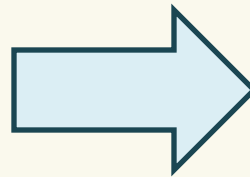
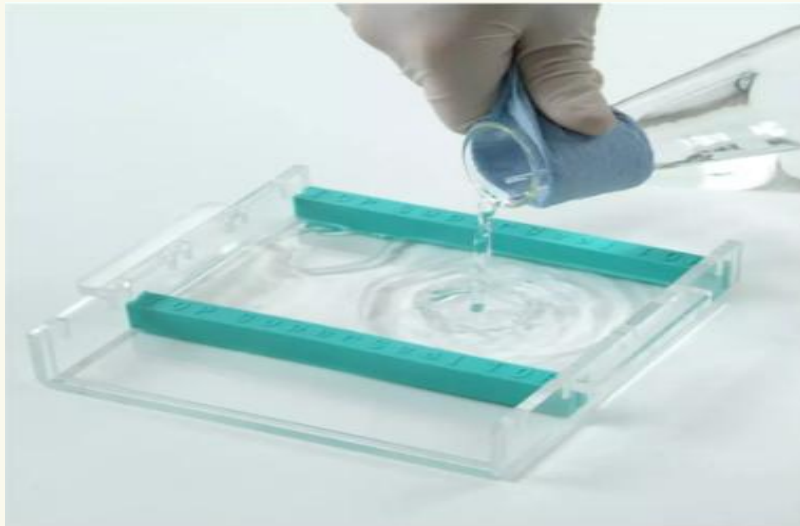
- According to the supporting media used (the matrix at which separation takes place) :

1) Agarose gel electrophoresis.

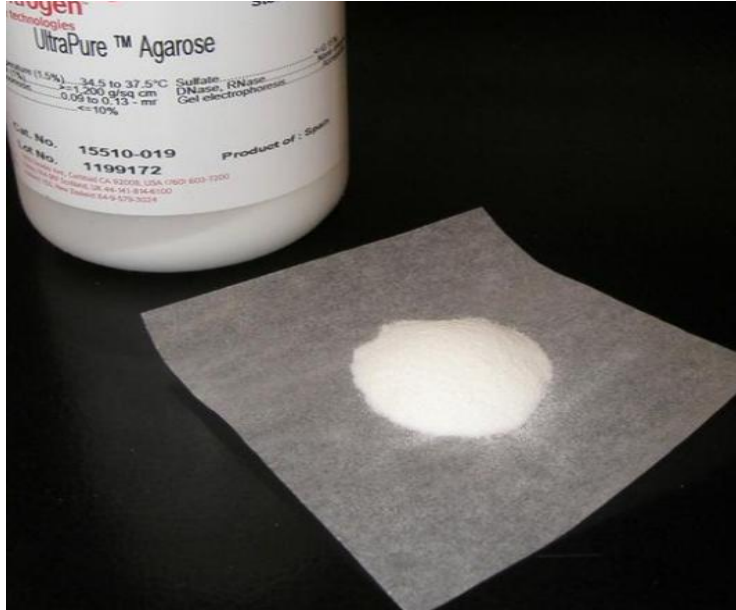
2) Polyacrylamide gel electrophoresis (PAGE).

Agarose Gel Electrophoresis

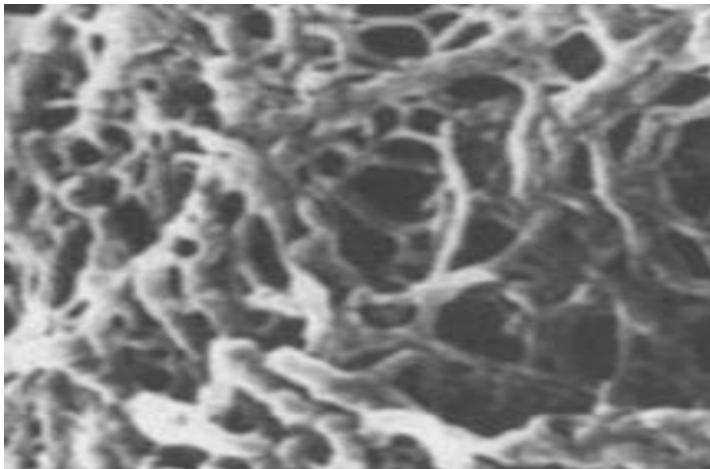
- Agarose is **hetero-polysaccharide** that forms viscous liquid when hot that **solidify to a gel on cooling**.
- The gel is prepared in the buffer , spread over a casting tray and allowed to cool.



Agarose gel used in Electrophoresis

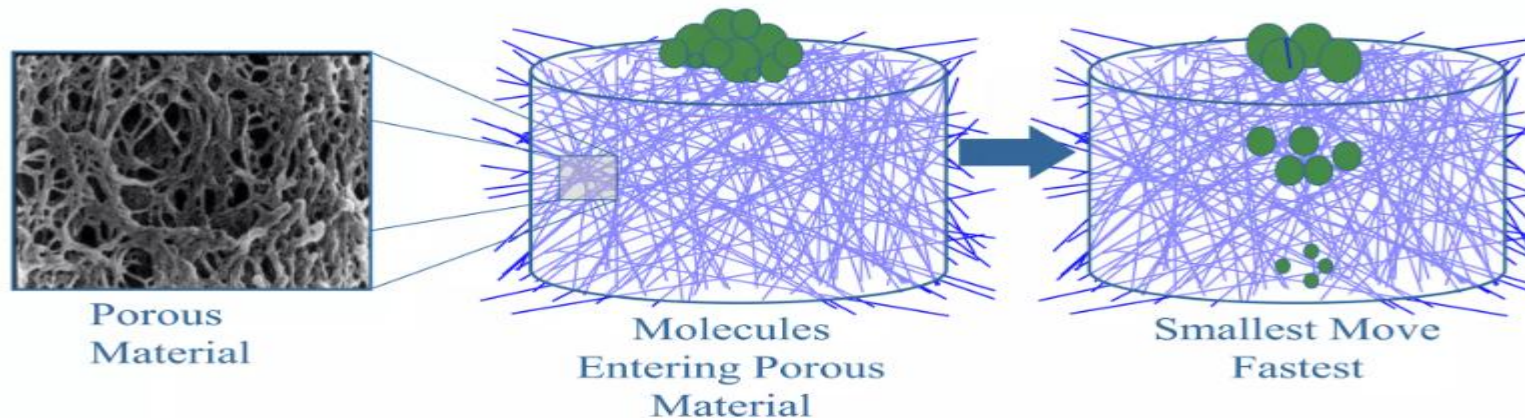


- Agarose is **porous**, allowing for the movement of DNA.
- The gel resembles **a sponge with holes**; DNA travels through "holes")

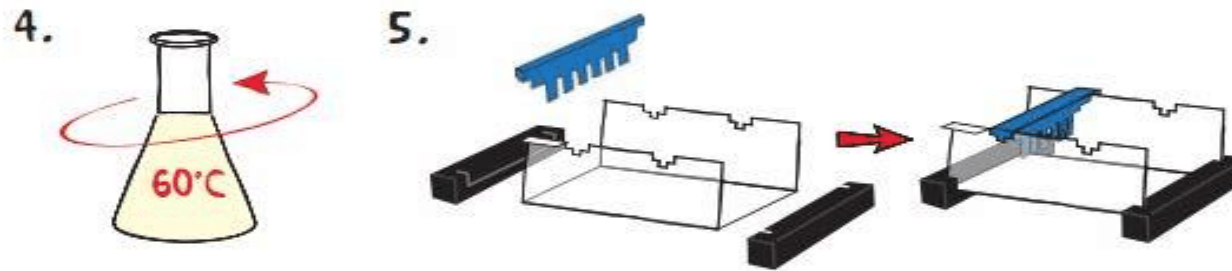
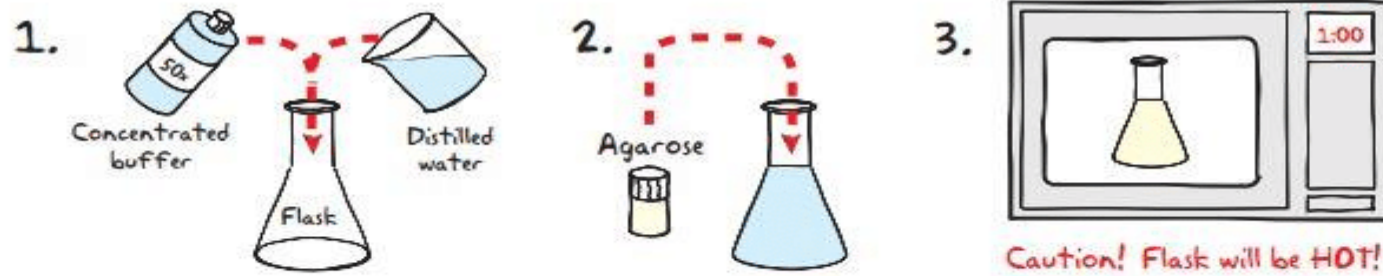


Agarose gel used in Electrophoresis

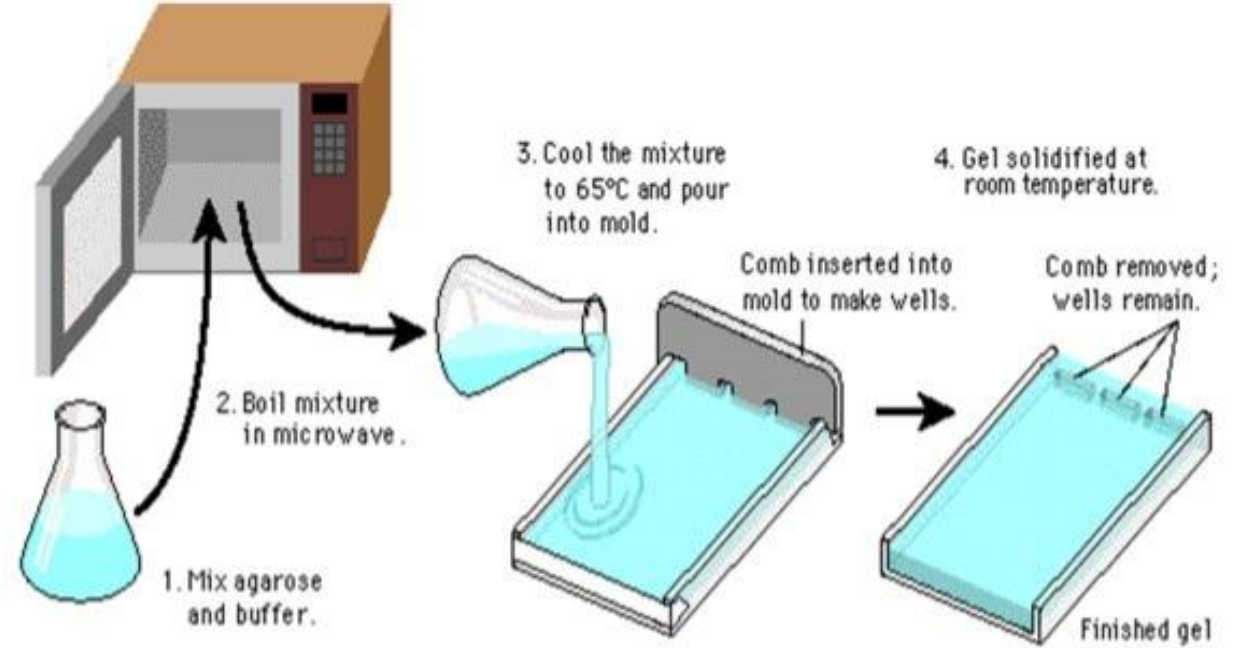
- Low conc. = larger pores → better resolution of **larger DNA fragments**
 - High conc. = smaller pores → better resolution of **smaller DNA fragments**
- So smaller molecules move faster and migrate farther than larger ones because smaller molecules migrate more easily through the pores of the gel. This phenomenon is called **sieving**.



Agarose Gel Electrophoresis: Steps



Agarose Gel Electrophoresis: Steps



An overview of gel electrophoresis

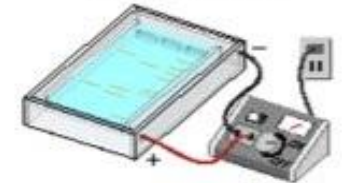
5 Obtain prepared DNA samples.



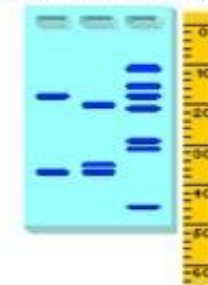
6 Load samples into gel.



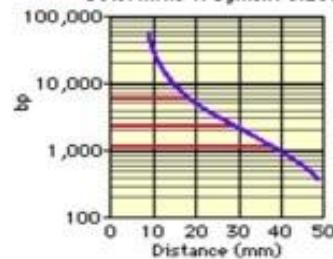
7 Separate fragments by electrophoresis.



8 Stain DNA fragments and measure distances.

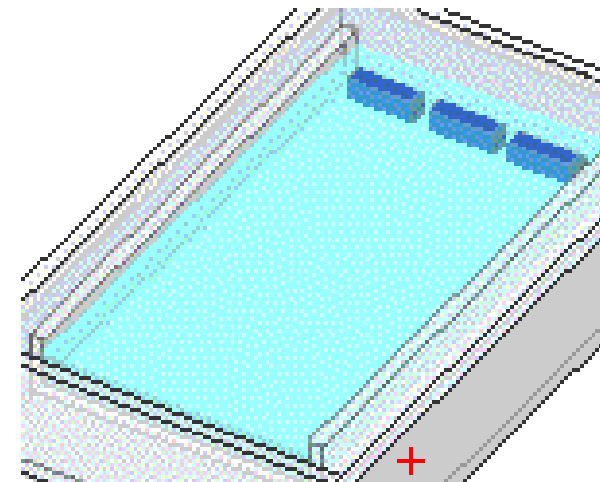
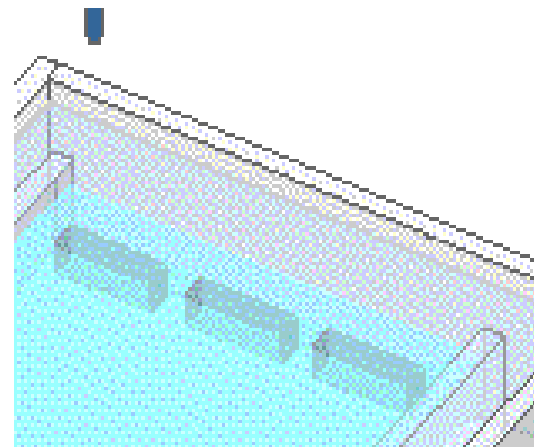
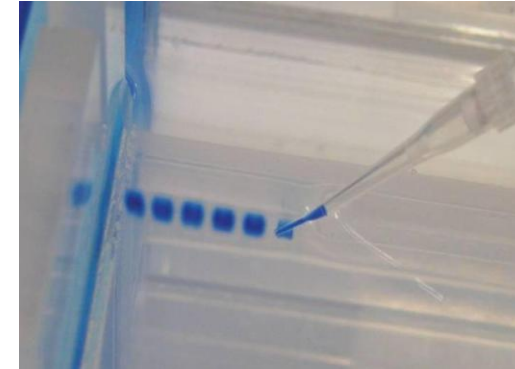
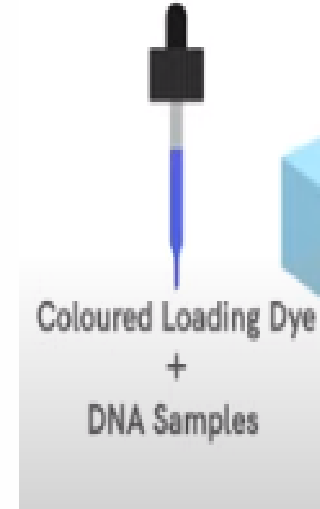


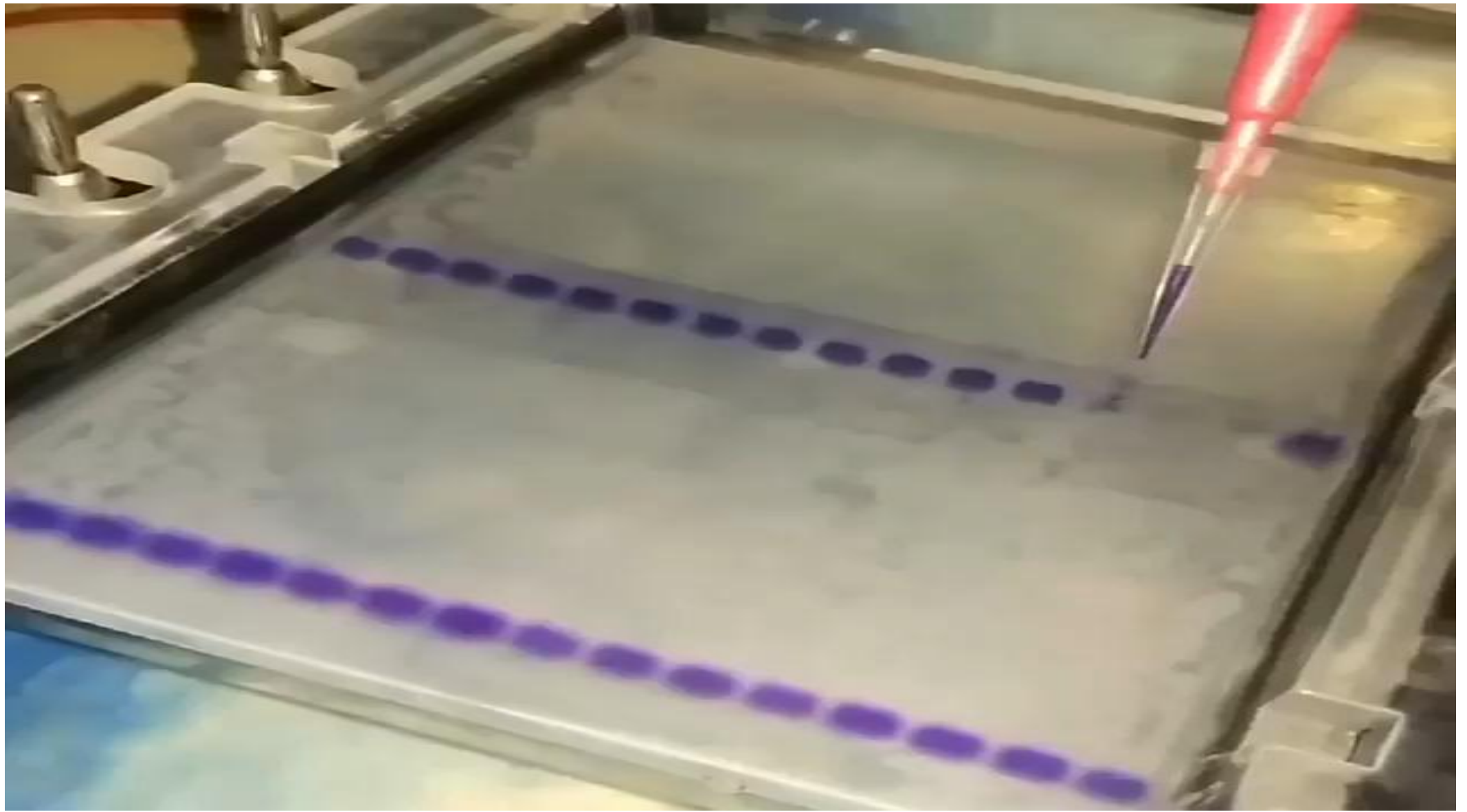
9 Prepare a standard curve. Determine fragment sizes.



Agarose Gel Electrophoresis: Steps

- ❑ A small sample (few μls) is applied into the gel wells.
- ❑ A colored loading dye (Bromophenol blue) is mixed to DNA sample to:
 - *Increase sample weight/density to help sample loading*
 - *Track the DNA migration (color)*



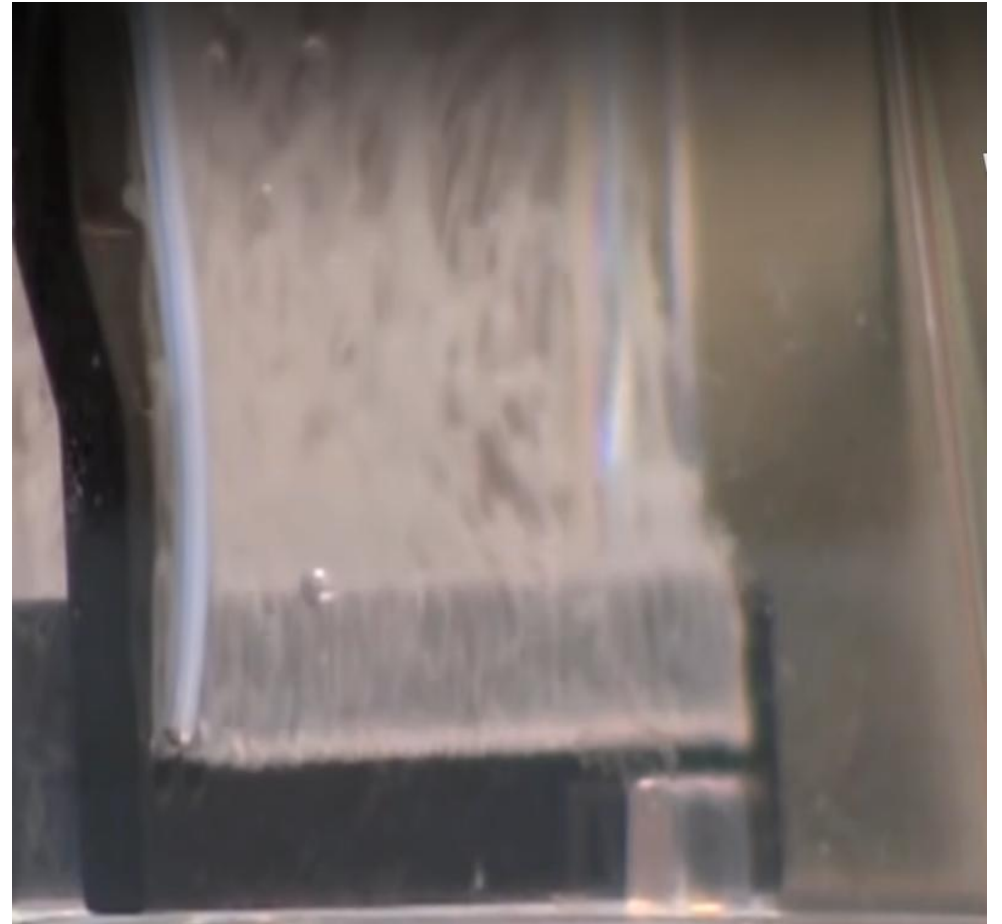
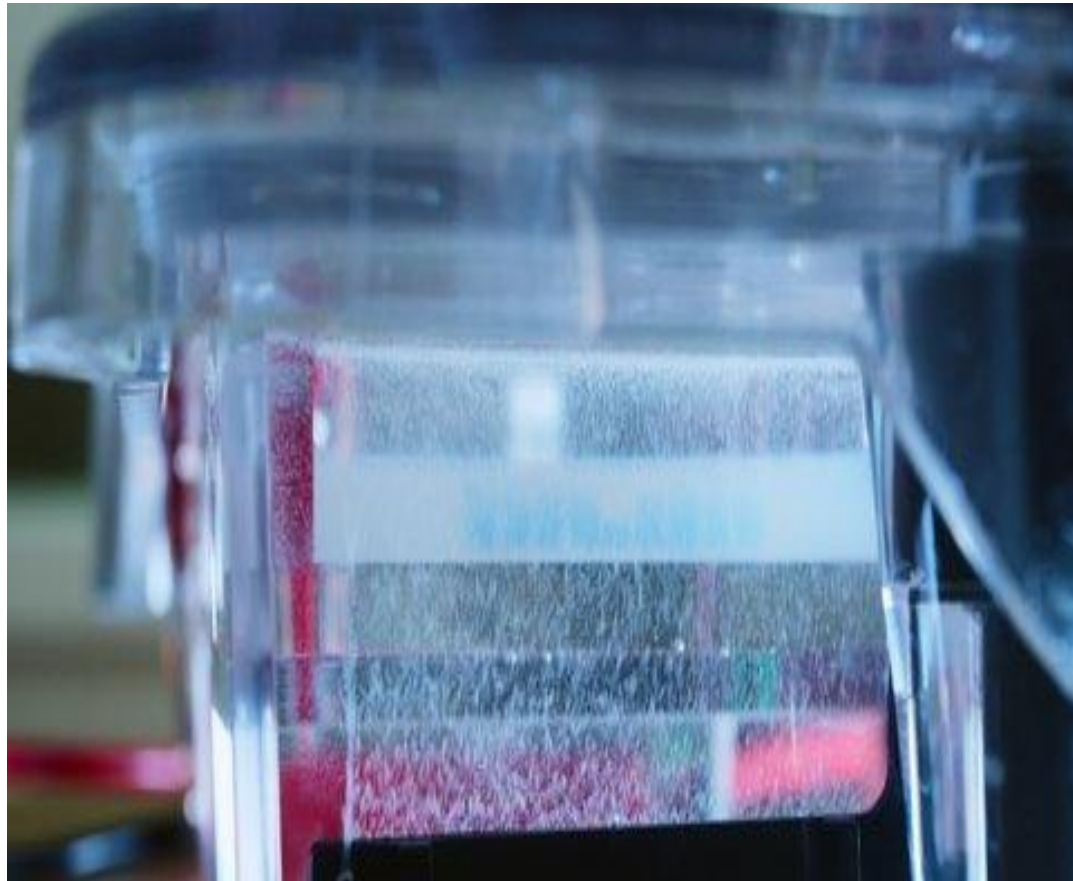


Running the gel (exposing it to an electric field)

- 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.

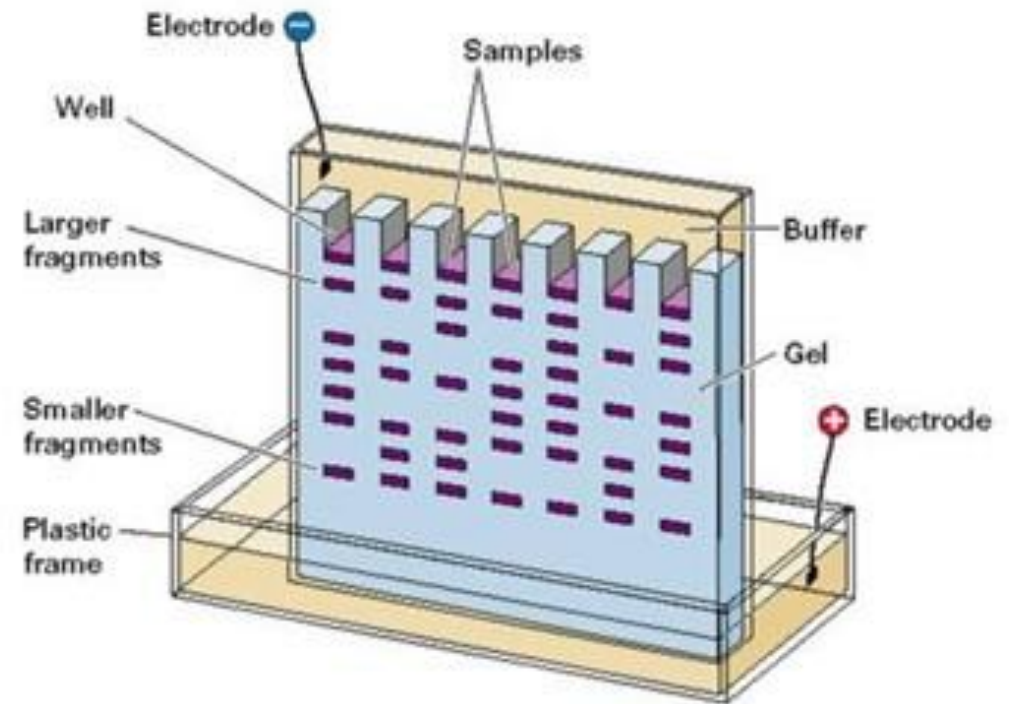


The presence of **bubbles** means that the electrodes are connected, plugged in, and that **current is flowing.**



Polyacrylamide gel electrophoresis (PAGE)

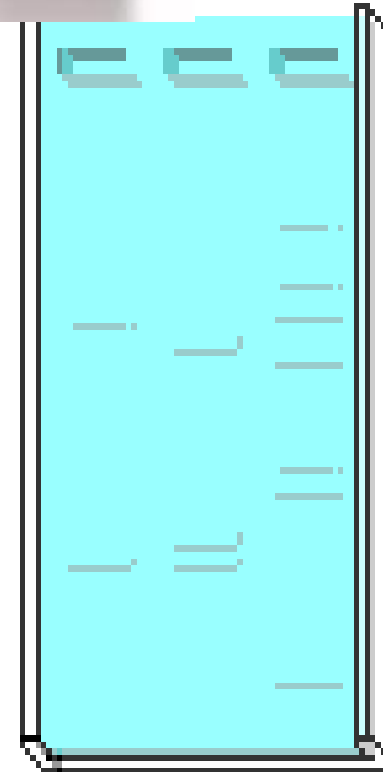
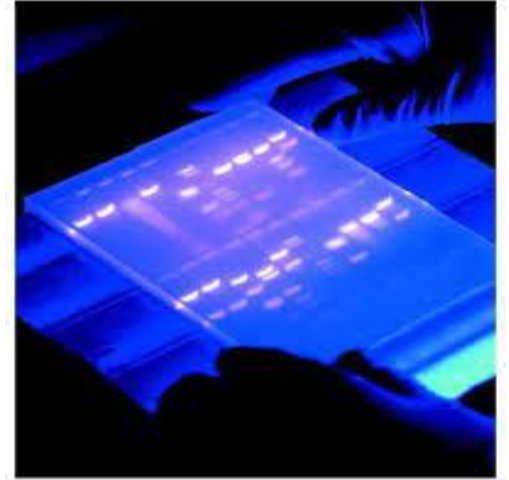
- Very efficient separation.
- Polyacrylamide gel separate **short DNA chains** that differ in length by only one nucleotide while agarose gel separate **chains of larger size**.



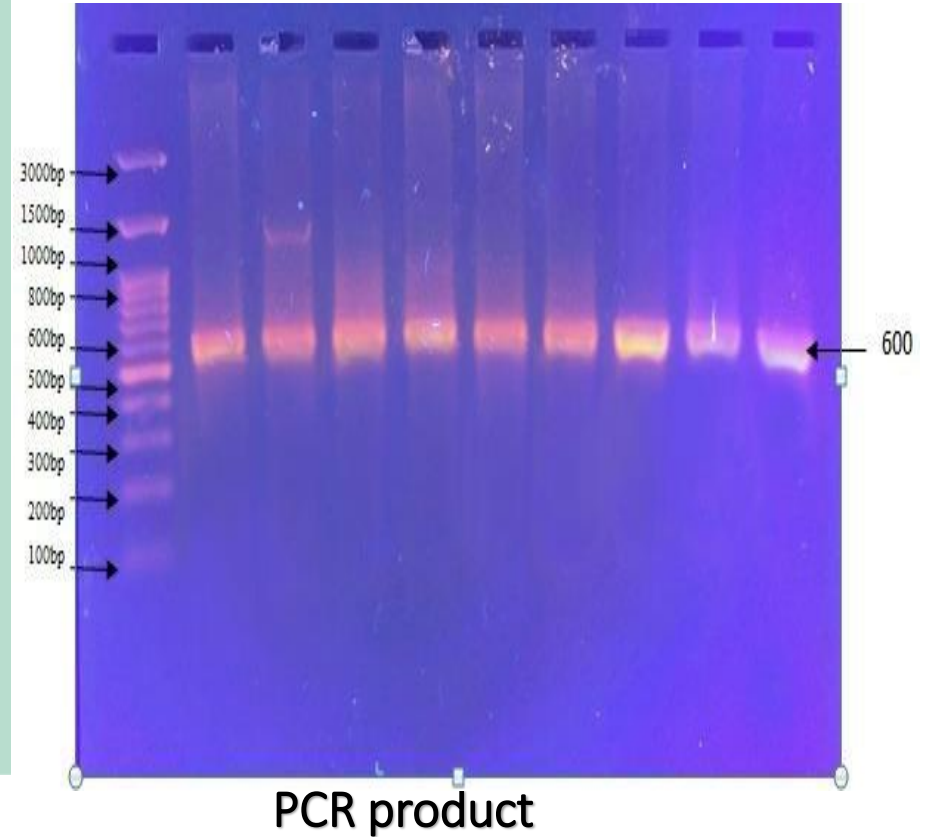
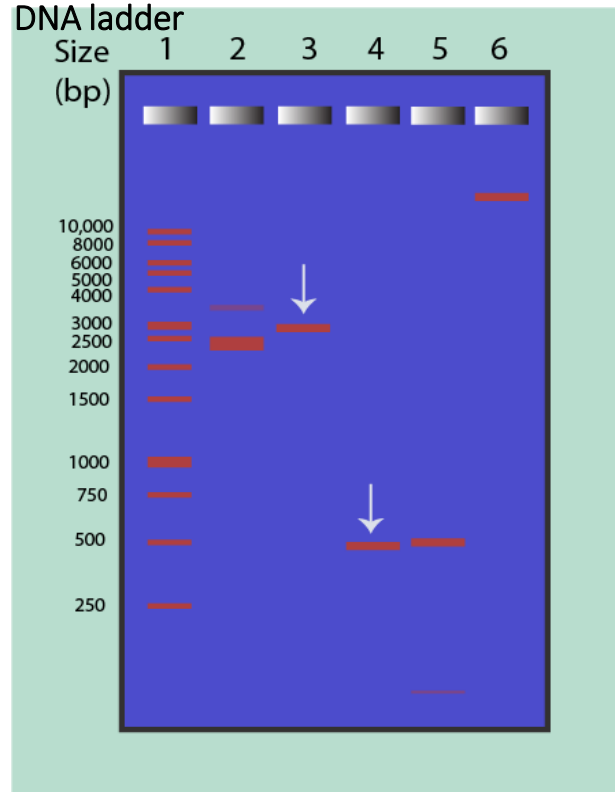
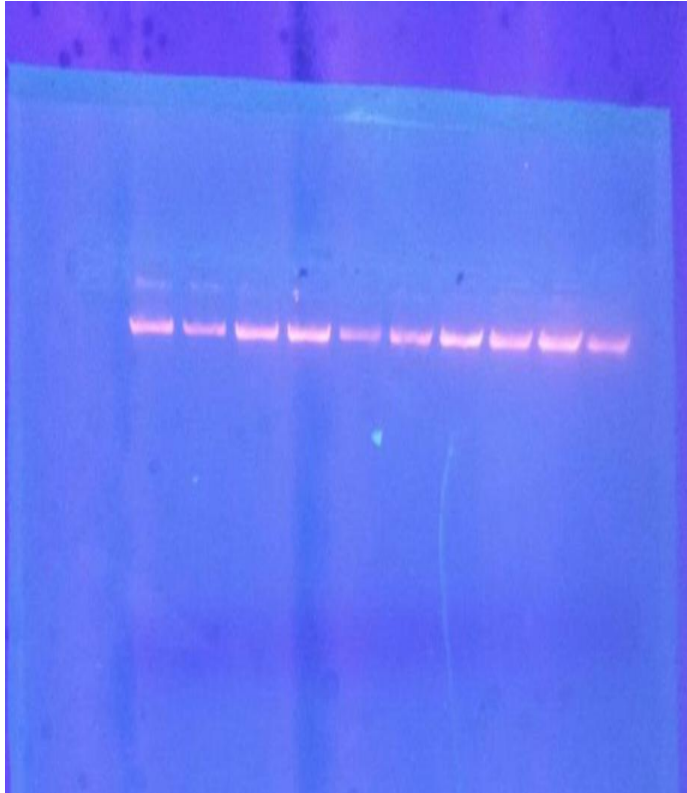
Visualization of DNA

□ 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

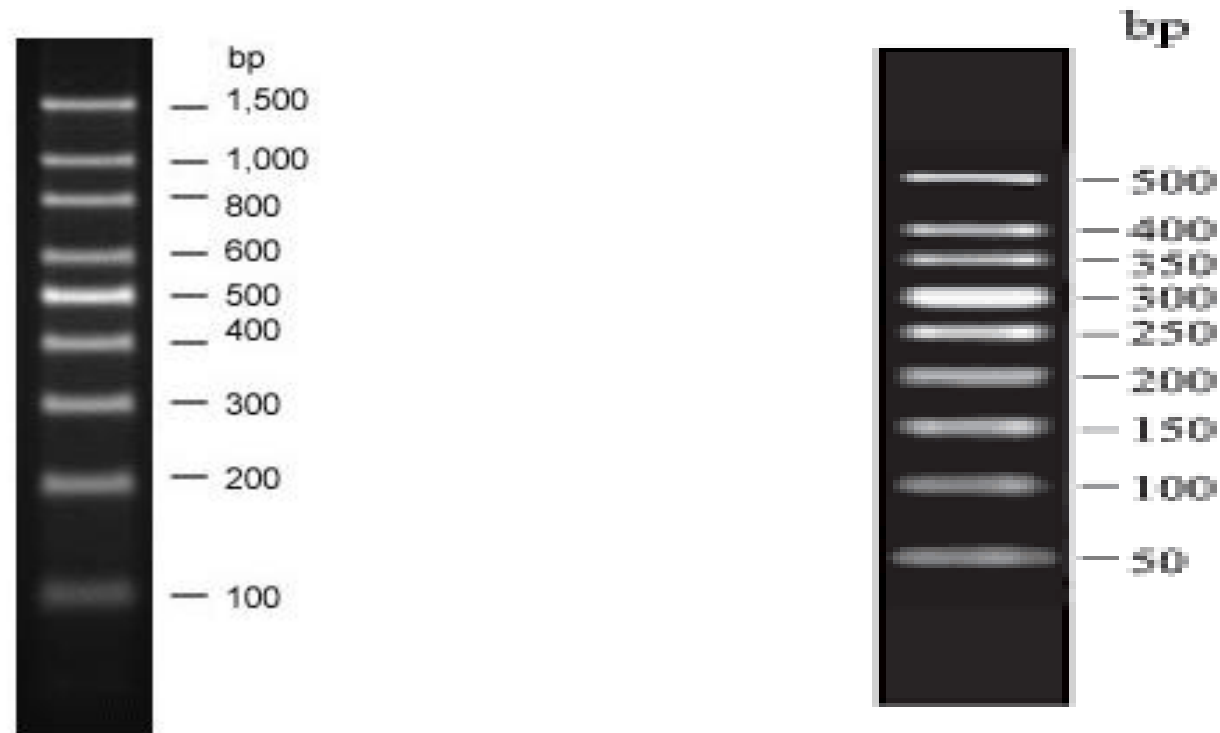


CAUTION! Ethidium bromide is a powerful *mutagen* and is moderately toxic. Gloves should be worn at all times

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

The image is a screenshot of a Microsoft PowerPoint presentation titled "electrophoresis - PowerPoint". The interface shows the "Animations" tab selected, with various animation options like Wave, Disappear, Fade, Fly Out, Float Out, Split, Wipe, and Shape. The main slide content features a detailed diagram of an agarose gel electrophoresis setup. The diagram includes a central circular well, concentric black circles representing the gel, and a blue DNA double helix at the bottom. On the right side, there are three curved black lines representing the gel lanes. A large, colorful, abstract graphic of a DNA double helix is overlaid on the diagram, with various colored spheres (purple, red, orange, yellow) representing nucleotides. The text "Agarose Electrop" is partially visible on the right side of the slide. The bottom status bar shows "Slide 1 of 23", "French (France)", and a search bar. The Windows taskbar at the very bottom shows the time as 16:48 on 08/07/2018.

<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

