Gel
electrophoresis
lab -4-

What is gel electrophoresis?

Electrophoresis is a technique used to separate and purify macromolecules that differ in size, charge or conformation.

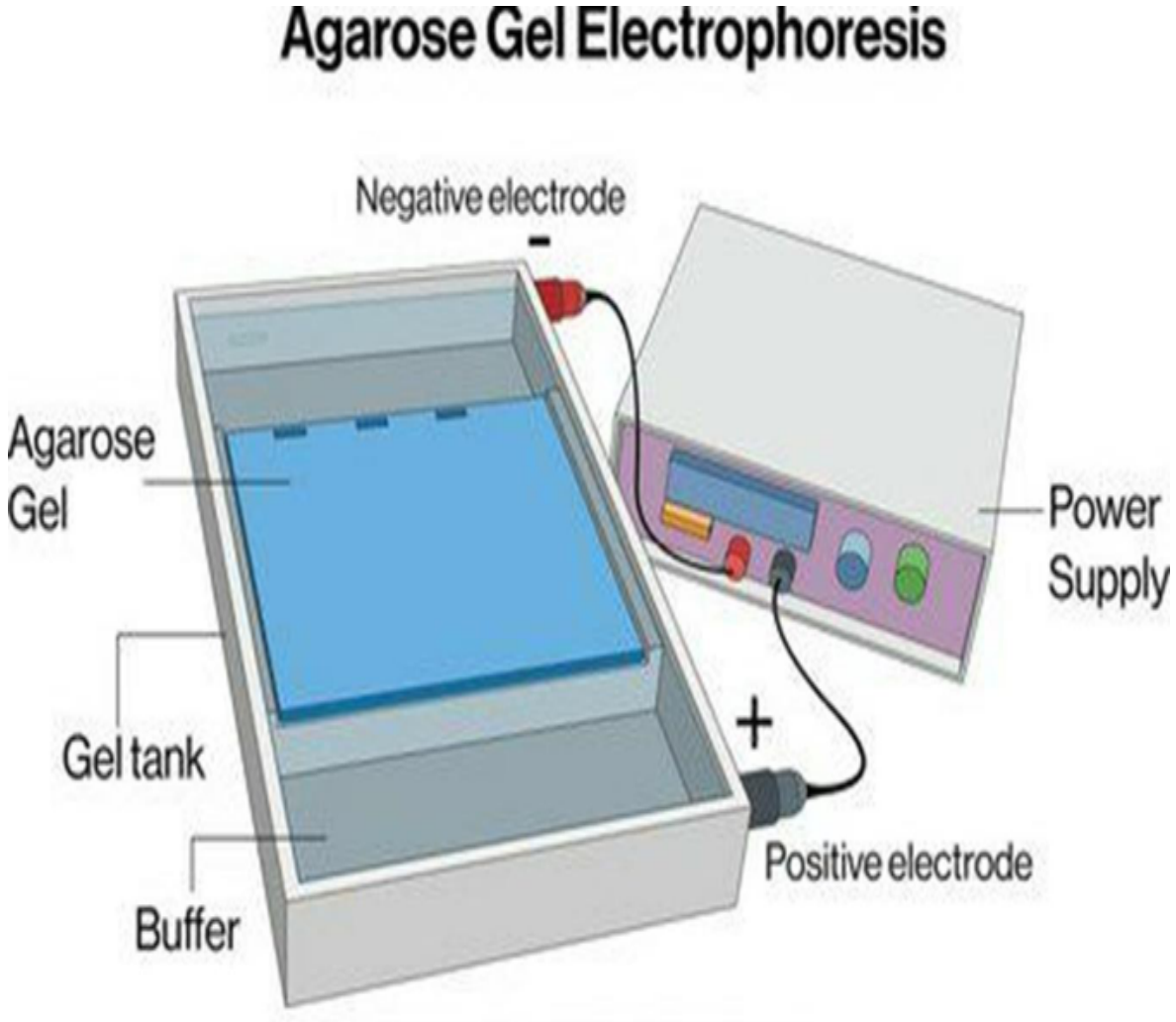
-the molecules will travel through the gel in different directions or at different speeds (Based on their size and charge)allowing them to be separated from one another.

The gels are porous and the size of the pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. Thus, separation not only depends on the charge but also on its size.-

Purpose of gel electrophoresis :

1. used to separate different size of:
-DNA
-RNA
-Proteins

2. To purifying DNA, RNA, Proteins.



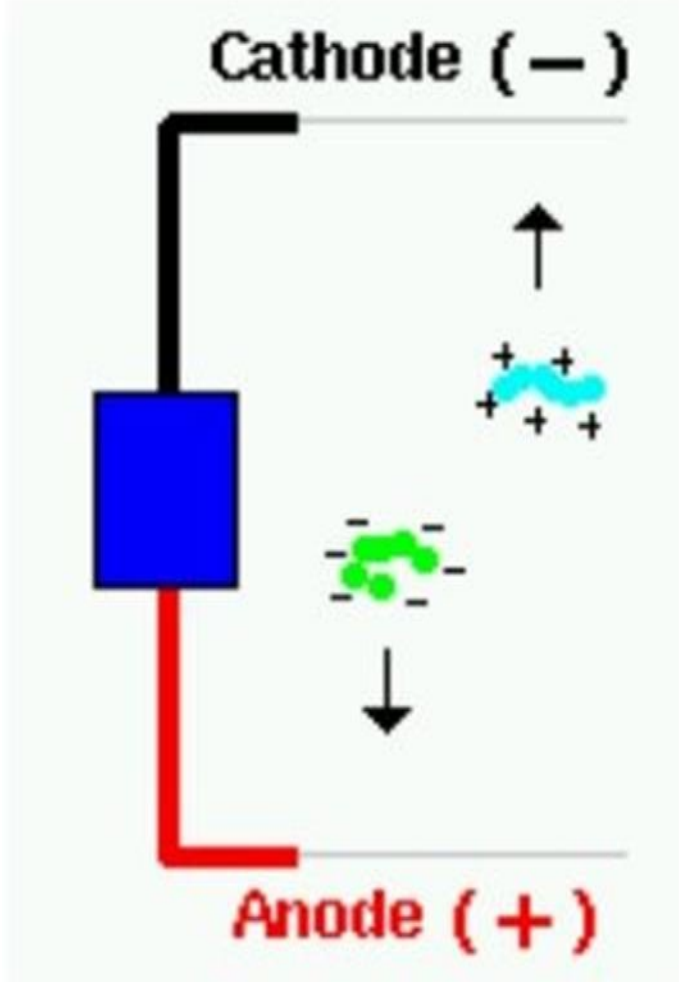
Specialized gel electrophoresis technique

1) Discontinuous (disc) gel electrophoresis
2) Gradient gel electrophoresis
3) High voltage electrophoresis
4) Isoelectric focusing
5) 2D gel electrophoresis
6) Pulse field gel electrophoresis
7) Immuno electrophoresis
8) Electrophoresis on cellular gels
9) Capillary electrophoresis

Principle of separation

- **According to charge:** When charged molecules are

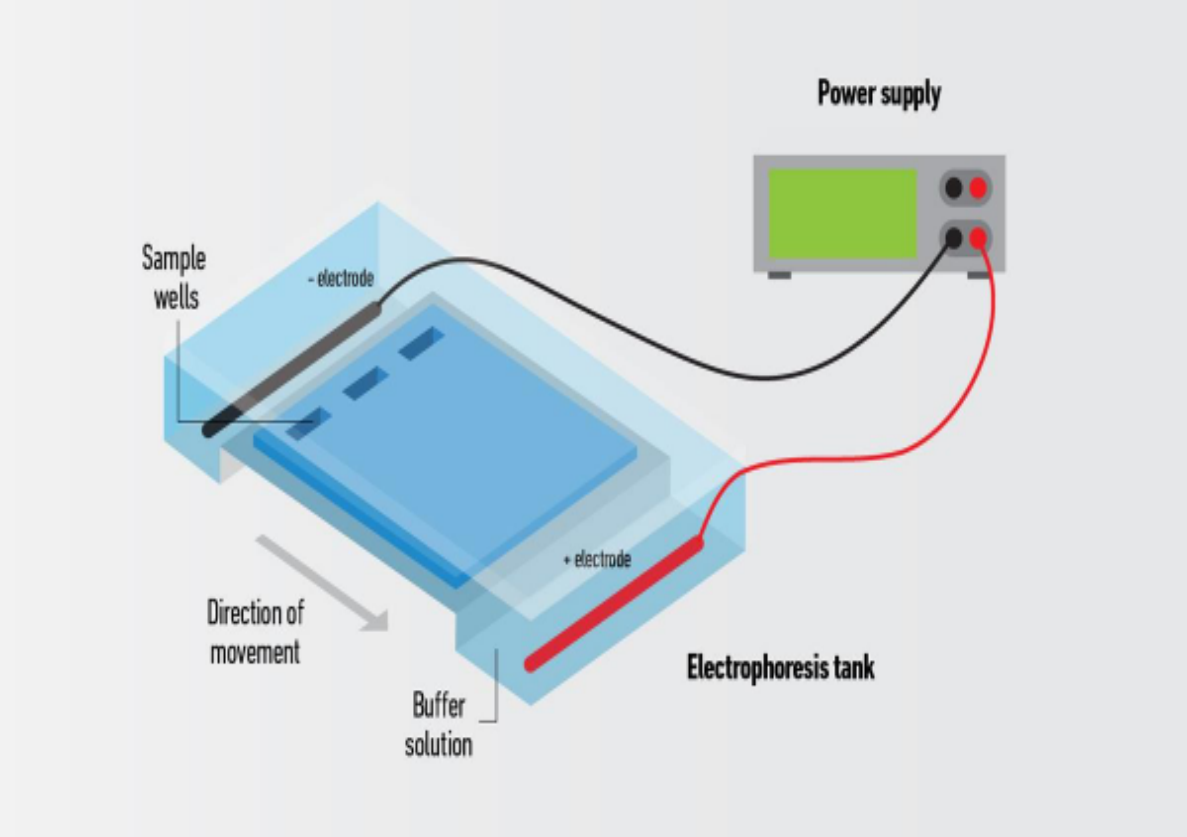
  placed in an electric field, they migrate toward the opposite side, either the positive (anode) or negative (cathode) pole according to their charge.



- **According to size:** The smaller molecules move faster than the larger sized ones, as they can travel through the pores more

Instrument and reagents

1. Electrophoresis apparatus
2. Buffer
3. Power supply
4. Supporting media
5. Detection and Quantification



Electrophoresis apparatus:

- The casting tray is made up of glass or plastic.
- The comb contains varying number of teeth in order to help in formation of well.

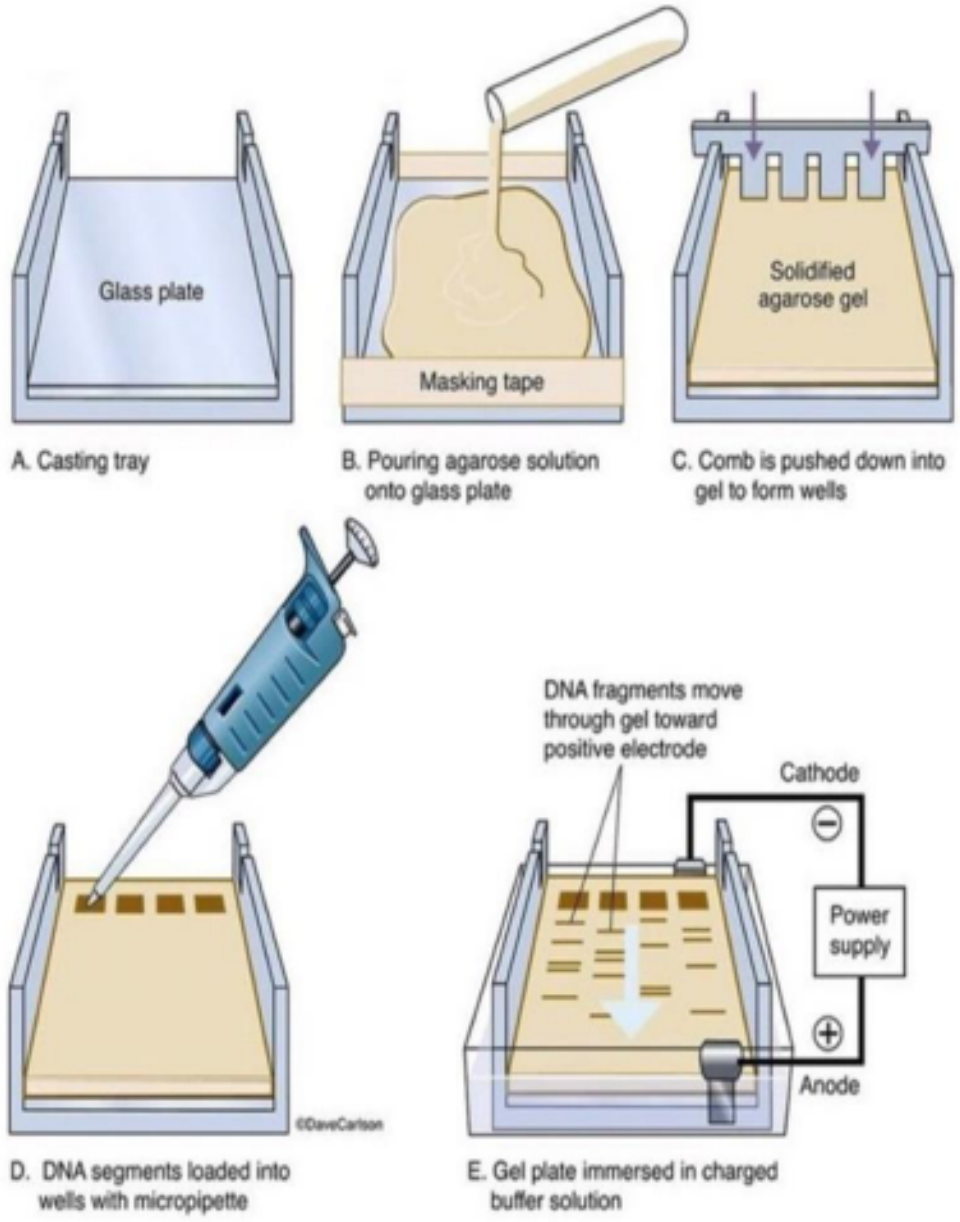
Buffer:

- Buffers in gel electrophoresis are used to provide ions that carry a current and to maintain the pH at a relatively constant value.
- **Tris-borate EDTA buffer (TBE):** It has a higher buffering capacity. It preferred for **longer electrophoresis time**. This buffer composed of (Tris base, boric acid and EDTA).
- **Tris-acetate EDTA buffer (TAE):** It has low buffering capacity and can easily become exhausted. It used for **short electrophoresis time**.

Supporting media: (Gel)

1. Starch
2. Agar/agarose
3. Cellulose acetate
4. Polyacrylamide gel

- The kind of supporting matrix used depends on type of molecules to be separated and the desired basis for separation: charge, molecular weight or both



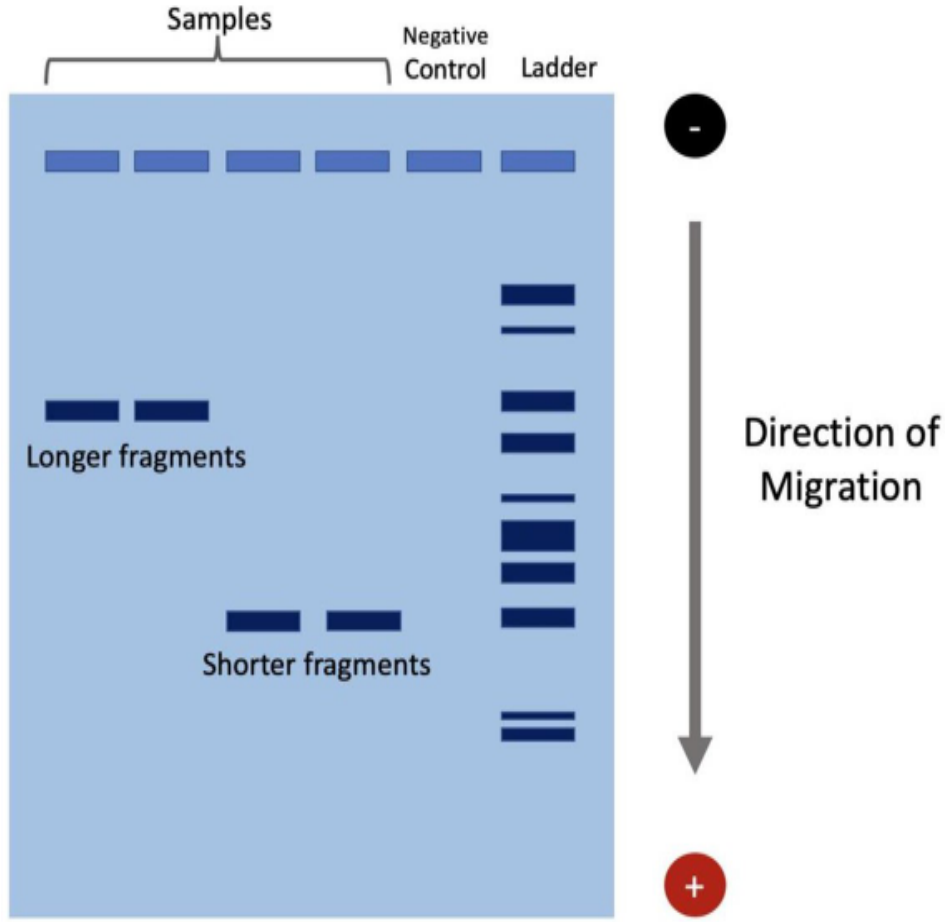
Detection and quantification:

- Stains
- Protein staining
- Ethidium bromide staining
- Blotting:

-Southern blotting (for DNA)

-Northern blotting (for RNA)

-Western blotting (for



visualizati on

- The molecules in the gel are stained to make them visible. DNA may be visualized using ethidium bromide which, when intercalated into DNA, fluoresce under ultraviolet light, while protein may be visualized using silver stain or Coomassie Brilliant Blue dye.

SYBR Green I is more expensive, but 25 times more sensitive and possible safer than ethidium bromide.

- SYBR Safe is a variant of SYBR Green, and show low level of mutagenicity and toxicity.

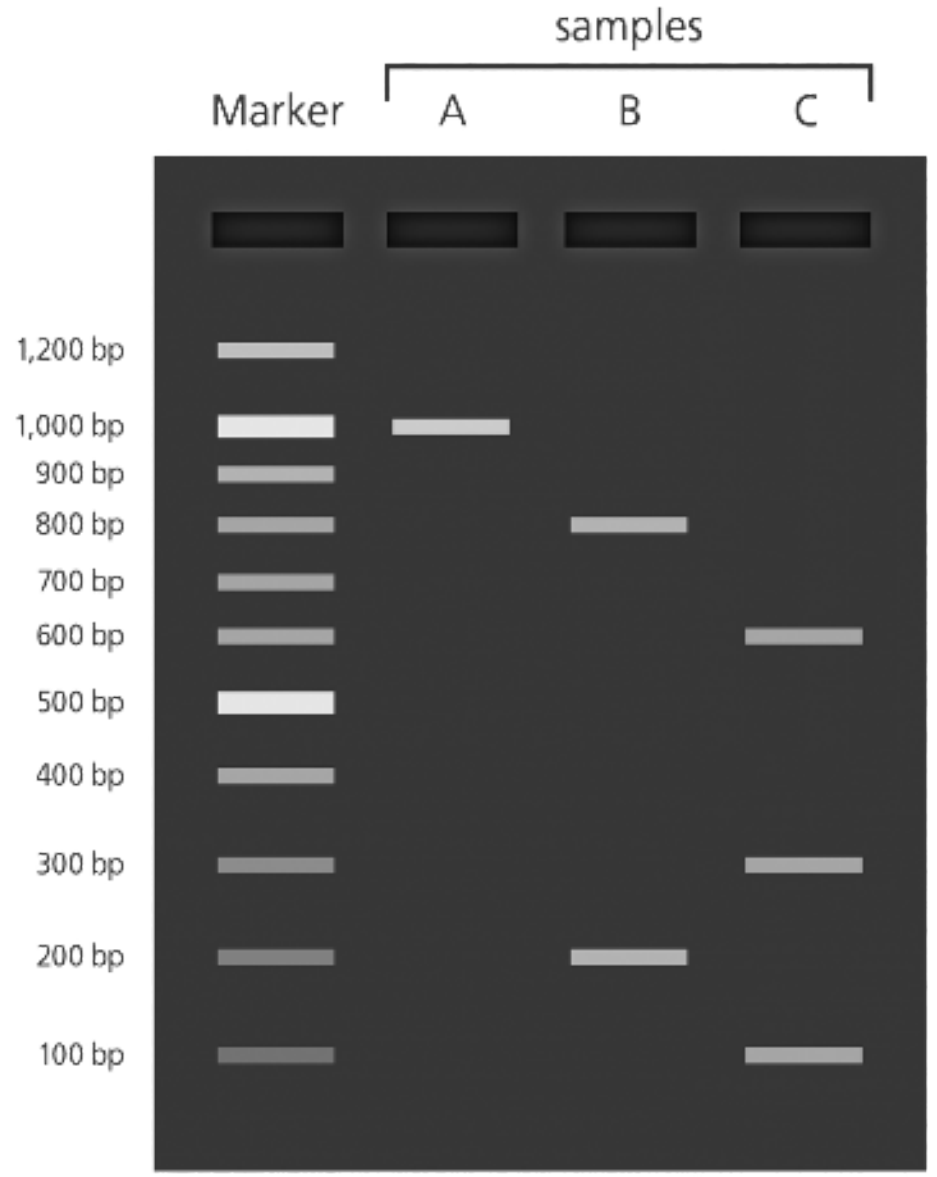
- Other less frequently used markers are Cresol red and Orange G.

Marker

- Marker is a standard reference that contains a mixture of DNA or proteins fragments of known sizes.

Function:

- Helps identify the size of unknown samples by comparing their migration distance wit that of marker bands.
- Provides a quality check to ensure the gel and electrophoresis process are functioning properly.



Advantages and disadvantage of Gel Electrophoresis

Disadvantage	Advantages
1-Time-consuming: the process can take up to an hour or more.	1-High resolution: can separate molecules based on size accurately.
2- Requires special equipment and reagents, which can be expensive.	2- Versatile: Can be used for DNA, RNA, and protein analysis.
3- DNA stains (e.g., Ethidium bromide) are hazardous.	3- Visualizes results clearly with staining methods.
4- Gel can degrade or distort results if not handle properly.	4- Easy to perform and set up in most labs.


Thank You!