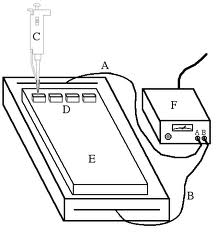
GEL ELECTROPHORESIS

**Gel electrophoresis** is a method used to separate nanoparticles by charge or size. **It is used to:**

1. Separate a mixed population of DNA and RNA fragments by length.
2. To estimate the size of DNA and RNA fragments or to separate proteins by charge.

# Gel electrophoresis apparatus

An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear. The negative terminal is at the far end, so DNA migrates from A to B wair. There are 2 types of gel was commonly used in electrophoresis apparatus: Agarose gel and Polyacrylamide Gel.



A. positive (+) electrode (anode)

B. Negative (-) electrode (anode)

C. DNA sample

D. pore

E. gel

F. electronic source.

# Agarose gel

* Agarose gels are easily cast and handled compared to other matrices.  Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator.
* Agarose gel electrophoresis can be used for the separation of DNA fragments ranging from 50 base pair to several megabases (millions of bases) using specialized apparatus.
* Most agarose gels are made with between 0.7% and 2% agarose dissolved in electrophoresis buffer.

# Polyacrylamide Gel

* Polyacralamide gel electrophoresis (PAGE) is used for separating proteins ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel.
* Pore size is controlled by controlling the concentrations of acrylamide and bis-acrylamide powder used in creating a gel.
* SDS-PAGE, Sodium Dodecyl Sulfate. Polyacrylamide Gel Electrophoresis: describes a technique used to separate proteins according to their electrophoretic mobility.

# Buffers

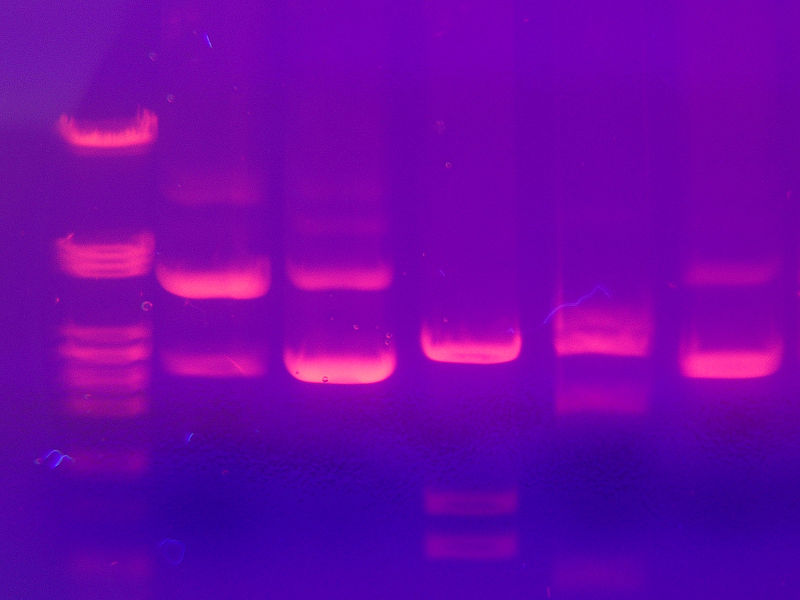
* A **buffer solution** is an aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. It has the property that the pH of the solution changes very little when a small amount of strong acid or base is added to it.
* Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications.
* There are a number of buffers used for electrophoresis. The most common being, for nucleic acids Tris/Acetate/EDTA (TAE), Tris/Borate/EDTA (TBE).

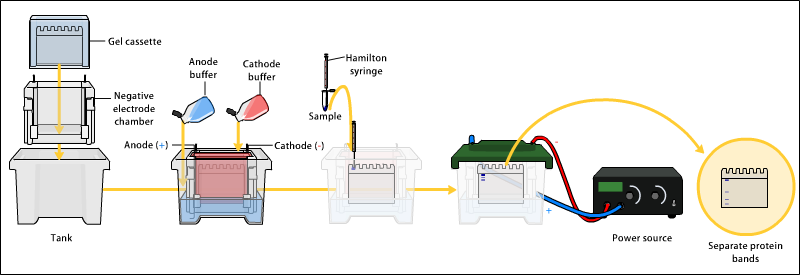
# Visualization and analysis

* After the electrophoresis is complete, the molecules in the gel can be stained to make them visible.
* DNA may be visualized using ethidium bromide which, when intercalated into DNA, fluoresce under ultraviolet light.
* Protein may be visualized using silver stain or Coomassie Brilliant Blue dye.
* Visualization can also be achieved by transferring DNA to a nitrocellulose membrane followed by exposure to a hybridization probe. This process is termed **Southern Blotting.**
* After electrophoresis the gel is illuminated with an ultraviolet lamp (usually by placing it on a light box, while using protective gear to limit exposure to ultraviolet radiation). The illuminator apparatus mostly also contains imaging apparatus that takes an image of the gel, after illumination with UV radiation.
* The ethidium bromide fluoresces reddish-orange in the presence of DNA, since it has intercalated with the DNA. The DNA band can also be cut out of the gel, and can then be dissolved to retrieve the purified DNA.

# Abstract of SDS- PAGE procedure:

1. **Sample preparation: Samples may be any material containing proteins**
2. **Mixing with SDS:** **The sample is mixed with SDS, anionic detergent.** To denatures secondary and non–disulfide–linked tertiary structures. To apply a negative charge to each protein.
3. **Heating: The samples are heated at 60°C.** To promote protein denaturation, helping SDS to bind.
4. **Addition of tracking dye:** A tracking dye may be added to the protein solution. As it has a higher electrophoretic mobility which allow the experimenter to track the progress of the protein solution through the gel.
5. **Preparing acrylamide gels:** Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells. After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis
6. **Electrophoresis:** Various buffer systems are used in SDS-PAGE depending on the nature of the sample and the experimental objective. An electric field is applied across the gel, causing the negatively-charged proteins to migrate across the gel towards the positive (+) electrode (anode)





Picture show techniques’ of separate the DNA in gel electrophoresis.