Agarose Gel Electrophoresis

Horizontal Separation of Charged Molecules

Agarose Gel Electrophoresis

- Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or RNA in a matrix of agarose
- Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool
- They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation

Agarose gel:

- Agarose is a linear polysaccharide (M.W. 12000 Da) made up of the basic repeat unit of agarobiose (which comprises alternating units of galactose and 3,6-anhydrogalactose)
- It is one of the components of agar, that is a mixture of polysaccharides from seaweeds
- It is used at a concentration between 1% and 3%
- Agarose gel is formed by suspending dry agarose in aqueous buffer and then boiling the mixture till it becomes clear solution, which is then poured and allowed to cool at room temperature to form rigid gel
- The gelling properties is attributed to inter and intramolecular H-bonding within and between long agarose chains

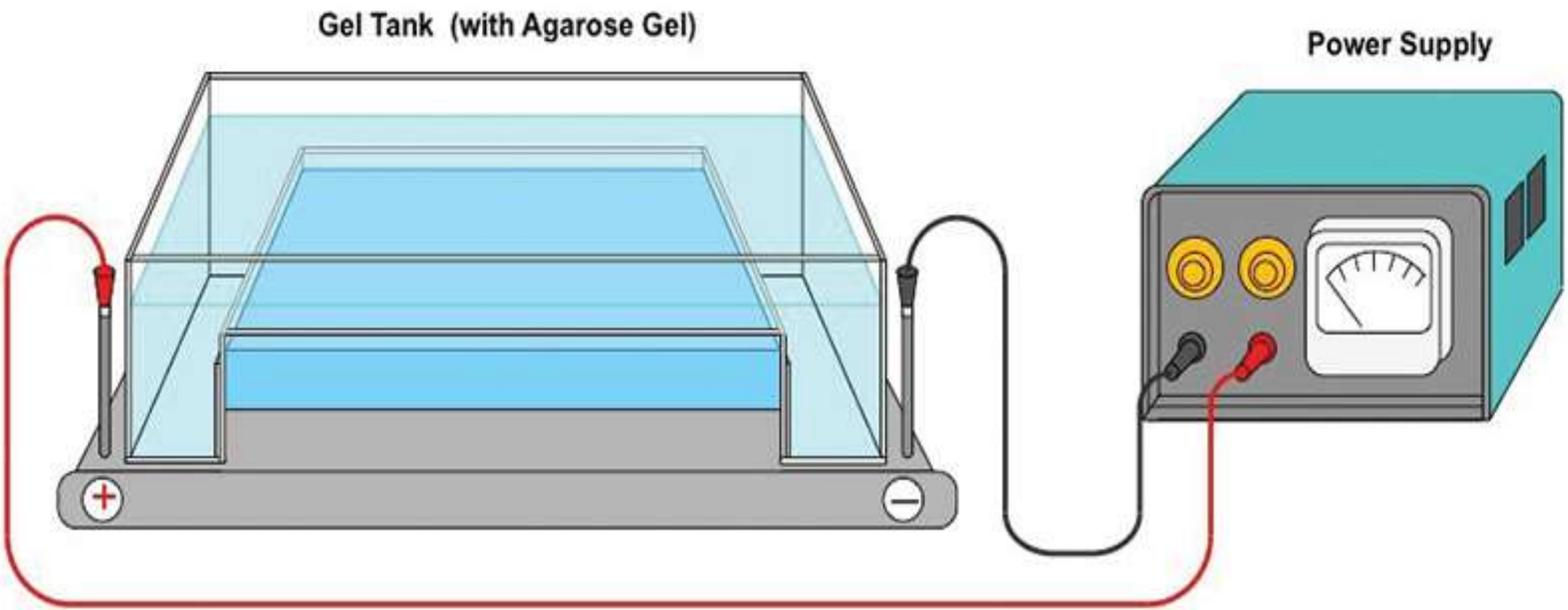
- **The pore size of the gel is controlled by the initial concentration of agarose, large pore size corresponds to low concentration and vice versa**
- **Such large pure gels are used to separate much larger molecules such as RNA and DNA, because the pore sizes are still large enough for RNA and DNA molecule to pass through the gel**
- **This gel can be reliquefied by heating to 65^{o}C and thus, for example DNA samples separated can be cut out of the gel, returned to solution and recovered**

Procedure

- Gel electrophoresis separates DNA fragments by size in a solid support medium such as an agarose gel
- Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current at the anodal, negative end which causes the negatively-charged DNA to migrate (electrophorese) towards the positive end
- The rate of migration is proportional to size: smaller fragments move more quickly, and wind up at the bottom of the gel
- DNA is visualized by including in the gel an intercalating dye, ethidium bromide

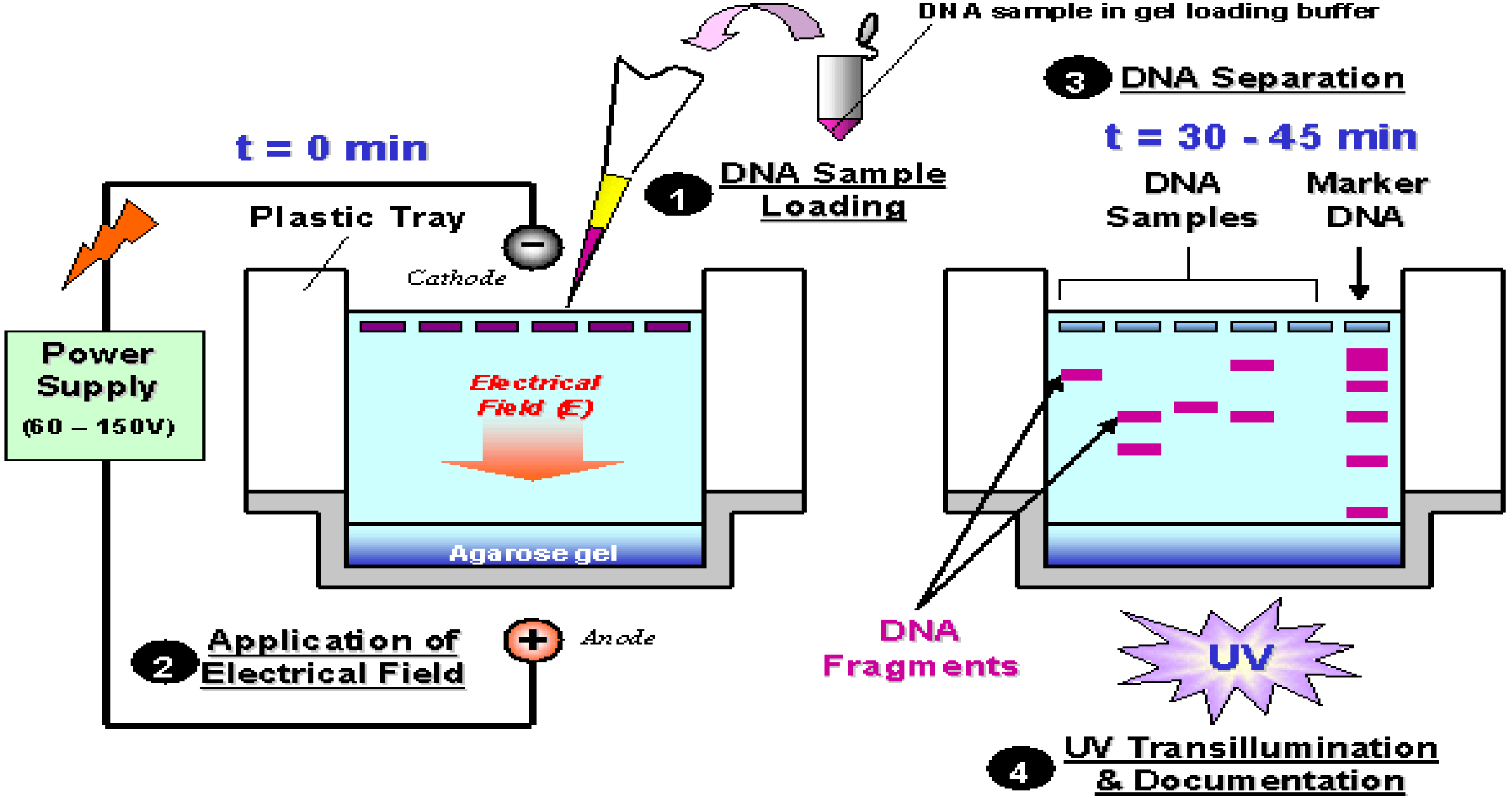
- **DNA fragments take up the dye as they migrate through the gel and Illumination with ultraviolet light causes the intercalated dye to fluoresce**
- **The larger fragments fluoresce more intensely and the smaller fragments include less mass of DNA, take up less dye, and therefore fluoresce less intensely**
- **A "ladder" set of DNA fragments of known size can be run simultaneously and used to estimate the sizes of the other unknown fragments**

Agarose Gel Electrophoresis

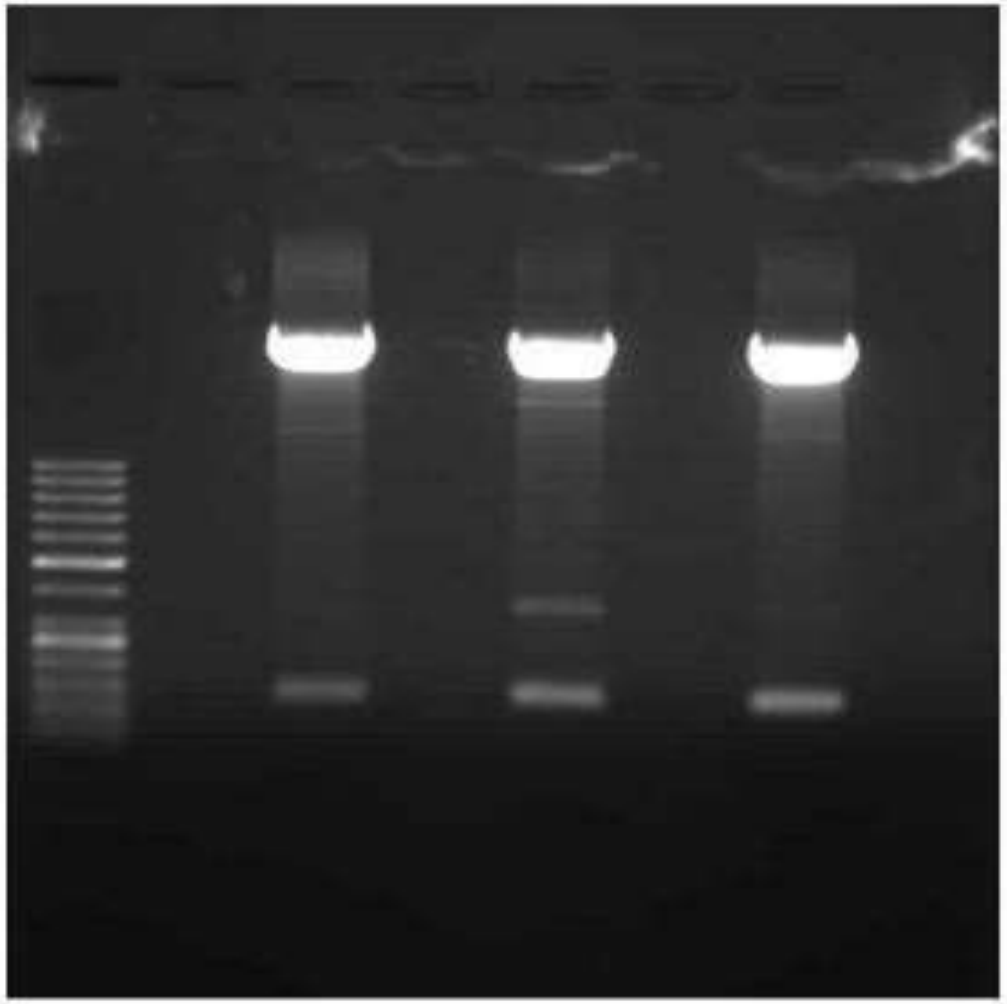


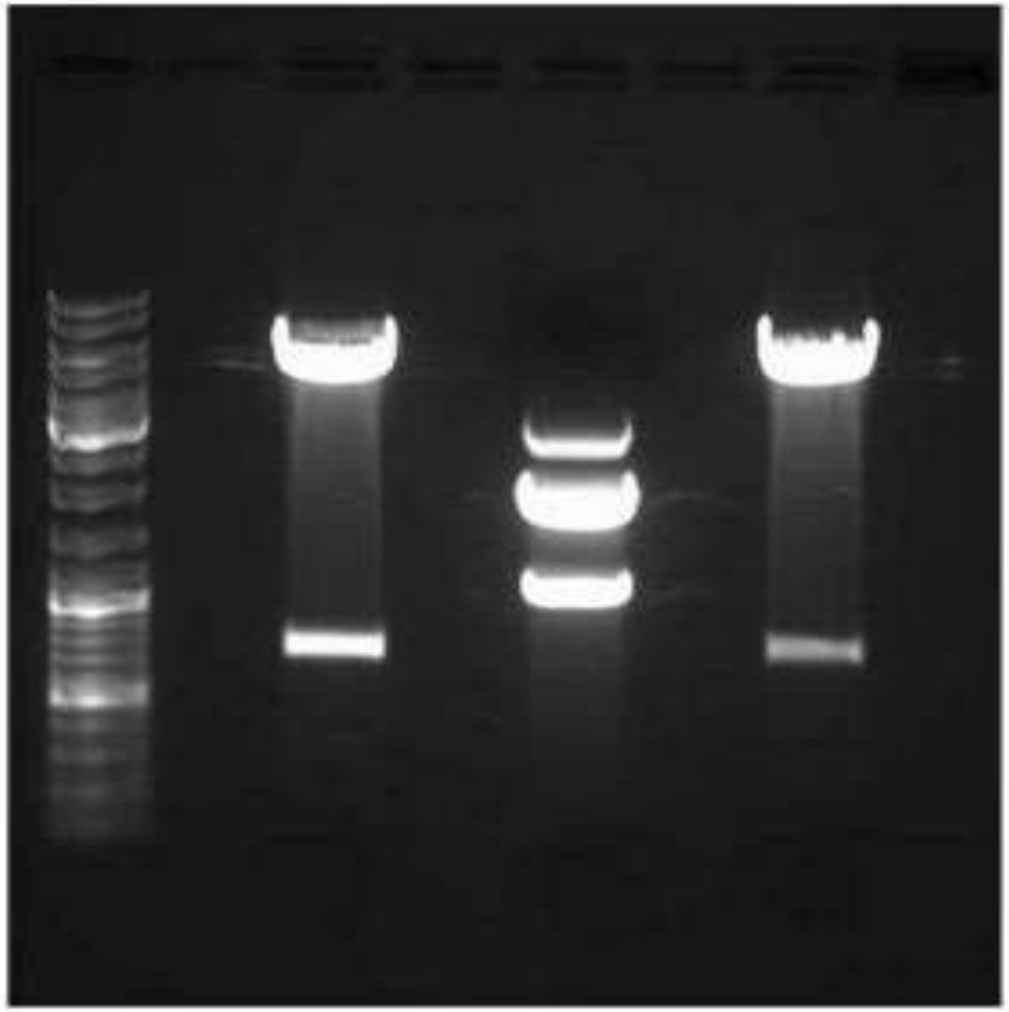
Requirements/ Instrumentation

1. An electrophoresis chamber and power supply
2. Gel casting trays available in a variety of sizes and composed of UV transparent plastic
3. Sample combs, around which molten medium is poured to form sample wells in the gel
4. Electrophoresis buffer, usually Tris-Acetate-EDTA (TAE) or Tris-Borate-EDTA (TBE)
5. Loading buffer with something dense (e.g. glycerol) to allow the sample to get settled into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring
6. Staining: DNA molecules are easily visualized under an ultraviolet lamp when electrophoresed in the presence of the extrinsic fluor ethidium bromide when intercalated into double stranded DNA, fluorescence of this molecule increases greatly
7. Transilluminator (an ultraviolet light box), which is used to visualize stained DNA in gels.


DNA sample in gel loading buffer
3 DNA Separation
t = 30 - 45 min
DNA Samples
Marker DNA
t = 0 min
1 DNA Sample Loading
Plastic Tray
Cathode
Power Supply
(60 – 150V)
Electrical Field (E)
Agarose gel
2 Application of Electrical Field
Anode
DNA Fragments
UV
4 UV Transillumination & Documentation

Bands on Agarose Gel under UV light





Applications

- Separation of DNA or RNA molecules
- Estimation of the size of DNA molecules
- Analysis of PCR products
- Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis
- The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes
- Agarose gel electrophoresis is commonly used to resolve circular DNA with different supercoiling topology
- Purification of DNA since purification of DNA fragments is necessary for a number molecular techniques such as cloning

Advantages of Agarose Gel Electrophoresis

- For most applications, only a single-component agarose is needed and no polymerization catalysts are required, therefore, it is a simple and rapid technique
- The gel is easily poured, does not denature the samples
- The samples can also be recovered

Disadvantages of Agarose Gel Electrophoresis

- Gels can melt during electrophoresis
- The buffer can become exhausted
- Different forms of genetic material may run in unpredictable forms