Agarose Electrophoresis

When charged molecules are placed in an electric field, they migrate through matrix or gel, towards either negative or positive pole depending on the charge of the molecule. Shorter molecules move faster than the longer ones due to their size, as the small ones can migrate more easily through the pores of gel. This phenomenon is known as sieving. The rate of migration depends on the charge, conformation and strength of the applied current.

Agarose gel electrophoresis is used to resolve DNA fragments on the basis of their molecular weight. Smaller fragments migrate faster than larger ones; the distance migrated on the gel varies inversely with the logarithm of the molecular weight. The size of fragments can therefore be determined by calibrating the gel, using known size standards known as DNA Markers, and comparing the distance the unknown fragment has migrated. The velocity of the DNA fragments decreases as their length increases and is proportional to electric field strength. Nucleic acids are generally separated by length, although the presence or absence of secondary structures such as those observed in plasmids with varying degrees of DNA supercoiling also affects the rate of migration during agarose gel electrophoresis. Supercoiled DNA plasmid migrates faster than the nicked and linear forms.

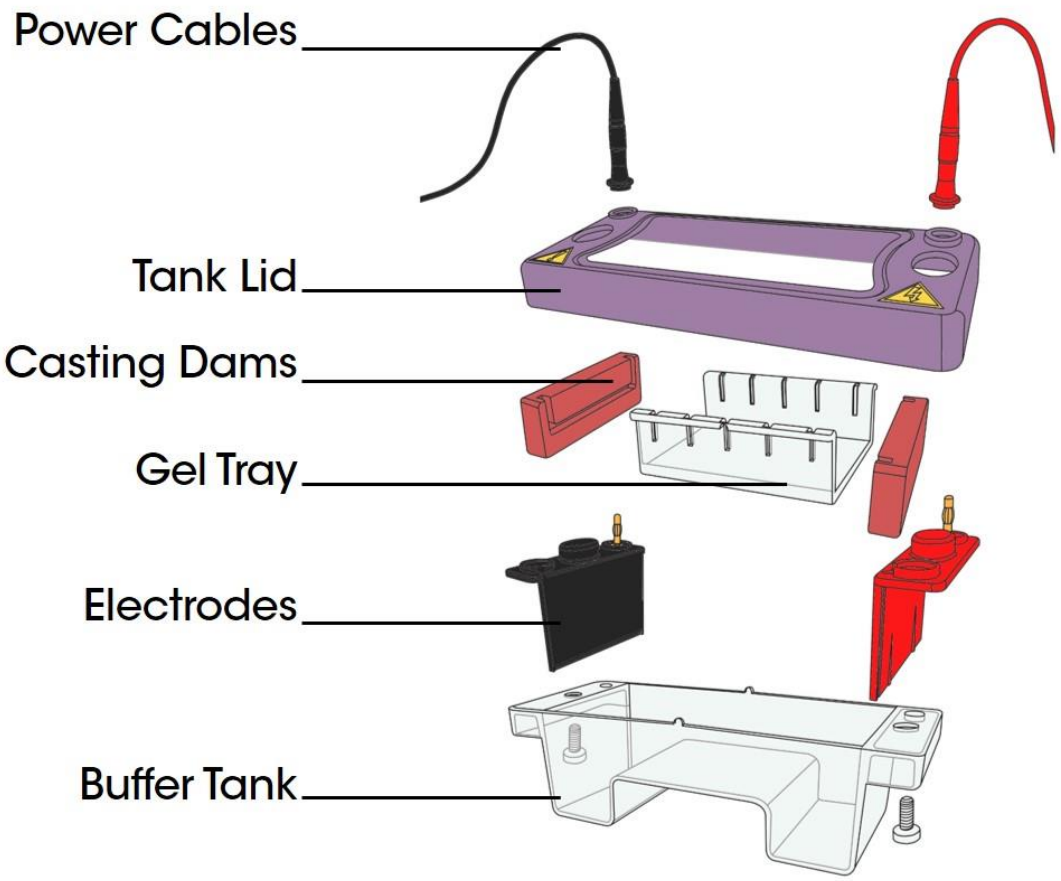
Chemicals required

- Agarose
- TBE/TAE buffer
- Bromophenol blue (loading dye)
- Ethidium bromide (EtBr)
- Deionized water

Apparatus

Basic apparatus consists of: Buffer tank, casting tray (to prepare agarose gel), coombs (to prepare wells), connecting wires (to connect the electrodes with the power supply) and powerpack.

Casting of an agarose gel: Agarose gel percentage can vary depending upon the size of the DNA to be separated. Agarose gels cast with low concentrations of agarose (0.1-0.2% w/v) are capable of resolving extremely large DNA molecules (5-10kb) and a 2% gel will show good resolution for small fragments with size range of 0.2-1kb.



Sample preparation

Sample is prepared in loading dye. The components and role of DNA loading dye is as follows:

Bromophenol blue and Xylene cyanol- to track the migration of the sample (i.e., DNA). Also known as tracking dye.

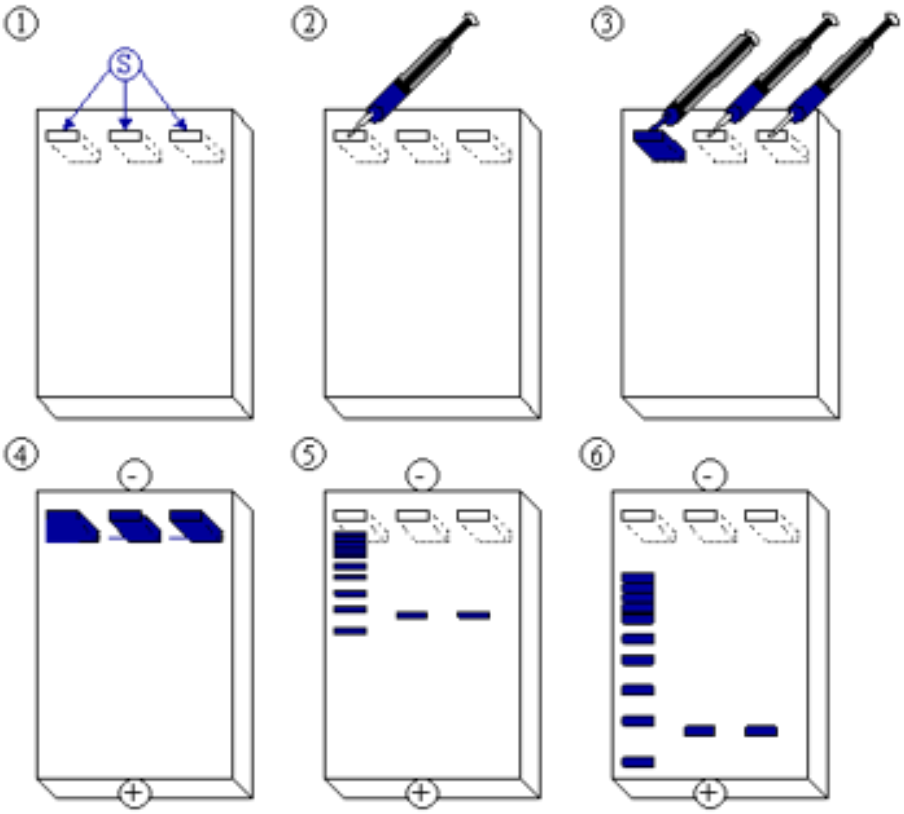
Glycerol- to impart density to the sample, so that it can settle in the well.

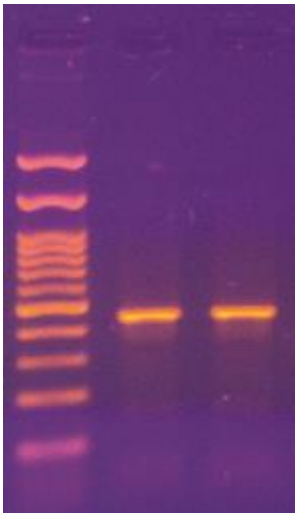
Tris EDTA- Tris is the buffer, which maintains the pH and EDTA is the inhibitor of the nuclease.

Running

1X TAE/TBE (Tris Acetate EDTA/ Tris Borate EDTA) Buffer is used as running buffer. It is filled in the tank. Sample prepared is loaded on cathode (negatively charged electrode) as DNA is negatively charged. It migrates towards anode (positively charged electrode) since opposite charges attract each other. After loading the sample in the wells, gel is run at 5V/cm.

The below mentioned figure depicts loading and movement of the sample in agarose gel.





Visualization- Once the tracking dye reaches the bottom of the gel, power supply is curtailed and gel is taken out to visualize it under UV transilluminator. DNA band fluoresces under UV light as EtBr intercalates between the nucleotides (as shown in the picture). Ethidium bromide (EtBr- 10mg/ml) can be added to the gel or running buffer.

Applications- Molecular Cloning, Genetic Fingerprinting, and Diagnostics.

You tube link: https://www.youtube.com/watch?reload=9&v=vq759wKCCUQ

References:

- Principles and Techniques of Biochemistry and Molecular Biology by Wilson and Walker
- https://www.cleaverscientific.com/applications/agarose-gel-electrophoresis-of-dna/
- https://vlab.amrita.edu/?sub=3&brch=77&sim=1375&cnt=1
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846332/pdf/jove-62-3923.pdf