Agarose gel electrophoresis

The term electrophoresis describes the migration of a charged particle under the influence of an electric field. Many important biological molecules such as amino acids, peptides, proteins, nucleotides and nucleic acids, possess ionisable groups and therefore, at any given pH, exist in solution as electrically charged species either as cation or anions. Under the influence of an electric field these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge. The equipment required for electrophoresis consists basically of two items, as power pack and an electrophoresis unit.

For the majority of DNA samples, electrophoretic separation is carried out in Agarose gels. This is because the DNA molecules and their fragments that are analyzed routinely are considerably larger than proteins and therefore, because most DNA fragments would be unable to enter a polyacrylamide gel, the larger pore size of an Agarose gel is required. For example, the commonly used plasmid pBR322 has a molecular weight of 2.4×10^6. However, rather than use such large numbers it more convenient to refer to DNA size in terms of the number of base pairs. So, the plasmid pBR322 has the size of 4.36 kb (4360 base pairs).

As all the DNA molecules are negatively charged at neutral and alkaline pH, under the electric field, the DNA molecules would be moving towards the anode. The separation in Agarose gels is achieved due to resistance to their movement caused by the gel matrix. The largest molecules will have the most difficulty passing through the gel pores (very large molecules may even be blocked completely), whereas the smallest molecules will be relatively unhindered. Consequently the mobility of the DNA molecules during gel electrophoresis will depend on the size, the smallest molecules moving fastest. This is analogous to the separation of proteins in SDS.

Once the gel has been run, the DNA in the gels needs to be stained and visualized. The reagent most widely used is the fluorescent dye ethidium bromide. The gel is rinsed gently in solution of ethidium bromide (0.5µg per ml) and then viewed under ultraviolet light (300 nm wavelength)*. The ethidium bromide is a cyclic planer molecule that bind between stacked base-pairs of DNA (i.e. it intercalates). The ethidium bromide concentration therefore buildup at the site of DNA bands and under ultraviolet light the DNA bands fluoresce orange-red. Al little as 10 ng of DNA can be visualized as a 1 cm wide band.

** Generally the ethidium bromide is added into the gel during its preparation. The concentration of ethidium bromide is used @ 0.5µg per ml of gel.*

Stock solution:

Agarose Gel Electrophoresis buffer: For Agarose gel electrophoresis, either 1×TAE or 0.5×TBE can be used. For better resolution of DNA bands TAE is preferred to TBE. However, for routine gel runs TBE is preferred due to its long keeping quality. The stock solutions of TAE and TBE are 50×TAE and 10×TBE.

A. Tris Acetate EDTA (TAE) 50X buffer

Tris base	: 24.2 g
Glacial acetic acid	: 5.71 ml
0.5M EDTA (pH 8.0)	: 10 ml

Make the volume up to 100 ml with double distilled water. Autoclave and store at RT.

Tris Borate EDTA (TBE) 10X buffer,

Tris base	: 10.8 g
Boric acid	: 5.5 g
0.5M EDTA	: 4 ml (pH 8.0)

Make the volume up to 100 ml with double distilled water. Autoclave and store at RT

B. 6X Gel loading buffer:

Bromophenol blue	: 0.25%
xylene cyanol	: 0.25%
Sucrose	: 40%

Store at 4°C.

C. Ethidium bromide solution (10 mg/ml) (Stock solution)

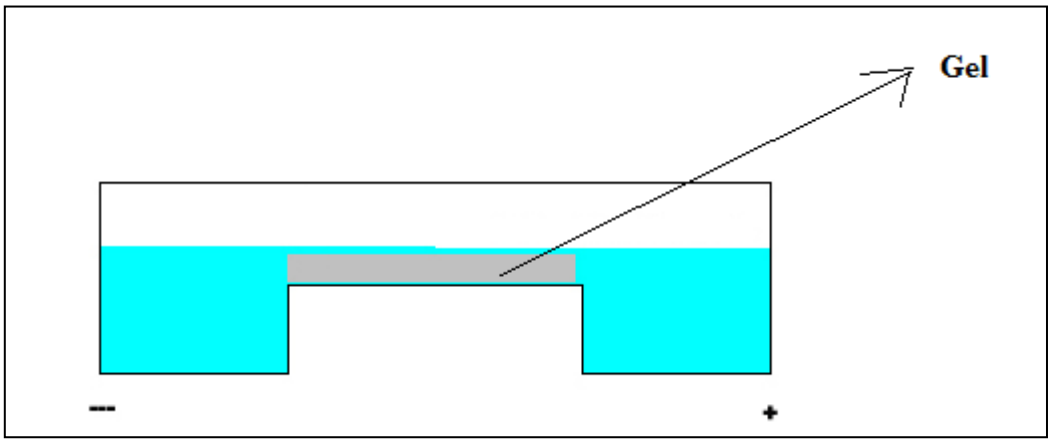
Ethidium Bromide	: 10 mg
Triple glass distilled water	: upto 1 ml

Working solution of EtBr is 1 mg per ml that is obtained by diluting the stock solution ten times.

The Ethidium Bromide is carcinogenic in nature. So, it is to be handled using gloves.

PROCEDURE FOR THE AGAROSE GEL ELECTROPHORESIS:

1. Preparation of Agarose gel (0.8%) 100 ml:
    a. Weigh 0.8 g of Agarose gel base and pour it into the 250 ml flat bottom flask.
    b. Add 1XTAE upto 100 ml.
    c. Heat the suspension with continuous stirring till a clear solution is obtained.
    d. Add working solution of EtBr (0.5µg per ml gel) when the gel solution has the temperature of 60°C and mix the gel solution by stirring.
    e. Caste the gel on the horizontal gel electrophoresis casting unit with the comb placed in it.
    f. Mix the DNA solution in 6X loading dye in the ratio of 2µl of dye with 10 µl of DNA solution.
    g. Load the dye-mixed DNA solution into the well of gel.
    h. Run the electrophoresis @ 5 volts per cm.
2. Viewing the DNA bands.
    a. The DNA bands can be visualized by placing the gel in U.V transilluminator.



Horizontal electrophoresis assembly