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, a power pack and an electrophoresis unit.

In an agarose gel (or in polyacrylamide gel), the negatively charged DNA fragments will move toward the positive electrode at a rate inversely proportional to their length. After the electric field is applied for a certain period, DNA fragments with different lengths will be separated, which can be visualized by autoradiography or by treatment with a fluorescent dye (e.g., ethidium bromide).

Agarose is a linear polymer composed of alternating residues of D- and L- galactose joined by α-(1-3) and β-(1-4) glycosidic linkages. Commercially prepared agarose polymers are believed to contain ~800 galactose residues per chain. However, agarose is not homogeneous. The average length of the polysaccharide chains varies from batch to batch and from manufacturer to manufacturer. The rate of migration of DNA through agarose gels is regulated by following factors.

1. The molecular size of the DNA: Molecules of double-stranded DNA migrate through gel matrices at rates that are inversely proportional to the \( \log_{10} \) of the number of base pairs. Larger molecules migrate more slowly because of greater fractional drag and because they worm their way through the pores of the gel less efficiently than smaller molecules.
2. The concentration of agarose: A linear DNA fragment of a given size migrates at different rates through gels containing different concentrations of agarose. There is a linear relationship between the logarithm of the electrophoretic mobility of the DNA ($\mu$) and the gel concentration ($I$) that is described by the equation:

$$\log \mu = \log \mu_0 - K r I$$

Where $\mu_0$ is the free electrophoretic mobility of DNA and $Kr$ is the retardation coefficient, a constant related to the properties of the gel and the size and shape of the migrating molecules.

3. The presence of ethidium bromide in the gel and electrophoresis buffer: Intercalation of ethidium bromide causes a decrease in the negative charge of the double-stranded DNA and an increase in both its stiffness and length. The rate of migration of the linear DNA-dye complex through gels is consequently retarded by a factor of $\sim 15\%$.

4. The applied voltage: At low voltages, the rate of migration of linear DNA fragments is proportional to the voltage applied. However, as the strength of the electric field is raised, the mobility of high-molecular-weight fragments increases differentially. Thus, the effective range of separation in agarose gels decreases as the voltage is increased. To obtain maximum resolution of DNA fragments $>2$ kb in size, agarose gels should be run at no more than $5 - 8$ V/cm.

5. The electrophoresis buffer: The electrophoretic mobility of DNA is affected by the composition and ionic strength of the electrophoresis buffer. In the absence of ions (e.g., if water is substituted for electrophoresis buffer in the gel or in the reservoirs), electrical conductivity is minimal and DNA migrates slowly, if at all. In buffer of high ionic strength (e.g., if 10x electrophoresis buffer is mistakenly used), electrical conductance is very efficient and
significant amounts of heat are generated, even when moderate voltages are applied. In the worst case, the gel melts and the DNA denatures. Generally 1x TAE or 0.5x TBE buffer used for agarose gel electrophoresis.

Following protocol is for preparing either 1% or 2% Agarose Gel used for checking the presence and quality of DNA.

1. Mix the appropriate weight of agarose with buffer (1 x TAE or 0.5 x TBE).
2. Place the container in the microwave at full power for 2 min.,
3. The agarose should get dissolved, but not it boil
4. Place at 55°C water bath to allow it to cool without setting
5. Add 100μl ethidium bromide solution (10mg/ml) per liter of gel.
6. Decant it in the gel-casting try having eadges sealed and comb insertion in to that try.
7. After solidify, remove the comb and eadge seal and put the gel in electrophoresis tank having tank buffer (1 x TAE or 0.5 x TBE). Ensure that the gel is submerged.
8. Add the 2μl samples (mixing with 1 μl of 1x xylene-cyanol lodging dye) to the sample wells and run at 80V for about 30 minutes or until front dye is near the bottom of the gel (check that properly anode and cathode in appropriate place).
9. Check electrodes are bubbling so you know the circuit is complete (or check that you have amps as well as volts by pressing the button on the power pack).
10. After the electrophoresis, photograph gel under UV (300nm) transillumination.