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# DEPARTMENT OF BIOTECHNOLOGY
# FACULTY OF ENGINEERING & TECHNOLOGY

# LT 17. Agarose gel electrophoresis

**Content Outline**

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# Agarose Gel Electrophoresis

•Agarose gel is used as support medium to separate DNA instead of polyacrylamide gel because of smaller pore size of polyacrylamide gel. Since the charge per unit length (owing to the phosphate groups) in any given fragment of DNA is the same, all DNA samples should move towards the anode with the same mobility under an applied electrical field. However, separation in agarose gels is achieved because of 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 DNA molecules during gel electrophoresis will depend on size, the smallest molecules moving fastest.

•**Buffer and reagent for electrophoresis-** The purpose of each reagent used in horizontal gel electrophoresis are as follows-

1. **Agarose-**polymeric sugar used to prepare horizontal gel for DNA analysis.
2. **Ethidium bromide-** for staining of the agarose gel to visualize the DNA.
3. **Sucrose-**For preparation of loading dye for horizontal gel.
4. **Tris-HCl-** The component of the running buffer.
5. **Bromophenol blue-**Tracking dye to monitor the progress of the electrophoresis.

•Gels containing 0.3% agarose will separate double-stranded DNA molecules of between 5 and 60 kb size, whereas 2% gels are used for samples of between 0.1 and 3 kb. Many laboratories routinely use 0.8% gels, which are suitable for separating DNA molecules in the range 0.510 kb. Since agarose gels separate DNA according to size, the Mr of a DNA fragment may be determined from its electrophoretic mobility by running a number of standard DNA markers of known Mr on the same gel.

•DNA gels are invariably run as horizontal, submarine or submerged gels; so named because such a gel is totally immersed in buffer. Agarose is dissolved in buffer and allowed to set onto a glass or plastic plate, surrounded by a wall of adhesive tape or a plastic frame to provide a gel about 3 mm in depth. The gel is placed in the electrophoresis tank, covered with buffer, and samples loaded by directly injecting the sample into the wells. No stacking gel is needed for the electrophoresis of DNA because the mobilities of DNA molecules are much greater in the well than in the gel, and therefore all the molecules in the well pile up against the gel within a few minutes of the current being turned on, forming a tight band at the start of the run.

General purpose gels are approximately 25 cm long and 12 cm wide, and are run at a voltage gradient of about 1.5 V cm1 overnight. A dye such as bromophenol blue is also included in the sample solvent; it makes it easier to see the sample that is being loaded and also acts as a marker of the electrophoresis front.

Once the system has been run, the DNA in the gel needs to be stained and visualised. The reagent most widely used is the fluorescent dye ethidium bromide. The gel is rinsed gently in a solution of ethidium bromide (0.5 mg cm3) and then viewed under ultraviolet light (300 nm wavelength). The ethidium bromide concentration therefore builds up at the site of the DNA bands and under ultraviolet light the DNA bands fluoresce orange-red. As little as 10 ng of DNA can be visualised as a 1 cm wide band.

# Test your understanding

What is the function of Ethidium bromide in agarose gel electrophoresis?

a. Stains the separated DNA
b. Enables Visualisation of band under transilluminator or Gel doc
c. Used in elution of DNA from gel
d. Both ( a) and (b)

Gels containing 0.3% agarose will separate double-stranded DNA molecules of between

a. 5-60 kb
b. 0.1 and 3 kb
c. greater than 100 kb
d. None of these

Gels containing 2 % agarose will separate double-stranded DNA molecules of

a. 5-60 kb
b. 0.1 and 3 kb
c. greater than 100 kb
d. None of these

Electrophoresis of histones and myoglobin under non-denaturing conditions (pH = 7.0) results in

a. both proteins migrate to the anode
b. histones migrate to the anode and myoglobin migrates to the cathode
c. histones migrate to the cathode and myoglobin migrates to the anode
d. both proteins migrate to the cathode

Glycerol is added to protein samples before they are loaded to the wells of PAGE. The function of glycerol is to ……..

Stabilize protein structure
Provide density to sample

a. Helps to bind Sodium dodecyly sulphate (SDS) to protein
b. Helps to reduce disulfide bonds by beta-mercaptoethanol

# References & Further reading


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3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
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