Aim 53 To determine the Molecular Weight of DNA/Plasmid DNA by Agarose Gel Electrophoresis

Introduction

The more porous gel and gels having larger pore size than polyacrylamide gels are Agarose gels are used to separate large macro- molecules like nucleic acid.

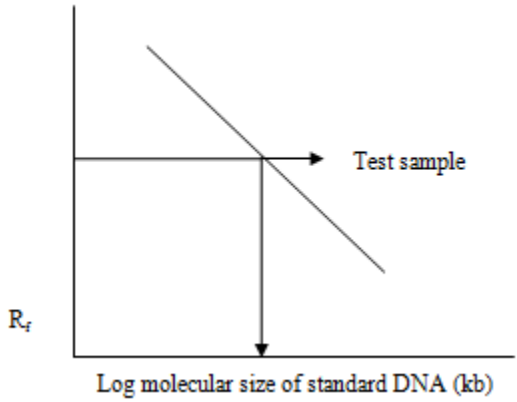
Agarose is a linear polymer of D-galactose and 3,6-anhydo-L-galactose. Porosity of the gel depend upon the concentration of Agarose. If, the concentration increases, size of pores decrease, small DNA molecules can be separated at higher concentration of Agarose. At alkaline pH, DNA is negatively charged and charge mass ratio is one. Due to negative charge, they migrate towards the anode when electric current is applied. Small molecules will move fast as compared to large.

The migration rate of various form of DNA is:

Supercoiled DNA > Linear double stranded DNA > Open circular DNA.

DNA molecules of same size travel at different rate, if they have different conformation.

The molecular size of unknown DNA is determined by comparing its electrophoretic mobility with standard DNA on agarose gel, the test sample and standard molecular weight DNA marker are run on agarose gel. Then, distance (migrated by them) are measured and a graph is plotted between their electrophoretic mobility as well as log molecular weight. From the graph the size of the test DNA sample can be obtained.



Depending upon the concentration of gel 200 bp to 50 kb DNA molecules can be separated, 1 % gel separates 0.527 kb DNA molecules.

Requirements

1. Agarose gel electrophoresis unit (horizontal)
2. Agarose - 1%
3. Tris-acetate buffer (TAE) stock solution – 5 X
   - **a.** Tris 24.2 g
   - **b.** Glacial aacetic acid 5.7 ml
   - **c.** 0.5 EDTA 10.0 ml
   - **d.** Final volume 1 liter
   - **e.** pH 8.0
4. Gel loading solution
   - **a.** Xylene cyanol FF 0.25 %
   - **b.** Bromophenol blue 0.25 %
   - **c.** Glycerol 30 %
   - **d.** Total volume 100 ml

   Bromophenol blue moves faster than xylene cyanol FF.
5. *Ethidium bromide* - 10 mg/ml of 1X TAE buffer
6. Standard molecular weight DNA marker (add 10 µL of gel loading solution to 20 µL of marker and mix well).
7. Test sample 1, 2, 3 (add 10 µL of gel loading solution to 20 µL of test sample and mix well).

8. UV- transilluminator with camera.

Procedure

1. Take a clean gel casting plate, seal it from sides by using adhesive tape so that the agarose gel solution should remains with in plates for polymerization.
2. Prepare 1 X TAE by diluting 5 X TAE buffer with double distilled water (to suppress the activity of DNase EDTA is added in buffer).
3. Take 50 ml of 1 X TAE and add 0.5 g of agarose in conical flask, then boil it to dissolve agarose till a clear solution is obtained.
4. Allow it to cool to 60°C.
5. In electrophoresis unit the comb is adjust in such a way that it is about 2 cm from one end.
6. Pore the gel when its temperature is around 60°C into the gel tank without creating bubbles. Leave the set undisturbed till the gel solidifies.
7. Remove the comb and tape carefully transfer the gel plate to electrophoresis tank. Wells should be towards the cathode.
8. Pour 1 X TAE buffer dropwise into the tank till the buffer level reach 0.5-0.8 cm above the gel surface.
9. Before loading the sample, connect the electrophoresis unit to power supply and the cords are connected between the electrophoresis set and power supply before loading the samples.
10. In different wells load the standard DNA markers and test sample with the help of micropipette.
11. Start the power and run at 100v after loading.
12. The sample is run till blue dye has reached near opposite edge of the gel.
13. Switch off the power supply, disconnect electric cord from electrode remove the gel from gel tank and invest the gel tank on the staining tray.

14. Pour ethedium bromide solution and stain for 20 min. Keep the gel in water for 15 min for destaining the gel.
15. On UV–transilluminator place the gel and observe the orange colour band of DNA in UV light.
16. Measure the distance travelled by each band from edge the loading well in cm.
17. Measure the R_f value.

    R_f = Distance travelled by the DNA molecule / Distance travelled by the dye.
18. Draw the graph between log molecular weight of standard DNA markrs v/s R_f values.
19. Measure the R_f values of test sample and obtain the corresponding molecular size from graph.

Precautions

1. High quality chemicals and double distilled water should be used.
2. Ethidium bromide should be handled with extreme care because it is carcinogenic.
3. Wear UV protective glasses while viewing gel because UV light is injurious to eyes.
4. Comb should be lifted gently from the gel to avoid damage.
5. Avoid air bubbling in gel before starting electrophoresis check that the gel is completely emerged in buffer.
6. The tracking dye should not run out of the gel.