Agarose gel electrophoresis for DNA

Introduction →

Agarose gel electrophoresis remains the most widely used technique for separating nucleic acid fragments because it is a simple technique that is non-toxic and offers a broad separation range. The size of the gel pores can be controlled by simple adjusting the agarose conc. to prepare gels appropriate for the separation of nucleic acids in agarose gel is also affected by the choice of running buffer and the applied voltage. Electrophoresis used on electric field to move the negativity charged DNA towards a positive electrode through on agarose gel matrix. The length of the DNA segment can be accurately determined by running it on an agarose gel along side a DNA ladder. Agarose extracted from seaweed is a polymer composed of a reapiting disaccharide unit called agarobiose which consist of galactose and 3.6 anhydrogalactose. chains of agarose from helical structures that radius of 20-30 nm. Agarose is purified from agar by removing agar's other components like agaropeatin.

Principle :-

When changed molecules are placed in an electric field, they migrate towards either the positive or negative pole according to their change. Nucleic acids have a consistent negetive changes imported by their phosphate

backbone and migrate towards the anode. Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the log 10 of their molecular weight. Circular forms of DNA migrate in agarose ~~distrin~~ distinctly differently from linear DNAs of the same mass. Higher conc. of agarose facilitates separation of small DNAs while low agarose conc. allow the resolution of larger DNAs. Voltage applied when increased, larger fragments migrate proportionally faster than small fragments.

● Requirements :-

Ⓐ Reagents :

① Agarose solution → 2%, 0.8 gm of agarose in 60 ml of TAE.

② 50 × TAE buffer (for 1 lt solution) →

Tris base = 242 gm.

Glacial acetic acid = 57.1 ml.

EDTA (500 mM, PH 8) = 100 ml.

Water = volume adjustment.

for, 150 ml of 1 × TAE working buffer, 3 ml of TAE added to 147 ml of disfilled water.

③ Ethidium bromide → 0.5 Mg/ml. It is bi fluoresaement dye that intercalates between bases of nucleic acids and allows very convenient detection of DNA fragement in gels.

(iv) Gel loading buffer – 5 µl of sample + 2 µl of bromophenol blue and glycerol.

Bromophenol blue → Strains the DNA, indicates how for samples have migrated on the gel. The sample remain just behind the stain also prevents backflow, its a tracking dye. Provide colour and simplify the loading process.

Glycerol → Gives the sample its density. This makes the sample sink throught the buffer and remain in the wells.

(B) Equipments :-

(i) Gel castor and comb.
(ii) Electrophoric chamber.
(iii) Micropipette and tips.
(iv) Epperderts.
(v) Parastine paper.
(vi) Gel DOC.

(C) Protocol :-

(i) The edges a clean dry glass plate were sealed with tape to form a mould.

↓

(ii) A solution agarose was prepared in electrophoresis buffer. (0.8 gm of agarose powder in 60 ml of TAE).

↓

P.T.O →

(iii) The slurry was heated in a microwave until the agarose was dissolved.

↓

(iv) When the molten gel was cooled, 1 ul of ethidium bromide is added to a concentration of 0.5 ug / ml.

↓

(v) An appropriate comb is placed.

↓

(vi) The agarose solution was poured into mould.

↓

(vii) The gel was allowed to set completely.

↓

(viii) The combo was carefully removed.

↓

(ix) The gel placed in the electrophoretic tank.

↓

(x) TAE buffer was poured in well to cover the gel.

↓

(xi) 2 ul of gel loading buffer is added to the paraffin and 5 ul of DNA sample was mixed with it.

↓

(xii) The sample mixture were slowly loaded into the slots of the submerged gel using a micropipette.

↓

(xiii) Running buffers, TAE was added to the chamber until

the buffer just covers the top of the gel.

↓

xiv) The lid of the gel tank was closed and the electrical leads were attached.

↓

xv) The gel was run at a voltage of 80 volts for 30 mins.

↓

xvi) Electrophoresed until the bromophenol blue had migrated to within 3/4 of the positive electrode end of the gel.

↓

(xvii) The gel casting tray was lifted from the chamber.

↓

(xviii) The gel was gently scraped out and observed in the gel DOC.

Observation :-

Bands were observed and photographs were taken in Gel DOC using Software quantity one.