Date 27/10/18

Expt. No. 11

Page No. 27

5

Experiment No. - 11

Object ⇒ To perform the gel electrophoresis by nucleic ~~protein~~ acid.

Reference ⇒ Walker John, Wilson Keith, "Practical Biochemistry principle and technique", 5th edi., published by cambridge university, pp no. - 580-590.

Requirements ⇒

Chemical ⇒ TAC, Ethinium bromide, Agarose, Bromo phenol blue staining agent, etc

Apparatus ⇒ Transilluminator, castic tray, Gel combs,

Equipment ⇒ Beaker, glass rod etc.

Theory ⇒ Gel Electrophoresis

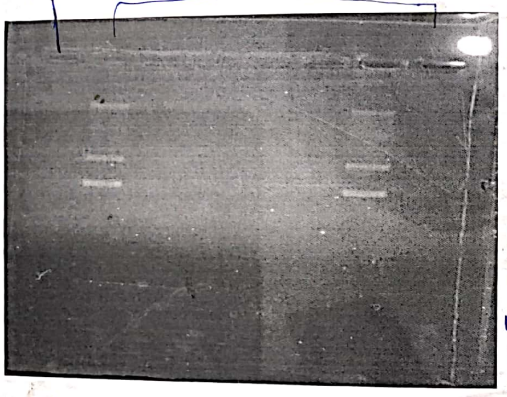
→ The migration of charge particle under the influence of electric or magnetic field is k/as electrophoresis. and when this technique uses then gel (agarose) then the name this technique agarose gel electrophoresis.

Agarose gel ⇒ Agarose is a highly purified polysaccharide derived from agar a natural product of red seaweed.

→ Gel electrophoresis a is a technique used to separate DNA fragment according to their size DNA. Sample are located into wells at one end of gel and an electric currents is applied to pull then through the gel

Teacher's Signature _____

Scanned with CamScanner



DNA sample are loaded into well

↓

Power is turned on & DNA fragments migrate through gel.

→ If we want the exact pattern of DNA we can use gel DOC system.

Date

Expt. No. 11.

Page No. 28

Types of gel electrophoresis - Agarose gel, polycrylamide gel

→ Poured vertically, seperate small molecules, neurotoxic is used for protein and DNA separation

Types of buffer - TAE - Tris acetate EDTA buffer
TBE - Tris borate EDTA buffer

Analysis of DNA ⇒

→ Quantitative :- To check or determine the Qty of DNA / agarose gel

→ Qualitative :- To check the Quality of then DNA / Agarose gel

Principle ⇒ When we place any charge molecule in an electric field, they move toward the positive or negative pole according to the charge they are having protein don't have any net charge where nucleic acid have a negative charge so they move towards the anode when electric field applied.

Staining agent ⇒ Ethidium bromide is a intercalating agent which is use to stain the gel during electrophoresis and visualize DNA band.

Dye - Bromophenol blue is dye & tracking system it track the DNA

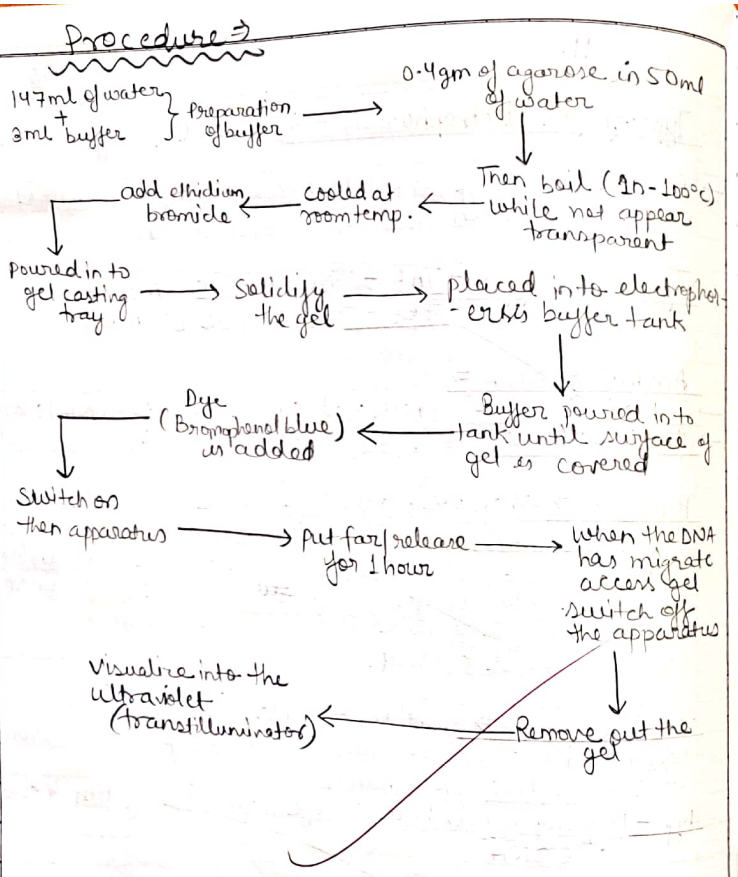
Procedure / methodology ⇒

Preparation of gel - To make a gel 0.4gm of agarose is dissolve in 50 ml of water.

Teacher's Signature

Scanned with CamScanner

Procedure ⇒



Expt. No. 11. Date

Page No. 29

Preparation of buffer ⇒ Take 147 of distilled water & dissolve in 3ml of buffer

→ To make a gel agarose powder is mixed with an electrophoresis buffer and heated to a high temp. until all of agarose powder has melted.

→ The melted gel is then poured in a gel casting tray & comb is placed at one end to make well.

→ Once the gel has cool & solidify the comb is removed.

→ The gel is then placed in electrophoresis tank & electrophoresis buffer is poured into the tank until the surface of the gel is covered. The buffer conduct the electric current.

Preparing DNA for electrophoresis = A dye is added to the sample of DNA prior to electrophoresis to ↑se the viscosity of the sample which will prevent it form floating out of well so migration of the sample can seen

→ A DNA marker (ladder) is loaded into the first well of the gel.

→ Well this is done the lid is placed on the tank making sure that the orientation of gel +ve & -ve.

Separating the fragment ⇒ The electric current is then turned on so that negatively charge DNA move through the gel toward the positive side of the gel

→ Shorter length / low m.w DNA moves faster than the higher m.w / longer length so move further in the time current is run.

Teacher's Signature

Scanned with CamScanner

Expt. No. 11. Date

Page No. 30

→ The distance the DNA has migrate in the gel can be judge visually by monitoring the migration of the loading buffer dye.

→ The electric current is left on long enough (1 hr) to ensure that the DNA fragment move you enough across the gel to say separate them;

Visualizing the result ⇒ Once the DNA has migrated for enough across the gel, the electrical current is switched off and the gel is removed from the tank

→ To visualize the DNA, the gel is stained with fluorescent dye that bind to the DNA & is placed on an ultraviolets transillminator which will show up the stained as bright band.

Result ⇒ We have successfully observed the DNA bonding pattern which was given (plasmid DNA.)

Teacher's Signature

Scanned with CamScanner