AIM: To detect DNA by gel electrophoresis.

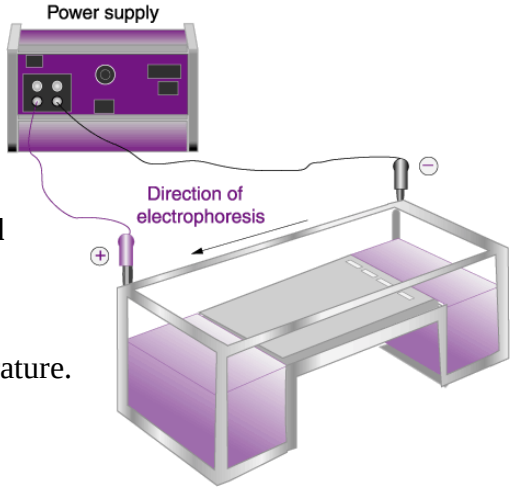
PRINCIPLE: Electrophoresis is a technique used to separate and purify macromolecules especially proteins and nucleic acids that differ in **charge, size,** and **mass**. Agarose gel electrophoresis is used to separate nucleic acids especially DNA on the basis of their charge:mass ratio represented as **q/m ratio.** Migration of DNA under influence of electric field provided by the Direct Current (DC) supply is towards anode because of the negative charge imparted by the phosphate backbone of DNA. DNA with lower molecular weight moves faster than high molecular weight when observed under UV. The pore size of the gel is inversely proportional to the concentration and hence to separate small molecular weight DNA higher concentration should be used.

REQUIREMENTS:

- 0.8% agarose gel, 1X TAE buffer, Ethidium bromide, Autoclaved distilled water, Loadind dye (Bromophenol blue).
- Gel casting tray, Electrophoresis chamber, microwave or hotplate, DC power pack, UV transilluminator.

Preparation of solutions

Stock solutions of the following should be prepared, autoclaved and stored at room temperature for further preparation of various working solutions:



i) *Tris Acetate EDTA Buffer (TAE)*: Stock (50X) For 1 litre:

Tris - 242g

Glacial acetic acid - 57.2ml

EDTA 0.5M (pH 8.0)-100ml

Make the final volume to 1 litre. Autoclave and store at room temperature.

ii) *Tris Borate EDTA Buffer (TBE)*: Stock (5X) For 1 litre:

Tris - 54g

Boric acid - 27.5g

EDTA 0.5M (pH 8.0)-20ml

Make the final volume to 1 litre. Autoclave and store at room temperature.

iii) *Running buffer for Electrophoresis:* any of the two above mentioned can be used for electrophoresis after dilution to **1X.**

For **1X TAE** dilute **20ml of the stock** with autoclaved distilled water to make up to 1 litre.

For **1X TBE** dilute **2ml of the stock** with autoclaved distilled water to make up to 1 litre.

iv) ***6X Gel-loading Buffer:*** 0.15% bromo-phenol blue, 0.15% xylene cyanol, 30%(v/v) glycerol in autoclaved water.

v) ***Ethidium Bromide Solution:*** Dissolve 10mg/ml in water. Wrap it with aluminium foil and store it at 4 ^{o}C. **While preparing and handling wear gloves and discard the gloves after use as ethidium bromide is strong mutagen.**

vi) ***1X Tris EDTA (TE) Buffer*** **:** For **100ml volume** add the following:
1ml of 1M Tris pH 8.0 (Final concentration 10mM)

0.2ml of EDTA 0.5M pH 8.0 (Final concentration 1mM) Make it up to 100ml, autoclave and store at room temp.

PROCEDURE:

Preparation of 0.8% gel and electrophoresis of the isolated genomic DNA: It has the following steps: (Fig 13)

1. Weigh 0.4g of agarose and transfer it to a 250 ml conical flask.
2. To this add 50ml of 1x TAE or TBE buffer and dissolve it evenly by heating.
3. Cool down to 60 ^{o}C and add ethidium bromide (10mg/ml stock) to get a final concentration of 0.5µg/ml of the gel and mix well by gently rotating the flask.
4. Place the gel comb in the casting tray and gently pour the gel solution into it without allowing air bubble to enter. Allow the gel to solidify. Gel should be 0.5-0.8 cm thick.
5. Gently remove the comb without breaking the gel, place it on the electrophoresis chamber with the wells on the cathode end and fill the tank with 1x TAE or TBE buffer. (Buffer used should be the one in which the gel was prepared).
6. Carefully load the 3-4µl of the sample mixed with 1µl of the 6xgel loading buffer (tracking dye) with micropipette and microtip. Keep the record of the lanes in which well what sample is being loaded as Lane1, Lane 2 etc.
7. Connect the instrument to the DC power pack and set the voltage to 60 V.
8. Run the gel till the blue dye reaches three fourth of the gel. Disconnect the apparatus and observe the gel under UV Transilluminator.

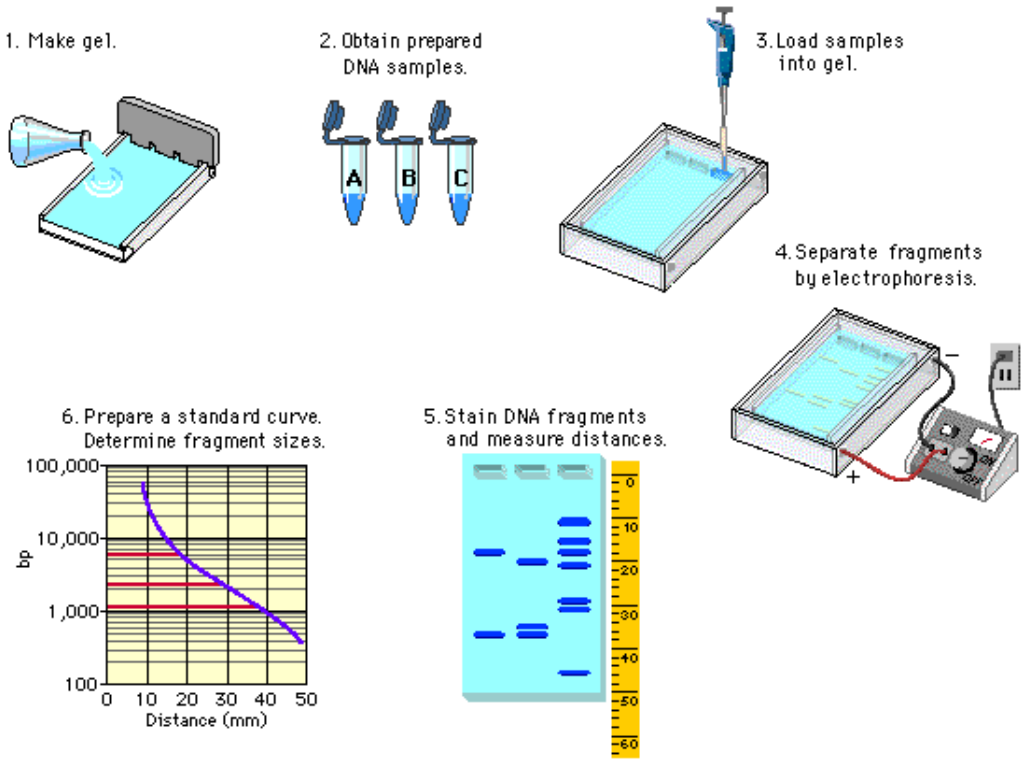


Fig 13. Showing the process of casting and loading the gel

RESULTS: On observing under UV transilluminator you will see a very high molecular weight band corresponding to the first band of λHind III digest marker (Fig 14)

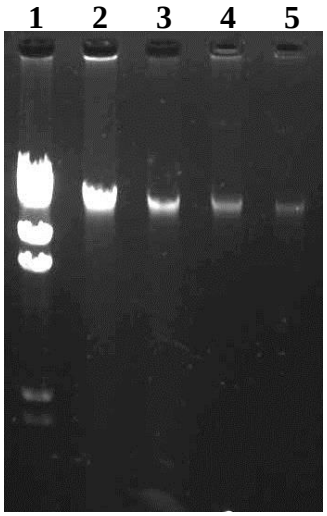


Fig 14. Showing DNA as visualized under UV Transilluminator after electrophoresis. Lane 1- λHind III digest marker, Lanes 2 to 5 DNA of different samples.

PRECAUTIONS:

1. All the solutions, glassware, plasticware should be autoclaved.
2. Tips should be discarded after every use. Same tip should not be inserted into same solution.

3. If tips touch any surface accidentally, discard it.
4. Solutions once pipetted out should not be transferred back to the reagent bottles.
5. While handling ethidium bromide always wear gloves and discard the gloves after use.
6. Always wear full sleeves white coat and UV protective glasses while observing on the transilluminator.
7. Sample should be carefully loaded without puncturing the wells.
8. As the electric supply is of DC source it should be carefully operated.
9. After visualizing and capturing the picture the gel should be carefully discarded.