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AGAROSE GEL ELECTROPHORESIS

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Gel Electrophoresis is widely used technique for analyzing Nucleic acids and Proteins. Agarose is a polysaccharide purified from sea weed Gelidium. An agarose gel is formed by mixing agarose powder in buffer solution.

PRINCIPLE

Agarose gel electrophoresis involves the use of electric field to move the negatively charged DNA towards a positive electrode through an agarose sieved gel. The shorter fragments migrate faster than smaller ones. By this we can determine the length of DNA fragment and presence or absence of PCR products by running it on agarose gel along with a DNA ladder (Collection of DNA fragments of known lengths) and DNA can be seen under UV Detector using ethidium bromide as staining reagents.

MATERIALS

- **DNA electrophoresis apparatus**
- **Loading tips,pippet**
- **10X loading buffer,**
- **DNA ladder**
- **Agarose**
- **Ethidium bromide**
- **Gel casting tray**
- **Combs**

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REAGENTS

Running buffer: 0.5X TBE (TRIS BORATE EDTA)

5.4g Tris Base

2.75g Boric Acid

2ml of 0.5M EDTA

In 1 litre water(ph =8)

PROTOCOL

PREPARING THE AGAROSE GEL

A) Weigh 1g of agarose powder and add it to microwavable flask.

B) Add 100 ml of 1X TBE.

Note:TAE(Tris acetate EDTA) can be used also instead of TBE.

C) Microwave for 1-3 minutes or place it in hot water bath until the solution becomes clear.

D) Let the solution to cool down for 5 minutes.

E) Add Ethidium Bromide (usually 2-3µl per 100 ml gel).Etbr is added as it binds to DNA and allows to visualize DNA under UV light.

Note:Etbr is highly carcinogenic so it should not be handled with naked hands.

F) Pour the agarose into Gel tray and insert the well comb.

Note: It should be poured slowly to avoid bubbles,which will disrupt gel.The bubbles can be pushed away or to edges with a pipette tip.

G) Allow it to solidify at room temperature for 20-3- minutes.

LOADING OF SAMPLES AND RUNNING OF AGAROSE GEL

A) Add loading buffer to sample.

Note: It provides a visible dye that helps to know how far the gel has run and also it contains glycerol ,so when it is added to the sample it becomes heavier and thus settle down of gel instead of diffusing in buffer.

B) Place the gel into electrophoresis unit.

C) Fill the unit with 1X TBE or TAE, cover the wells with buffer properly.

D) Carefully load your samples into wells.

E) Carefully load Ladder into well.

F)Run the gel at 80-150V until the dye is 70-80% down the gel.

Note:Black is negative,red is positive(As DNA is negatively charged,so it will run towards positive electrode)

G)Run time is usually 1-1.5 hours,depending on the concentration of gel and voltage.

H) Turn off power,disconnect the electrode ,carefully remove gel from gel box.

I)Using UV Transilluminator,visualize DNA fragments.