Agarose Gel Electrophoresis

Materials

- DNA ladder of 1 Kb
- DNA that was digested with EcoR1 and Spa1
- Ethidium bromide
- Agarose
- Buffer TAE 1X
- Dye blue
- Parafilm
- Micropipettes 1000 uL, 100 uL, 10 uL
- (1) Erlenmeyer flask 100 mL

Equipment

- Electrophoresis chamber
- Gel tray and comb
- Power pack
- Transilluminator

Proces

1. Prepare 1000 mL of TAE buffer 1 X from the stock
    a. Example of preparation of buffer TAE IX from a stock 10X

$$C_1V_1 = C_2V_2$$
$$C_1 = 1X$$
$$C_2 = 10X$$
$$V_1 = 1000mL$$
$$V_2 = ?$$

$$V_2 = \frac{C_1V_1}{C_2} = 100mL$$

2. Prepare the agarose gel depending on the size of the linear DNA

RESOLUTION OF LINEAR DNA ON AGAROSE GELS.

Recommended % Agarose	Optimum Resolution for Linear DNA
0.5	1,000–30,000bp
0.7	800–12,000bp
1.0	500–10,000bp
1.2	400–7,000bp
1.5	200–3,000bp
2.0	50–2,000bp

a. Weight the grams of agarose needed to achieve the desired concentration. Calculate for 50 mL of TAE buffer
b. Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g., 2 g of agarose in 100 mL of TAE will make a 2% gel).

3. Suspend the agarose powder in 50 mL of TAE buffer in a erlenmeyer flask.
4. Microwave the solution for 2 minutes (for a small gel)for 30 second bursts and 10 seconds burst at the end). Make sure that the agarose is completely dissolved by swirling the heated mixture roughly every 30 seconds. Allow it to cool for 3 minutes.
5. Wipe a plastic gel tray and comb with 70% ethanol and place in the electrophoresis tank so that the rubber tubing forms a seal with the sides of the tank. Add distilled water over the gel tray to ensure there is no leakage.
7. When the agarose is cool Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2-3 µl of lab stock solution per 100 mL gel). this was done with gloves and a lot of caution because said agent is carcinogenic, the tips used to pour it were deposited in a red bag

a. Add 1.5 uL to the 50 mL flask

8. Pour the melted agarose into the gel trays. Place the comb into the right position and allow it to set for approximately half hour (this can be done faster by placing the gel tray in the refrigerator.
9. Carefully remove the comb from the gel. Rotate the gel tray so that the wells are toward the negative (black) terminals (the top of the tank,

assuming that the electrodes are on the right hand side). Cover the gel with 1X TAE running buffer

10.The parafilm is used to mix the samples with the blue dye, which contained bromophenol blue, orange G and Xylene blue. With the help of a micropipette, small points of blue juice solution are placed on the paper as the samples are added to each of the lanes, they need to be mixed with the blue dye.

if the volume of dye added is “x” :

x + Volume of DNA = 6x

11. Load first the molecular weight marker, learn if it already has loading dye.

For the whole process, use a micro pipette and white tips. To download the samples in the gel with the micropipette,visualize the camera from above and maintain a constant support with an elbow on the table so as not to pour the material in a non-corresponding place.

12. Connect the camera through two electrodes (+/-) to its respective current system, adjusted to 80 volts. Then allow the gel to run for the necessary amount of time (about 1 hour however, check that the dye front has almost run through the gel).

13. Switch off the power pack and take the gel to the transilluminator.

For more information:

https://di.uq.edu.au/files/3190/MolBiolWS01DNATech.pdf

https://www.addgene.org/protocols/gel-electrophoresis/#faq

16 Agosto

Vacio-escalera-1-vacio-4-7-8

16-Agosto segundo intento

vacio escalera-2(DNA)-3(primers)-5(dna y primers)-6-buffer

17-Agosto

Vacio-escalera