Agarose Gel Electrophoresis

Introduction

- Aim: Check fragment size.
- Timeframe:
    - Preparation: 15 minutes.
    - Wait-time: 30 min, 30 min.
    - Overall: 2 hours.

Materials

- Agarose powder: 0.5 g in 50 mL LiAcO for 0.5% agarose gels
- 1X LiAcO buffer (0.66g in 1L dH2O)
- Microwave
- 500 mL conical flask with a removable lid
- Well comb
- Gel tray
- 1% Ethidium bromide
- NEB 1 kb QuickLoad ladder
- 4 µL NEB bromophenol loading dye
- 5-20 µL of your DNA samples
- Gel electrophoresis chamber
- Power pack

Procedure

- Agarose Gel

    1. Weigh 0.5g of agarose (to make a gel of 0.5%) and mix with 50 mL 1x LiAcO in a designated 500 mL Duran bottle with a lid.
    2. Microwave for 1-3 min until the agarose is completely dissolved. It is best to heat 30 seconds, stop and swirl towards a boil. Do not over boil and be careful stirring as eruptive boiling can occur. Placing cling film or a lid over the top can help (do not secure the lid, just place it on top).
    3. Let agarose solution cool down until you can comfortably use the flask, if necessary, use heat-proof gloves. If it begins to solidify re-boil it. It should take about 5 minutes to cool.
    4. Add 5 µL of ethidium bromide. N.B.: Caution the dye is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.
    5. Pour the agarose into a gel chamber with the well comb in place. Pour slowly to avoid bubbles. Bubbles can be popped or pushed to the side using a pipette tip or the comb end.
    6. Let sit at room temperature for 20-30 minutes until completely solidified.
    7. Once the gel is set, remove the comb by carefully and slowly pulling it up.

- Visualize DNA

8. Add 4 µL bromophenol blue with 20 µL of each of your DNA samples into a PCR tube and mix well by moving volume up and down your pipette tip.
9. Put gel inside the chamber. Black is negative (cathode), red is positive (anode) - the wells should be facing towards the cathode and DNA will migrate towards the anode. Fill the electrophoresis chamber with 1X LiAcO buffer stock, so that the buffer covers up the gel.
10. Gently load 10 µL of 1kb molecular weight ladder (NEB QuickLoad Ladder) into the first lane. N.B.: Maintain positive pressure once sample is pushed out and only release once the pipette is out of the buffer to prevent bubbles dislodging the DNA.
11. Gently load your samples into the well. It is highly advised to draw what you are loading into each well, so that you can label the photo accordingly.
12. Run the gel at 180V for about 30 minutes or until the dye line is several cm from the bottom of the gel. N.B: Set yourself a timer and keep on checking on the gel, this will help you ensure that the bands will not over run.
13. Turn OFF the power, disconnect the electrodes and carefully remove the gel and place on a tray.
14. Using a UV light device (transilluminator) visualise the DNA fragments. Print the photograph and label wells exactly with the samples and the control names, write down the date of the gel. N.B: Use safety goggles, face shield and gloves to shield your eyes and skin from the UV light.