0.8% Agarose Gel Electrophoresis

Gel Preparation

1. Set up water bath to 37^0 C
2. Weigh 0.80g Agar on analytical balance and place in 250mL Erlenmeyer flask
3. Add 100mL 1X TAE Buffer to Erlenmeyer flask and swirl to mix
4. Heat the solution in microwave until fully dissolved. Swirl every 50s.
5. Let solution cool down for 2 minutes (before polymerization can start).
6. Add 15uL of 2.5mg/mL EtBr. Let cool for 1 more minute.
7. Turn the gel tray so that the gaskets are sealed with the walls of the chamber.
8. Pour agarose solution into the tray and insert the comb.
9. Cover the apparatus with tinfoil and wait for the gel to polymerize (30min-1h). Prepare samples in the meantime.
10. Once polymerized, remove comb, and turn the gel tray back to the side.
11. Fill the apparatus with 1x TAE buffer until the entire gel is covered with buffer
12. Add 5uL ethidium bromide to the cathode side of the chamber (comb side) near the wall.
13. Add 10uL ethidium bromide to the anode wall of the chamber.
14. Pipette 10uL DNA marker (Freezer B) into the first well.
15. Pipette samples into other wells.
16. Attach both wires (red to red and black to black) to the power supply and run at 60V.
17. Once samples can be seen at the edge of the gel, turn off the power supply.

Sample Preparation

1. <u>Undigested Sample</u>
    - In an Eppendorf add:
        1. 5uL DNA sample
        2. 1uL Loading Dye (6x) (Freezer B)
        3. 4uL milli-Q water
    - Mix by pipetting up and down
    - Store on ice until placed into well

2. <u>Double Digested Sample</u>
    - In an Eppendorf
        1. 5uL DNA sample
        2. 1uL Restriction Enzyme 1 (Freezer B)
        3. 1uL Restriction Enzyme 2 (Freezer B)
        4. 1uL Loading Dye (6x)
        5. 2uL milli-Q water
    - Mix by pipetting up and down
    - Place in water bath for 15 minutes
    - Store on ice until placed into wells

Gel Reading

1. Remove gel from cast and place in container.
2. Wash gel with di water and dispose of the liquid in the chemical waste. Repeat.
3. Place gel on UV tray (purple) and place inside EZ Imager.
4. On the computer: ImageLab Software→ New Protocol → Application → Nucleic Acids → Ethidium Bromide → run protocol.
5. To store gel: wrap in paper towel and place in container. Fill container with 1x TAE buffer.