[iGEM 2017] Gel Electrophoresis

Introduction

Determine, roughly, the size of a dsDNA fragment.

This protocol describes:
- Creating an agarose gel
- Running DNA samples on the agarose gel

The typical cloning pipeline is:
PCR -> **Gel** -> DpnI -> PCR Purification -> Gibson Assembly -> Transformation -> Colony PCR -> Inoculation -> Miniprep

Materials

- 1X TAE Buffer
- Ethidium Bromide (EtBr)
- Microwavable EtBr-safe Plastic Tubes
- Agarose
- Gel Comb
- Gel Box

Procedure

Agarose Gel Prep

1. Add 50ml (use the falcon measuring tube) of 1x TAE buffer to a plastic microwavable tube. Then measure out .5g of agarose and add to buffer.

   This makes a 1% agarose gel. If you want to change the concentration, change the mass of agarose rather than the volume of TAE.

2. Microwave for 1 minute, or 1:30 if doing two tubes at a time. Ensure that there are no undissolved pieces of agarose.

   If after microwaving you still see small particles of agarose in the solution, heat for more time.

3. Briefly let cool, then add 1 drop of ETBR to TAE buffer agarose solution swirling until the red has disappeared. Insert tray into gel-box and pour solution. Add 10 well gel comb to farthest gel comb slot.

4. Let cool until hardening, reorient gel tray so that wells are aligned vertically, then add 1x TAE buffer till top of gel is covered. Gel can be used anytime within the same day.

   Each 10 well comb can be used for 8 samples, if you need to run more samples, make more gels.
   You can also place a second row of wells if performing colony PCRs.

Agarose Gel Electrophoresis

5. Remove PCR products from thermocycler. Cut a strip of parafilm for each gel you are loading.

6. Place 5µl dots of 1.2x loading dye on parafilm for each sample you intend to run. Add 1µl of each PCR product to each dot.

   Use 2µl PCR product for colony PCRs, and 4µl of dye.

7. Make sure the gel is oriented correctly, with the electrodes facing your right side, the lanes should start on the side of the gel farthest from you.

   DNA is negatively charged so it will run towards the positive electrode.
   Red = +
   Blue = -
   DNA runs to red.

8. Load the gel, leaving lanes 1 and 10 empty.

9. Add 6µl of DNA ladder to lanes 1 and 10.

   Choice of ladder depends on the expected lengths of your DNA fragments:
   1kb ladder spans 0.5 - 10 kb
   50bp ladder spans 50-1350 bp (Basically should only be used for very small fragments)
   (We add ladder to the edge lanes because the gel can bend if the agar is not homogeneous within the gel. However, if you are only running 1-3 samples, you can just use one ladder lane if you'd like)

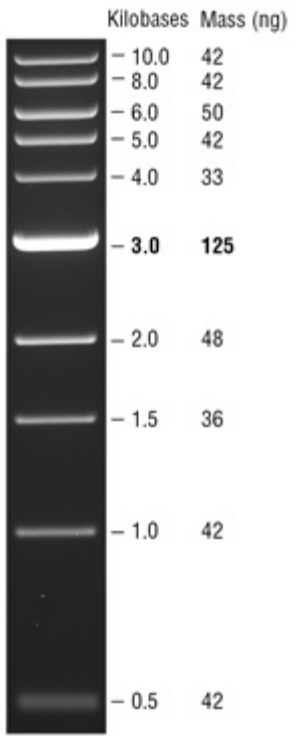
10. Add gel-box cover, making sure the DNA is loaded on the opposite side of the red (positive) cathode. Run gel at constant volts (160), for 30 minutes.

    This program is obtained by setting Program -> Run (screen should be on program #1)

11. Visualize using UV box when finished.

    CRITICAL Do not turn on box unless lid is closed. Ensure you take a photo of gel and upload to your notes. Throw away gels in gel waste container.
    Make sure to keep gel if you want to show to others.
    DNA fragment lengths are deduced by comparing to the known lengths given by the ladder. See the links above for your ladder type for these values.

12. 1kb Ladder from NEB



13. 50bp ladder from NEB