Gel Electrophoresis

Overview

This protocol covers the separation and imaging of nucleic acids via agarose gel electrophoresis. While this protocol is general purpose, it is written from the perspective of a DNA visualization after a PCR. For gel extractions, the gel extraction protocol should be used, but familiarity with this protocol is assumed.

Materials

- 1x TAE buffer
- Microwavable plastic flasks
    - In back room (note we only have two of these).
- ETBR dropper
- Nucleic Acid Sample
- DNA loading dye
- DNA ladder
- Agarose
    - Do not confuse with agar. Agarose is a white powder, agar is a yellowish brown.
- Gel comb
    - Typically 10 well, but 6 and 8 well varieties are sometimes used.[i]
    - Remember 2 of your lanes will be used for ladder, so 10 wells allows you to run 8 samples.
- Gel electrophoresis box
- Gel tray
    - Make sure not to use a broken one.

Procedure

1. Determine the agarose percentage for your gel. The standard percentage is 1% (w/v). For normal visualization, 1% should be used.
    - Higher agarose percentage allows better separation of low weight bands, while lower agarose percentage allows better separation of high weight bands.
    - Note that low agarose gels are very easy to break.
2. Weigh out the appropriate amount of agarose for each gel. Our gel trays are 50mL in volume, so each 1% agarose gel would need 0.5g of agarose.
3. Add agarose to flask(s)
4. Measure out 50mL of 1x TAE buffer from the carboy. Add to flask(s)
5. Microwave flask until agarose is dissolved. (50s for 1 flask, 1:30 for 2).
6. While flasks cool slightly, prepare for gel casting. Take gel tray and place sideways into gel box, making sure the seal is tight and no liquid will be able to

[i] Smaller numbers of wells allow for greater volumes to be loaded.

escape. Add gel comb into slots (make sure to use the one on the end, not the middle).

7. Places box(es) in the chemical fume hood. Ensure that boxes are not slanted.
8. Take flask(s) out of the microwave and into chemical fume hood. Add 1 drop of ETBR solution to each flask. Swirl to mix. Pour liquid into gel tray.
9. Rinse out flasks with water and hang on pegs above sink in back room.
10. Wait for gel(s) to set. ~30 minutes.[ii]
11. Once gel has set, remove comb by pulling straight up. Rinse comb with water.
12. Reorient gel tray so that wells are aligned vertically. Ensure that wells are correctly oriented. If you are facing the gel box head on and the electrodes are on the right side of the box, then the wells should be on the far side of the gel.
13. Add 1x TAE until top of gel is covered.
14. Prepare each sample by mixing DNA with gel loading dye and NFW.
    - Since usually 1µL of PCR product is run, we have a 1.2x gel loading dye which allows for 1µL of PCR product to be mixed with 5µL of dye. Steps 15-17 assume that this is the case.
15. To prepare samples, cut a thin strip of Parafilm and place wax side up on bench (do not remove paper).
16. (Assuming you are loading 1µL of PCR product): pipette a 5µl dot of 1.2x dye onto the Parafilm for each PCR product you intend to run. Then add 1µL of PCR product to each dot.
17. Set your pipette to 6µL and pipette up and load each dot. Don't forget that the first lane will have ladder.
    - Gels should be loaded from left to right with the wells away from you (towards the far end of the gel box).
    - Remember that the first and last lanes should have ladder loaded in them.
    - To load a gel, place pipette tip in liquid <u>above or just barely in</u> the well to be loaded and pipette down slowly to the first stop. The DNA loading dye mixture is heavier than solution and will sink into the well. Do not stab the gel.
    - Note that it is normal for the dots to be less than 6µL volume.
18. Load the first lane and the lane adjacent to the last sample with 6µL of ladder.
    - If you are only running a few samples (e.g. 3), it is ok to omit the second ladder lane.
19. Take gel box into back room and slide cover onto it. Make sure electrodes are connected well to wires. Plug in electrodes to current box.

[ii] If you are in a rush, consider pre-freezing the gel box, or hardening gel in freezer.

- Remember that DNA will run to red (move towards positive charge).

20. Choose the appropriate voltage, amperage and run time for your gel. In general we run at constant 160 volts, (250 mA) for 30 minutes. This can be selected by choosing program and then method 1 on the current box.
    - Longer run times and higher voltages lead to more separation. However, higher voltage/current leads to greater heat. It is not recommended to go higher than the voltage/current settings above.
    - Remember that smaller bands may run off the gel if run for too long.
21. After gel has finished running, shut off current box, slide off cover and bring gel tray to gel imager.
22. Place gel with wells facing away from you on gel imager image gel. Save image to a flash drive and store on Benchling.
    - Auto exposure usually works fine, but exposure times can be played around with for better images. Longer exposure means DNA is brighter, but also increases background brightness. Sometimes, after image is taken, shifting contrast can allow faint bands to be seen.[iii]
    - Images on Benchling should be annotated with information on the contents of each well as well as the interpretation of each well (i.e. whether the band is the right size or not).
23. Remove gel and clean gel imager with DI water.
24. Throw away gel in gel bucket in back room. Dump TAE down the drain.[iv] Rinse gel box with water to prevent electrode deterioration. Hang gel box on pegs to dry.

[iii] Contrast shifting in the opposite direction (eliminating bands) is a common method of academic fraud.
[iv] Technically TAE can be used twice. However, TAE will corrode the gel box electrodes over time. As such, TAE should not be left in for long periods of time (i.e. overnight).