

Protocol: Agarose Gel Electrophoresis using Bio-Rad mini sub cell

Preparation of a 1% agarose gel

1. Rinse and dry the gel casting tray (with 95% ethanol if available).
2. Tape the ends of the casting tray as demonstrated. Set the casting tray on a level surface; you may want to put a paper towel underneath in case it leaks. NEVER pour the gel with the casting tray *in* the electrophoresis chamber/box.
3. Adjust the level of the comb so it rests evenly with a few mm of space between teeth and the tray; this will allow wells to form in the agarose.
4. The 1% agarose has already been prepared (1 g agarose powder per 100 mL 1x TBE), and melted; it is stored in 68°C water bath next door. Gently swirl, make sure there are no particles. Using a 50 mL beaker, measure out about 30 mL. Return agarose stock to water bath.
5. Pour the 25-30 mL agarose into the casting tray.
6. Allow the gel to solidify (about 10 minutes); then remove the comb and tape.

Loading and running the gel

1. Add loading dye to each sample.
 - All loading dyes have a dense or viscous substance (like 40% sucrose, or glycerol) that gives your DNA sample weight so it sinks down into the well
 - Loading dyes also contain tracking dyes that visibly migrate, allowing you to monitor progress. In 1% agarose, bromophenol blue (which looks purple) moves like 300 bp dsDNA; xylene cyanol (which looks sky blue), migrates about as fast as 4 kb (4000 base pairs) DNAs.
2. Briefly centrifuge all samples to pool the contents.
3. Insert the casting tray (with the gel on it) into the electrophoresis chamber with the wells closest to the negative (black) electrode. DNA is negatively charged. During electrophoresis, it will migrate from the negative to the positive electrode.
4. Gradually add 1xTBE (Tris-Borate-EDTA; electrophoresis buffer) to the chamber until the buffer just covers the top of the gel.
5. Load samples, taking care not to puncture the well bottoms. Do not attempt to load a sample if there is an air bubble in your pipet tip. Also, if a well has an air bubble in it, push it out using a clean tip before loading a sample in it.
6. Attach lid, make sure cords are correctly plugged into the power supply (red to red, black to black)
7. Plug in the power supply.
8. Turn the power on and adjust the voltage control knob to 120 volts. You should not need to touch any other knob.

9. Electrophoresis (run) until the bromophenol blue has migrated to within $\frac{3}{4}$ of the positive electrode end of the gel.
10. Shut off the power supply, unplug the leads, unplug the power supply.
11. Lift the gel casting tray from the chamber.

Staining & photographing the gel (next door)

1. Wear gloves & lab coat.

2. Remove the gel from the casting tray and place it in a gently shaking solution of ethidium bromide. Use one of the small plastic boxes (lids from tip boxes). ****Ethidium bromide is a mutagen.****
3. Allow to stain for 5 minutes.
4. Carefully decant the staining solution back into the ethidium bromide stock bottle.
5. Destain your gel (remove EtBr that is in the gel but not bound to DNA) by soaking in distilled water. Do this once or twice, 3-5 minutes each time. Decant the water appropriately.
6. EtBr-stained DNA bands will light up under UV illumination. **Never observe EtBr fluorescence with unprotected eyes; short wave UV is damaging.** We have a computerized system for visualizing and photographing gels; you should not need to look at your gel directly. If you do, be sure to wear proper UV eye protection.
7. When finished, dispose of your gel in the pile of EtBr-contaminated gels (NOT in the trash). Clean the surface of the UV lamp with a paper towel.
8. *Never bring an ethidium bromide contaminated item back to your work space.*