Langerhans Lab Protocols

NC STATE UNIVERSITY

Agarose Gel Electrophoresis Protocol
(Making, Loading, Running, & Viewing)

CAUTION: Wear gloves, because SYBR Green binds to nucleic acid, which your cells have a lot of!

Agarose gels and TAE buffer can be re-used and gels can even be melted and re-cast. Wear gloves throughout, because of traces of SYBR Green in re-used gel and buffer. When excising bands for sequencing, use a new gel and new buffer. When gels need to be discarded, put them into the receptacle (bag) in the hood with Hazardous Waste label.

Making an Agarose Gel

1. Place gel tray with orange tubing touching the sides of the gel rig; it should be snug. Wet the orange gasket with TAE buffer to make it slide in easier.
2. Add the desired amount of gel combs.
3. Add 60 ml of 1x TAE Buffer to a 250 ml Erlenmeyer flask.
4. Measure out 0.6 grams of agarose on the scale and pour into flask
    a. Scale directions:
    b. Turn on scale
    c. Put measuring paper or weigh boat on scale
    d. Press the "Tare" button and make sure it reads 0.00
    e. Measure agarose, using spatula to dispense.
5. Twist and scrunch a piece of paper towel loosely into the top of the flask.
6. Place the flask in the microwave and run it for two minutes.
7. Using big white insulating gloves, remove the flask from the microwave and carefully remove the paper towel; carefully swish the liquid to mix and cool.
8. After a few swishes, hold up to the light and check for unmelted particles of agarose.
    a. If there are agarose bits, swirl the flask for a minute and re-check. If there are stubborn agarose bits that won't dissolve, return the paper towel to the flask and microwave it in increments of 15 sec until the agarose has melted.
9. Cool the agarose in the flask by setting it on the counter for about 5 minutes, swirling it gently and moving it to a new spot several times. If you need the agarose to cool more quickly, run the bottom of the flask under cold water for ~1-2 min while swishing the flask gently.
    a. You want the flask to be at a temperature where you can hold the flask with a gloved hand comfortably. If it gets too cool, the agarose will thicken and not pour into the mold smoothly; re-heat it in the microwave.
10. Pour the agarose into the gel tray, with combs in place. Remove any air bubbles.
11. Gel should be ready in about 10-15 min (cloudy and firm).

Running the Gel

1. After the gel has hardened, remove the gel tray, turn it 90 degrees, and replace it in the rig.
   Make sure the gel is set so that the samples will "run to red."
2. Pour in enough 1x TAE buffer to cover the gel (about 250 ml), and remove the gel combs carefully.
3. Aliquot 1.5 µl dots of [SYBR green + loading dye] (see below for recipe) onto strip of parafilm, one dot per sample + one for ladder.
4. Mix by pipette 5 µl of sample with one SYBR + dye dot and add to the well in the gel; change tip between samples. Load 5 µl of ladder and SYBR + dye in one well (center well is recommended).
   - The ladder already has loading dye, but it doesn't have SYBR green so won't fluoresce without it.
   - *note*: 3 or 4 µl of sample can be loaded, but if 5 µl are used, then concentration of PCR product can be estimated by comparing band intensity to that of 5 µl ladder band. Also, with 5 µl sample, the final conc. of loading dye is 1x.
5. Put lid on gel rig, matching red and black posts. Plug in red and black plugs to corresponding spots on the power supply.
   Rig is working if curtain of bubbles rises from each electrode wire in end trays.
6. Run at 95V-102V for about 25 min (20 – 40 min ok) Time depends upon size of fragments that need to be resolved; larger fragment = longer run time to separate.
   Some faint bands that are visible after 20 min may disappear after 30 min. This PCR product is still good enough to sequence (without diluting).

Viewing the Gel

1. Turn off power supply, remove rig lid, and lift gel out of frame (wear gloves!!).
2. Lift clear lid on FOTO Phoresis light box, then lay gel on the box.
3. To view:
   a. Close clear lid
   b. Put magnet against lid on the lower right corner.
   c. Turn on power. If the magnet is in the right spot, the red light on the front will light up and the UV light will be on.
   d. Look at the gel to find bands (or not).
4. To photograph:
   a. Remove magnet so UV light is off.
   b. Leaving clear lid open, place camera cone over gel.
   c. Turn on camera.
   d. Place your left hand against camera where it joins the cone, to block more light.
   e. Press camera Macro button (looks like a tulip), then take a photo. Turn off UV light as soon as photo has been captured.
   f. Connect camera to the computer using the gray cable to view gel image.
5. Turn off light box and camera when done.
6. If the gel and/or TAE buffer can be re-used, place them in the small bucket near the gel rig.

preparing SYBR Green + loading dye

Write date and concentration on all tubes.

SYBR Green 1: Shipped at 10,000x, stored in -20C freezer.

First dilution step: Mix 2 µl of 10,000x stock + 198 µl TAE buffer to make 100x stock. Store this at -20C.

To make working conc.: Mix 50 µl of 100x stock + 100 µl of 6x loading dye (Promega). Use this to mix with samples to load in gel. Store at -20C if not using regularly; if using often, store at 4C.

note: this gives a final conc. of 7x SYBR Green and 0.9x loading dye; working concentration could be played with to get 1x for each.

Loading dye: comes at 6x, ready to mix with SYBR Green as above.

making 1x TAE buffer

Purchase 10x TAE solution from Fisher (item # BP1335-1). Bottle is stored in cabinet next to PCR station in DCL 382.

to dilute 10x to 1x:

1. determine amount of 1x buffer needed, then calculate mixture: $C_1V_1=C_2V_2$
   ex.: to make 4 liters of 1x solution from 10x solution, mix 400 mL of 10x solution with 3600 mL water
2. Water: use miliQ H_2O, ddH_2O, or dIH_2O. Departmental miliQ water filter is in DCL 387.
3. Mix miliQ water + 10x solution in carboy beside sink in DCL 382.

see Dunn lab web site for help:
http://www.antmacroecology.org/~ambrosiasymbiosis/wiki/index.php/Main_Page