Gel Electrophoresis Protocol

This protocol should be used for verifying the quality (and sometimes concentration) of DNA extractions or PCR products.

Gel Preparation

1. 45X stock TAE buffer needs to be diluted to 1X with clean ddH2O. If the stock is not available, you can make 10X stock using this recipe (Tris= 10.8g, EDTA= 0.8g, Boric Acid= 5.6g, 100ml dH20, pH 8.0).
2. Combine 1.3 grams of agarose per 100ml of 1X TAE buffer in a narrow mouthed beaker, with ample space remaining in the beaker.
3. Microwave approximately 1 minute (do not let boil severely for more than 10 seconds, or it can overflow). Swirl in hand to check for complete dissolution of agarose.
4. Add a small amount of ethidium bromide (on the order of 1ul per 100ml of gel solution). Mix by swirling in hand.
5. Set up gel block and gel tray. Place comb(s) into gel tray. Pour gel into tray immediately. Allow gel to set in tray for 10-20 minutes.
6. When hardened completely, remove combs and orient gel tray with the wells closest to the negatively charged end.
7. Pour 1X TAE *running* buffer over gel, so that it is completely submerged.

Running samples

1. Find premixed 100bp ladder-6X loading buffer. Load 1.0ul in one lane of each comb.
2. Spot 1.5ul of 6X loading buffer on Parafilm, 1 spot per sample. Add the following amount of sample to each spot:
    A. If examining DNA extractions, use 1-2.0ul of extract.
    B. If examining PCR product, use 5ul of PCR amplification.
3. Load dye+sample mixture in gel. If well overflow occurs when loading, skip wells adjacent to overflow well. Note any leakage in notes.
4. Attach gel cover, so that the negative end is nearest to the wells. Attach electrodes to charge regulator. Double check to make sure that negative (black) is with negative, and positive (red) is with positive. The DNA will flow away from the negative charged end and towards the positively charged end. Thus, the gel should be oriented lengthwise away from the negative electrode.
5. Run approximately 25-30 minutes at 95-110 volts.

Checking results

1. Turn on computer and launch UVP gel imaging software.
2. When run is competed, turn off voltage regulator. Remove gel and gel tray, and place in UV detector. Position gel in center of detector.
3. Turn on exposure button, and acquire image using the Preview button.
4. Modify zooming, focus (using manual camera lens), and positioning of gel image (by moving it physically) until it is clearly positioned.

5. Choose and set an appropriate exposure duration (start with 2 seconds), then click on Capture.
6. Typically, adjust time until the individual fragments in the ladder are clearly visible. Over exposure will blur them together, underexposure will leave them faint and indistinct. Adjust exposure time if necessary to pick up image of faint bands.
7. Save photo into your user folder. You can email or print the image if you like.

Clean-up

1. Place gel into disposal box. Throw any heavily contaminated trash in the same box. Other trash goes in the regular trash bin..
2. Save 1X buffer from run by pouring into beaker with funnel. You can recycle the running buffer many, many times.
3. Rinse gel box, tray, comb, etc. and set aside to dry. Recycle gloves if doing many gels over consecutive days, otherwise dispose of gloves in uncontaminated waste bin unless heavily contaminated.

Expected Results

A. For DNA extractions, you should see a nice band or smear of DNA highlighted from the top of the well to around 500bp. If the smear is very faint (or only bright close to the well itself), you DNA extraction is very dilute and you will need to use a lot during PCR.
B. For PCR products, you should see a single band, and sized relative to the ladder according to the expected size of your gene fragment. Occasionally, you will see primer-dimers at 100bp, or other bands. This may mean that the conditions of your PCR have not been calibrated properly.

Note on Measuring Concentration

Typically, a gel image provides a relative measure of the concentration of PCR products or DNA extractions. If the concentration of the ladder is known (i.e. how much of fragment 600bp, vs. fragment 500bp, is in *x* ul of ladder+loading dye), then the brightness of your sample band (diluted with the same volume of loading dye) can be used as an estimate of sample concentration.

In Case of EtBr Spill

1. Prepare a decontamination solution: of 4.2 g of sodium nitrite (NaNO2) and 20 ml of hypophosphorous acid (50 percent) (H3PO2) in 300 ml of water.
2. Wash the area with a paper towel soaked in decontamination solution. Rinse the area five times with paper towels soaked in tap water, using a fresh towel each time. If the acid could damage the contaminated surface, use a few additional rinses with paper towels soaked in tap water.
3. Soak all the towels in decontamination solution for one hour. Then remove them, gently wring out excess solution back into the decontaminant container, and place in hazardous waste bin along with contaminated gloves.
4. Using chemical names, clearly label the container of decontamination solution (e.g., “Laboratory debris contaminated with ethidium bromide”), and contact EH&S and lab safety manager.