Electrophoresis of PCR Products – Instructor's Notes

Below are equipment, supplies, and prep instructions for colony-based PCR for a lab class of 24 students, assuming 6 lab groups of 4 students. We assume that each individual student conducts a colony-based PCR and each lab group conducts a negative and a positive control. In total, 36 PCR reactions will be done.

Equipment

- Gel boxes (1 per group) – assuming 8 lanes
- Electrophoresis power supplies (1 per two groups)
- P20 micropipeters (at least 1 per group)
- P200 micropipeters (at least 1 per group)
- Racks for microfuge tubes (1 per group)
- UV box for visualizing band on gel
- Water bath

Supplies

- Sterile P20 tips (1 box per group)
- Sterile P200 tips (1 box per group)
- GelRed (10,000x in water) Biotium #41003 (Fisher NC9594719 and many other vendors)
- 1% Agarose (recipe below)
    - 1x TAE (recipe below)
    - agarose
- DNA ladder (New England Biolabs 100 BP Quick ladder)
- Sample loading dye (if needed)
- Molecular grade water
- 1.5 mL sterile tubes
- 1X TAE buffer for gels (recipe below)
    - Tris
    - Disodium EDTA
    - Glacial Acetic Acid

Prepare tips and microfuge tubes

At least one day prior to lab, autoclave P20 tips, P200 tips, and 1.5 mL microfuge tubes on a standard dry cycle.

Prepare 50x TAE Buffer

1x TAE buffer is used in making 1% agarose and for running the gel. It is easier to make a 50x stock solution and then dilute it.

1. Add the 242g Tris and 18.61g disodium EDTA to approximately 700 ml of DI water, and stir until dissolved.
2. Add 57.1 ml glacial acetic acid.

3. Adjust the volume to 1 liter with DI water.

Prepare 1x TAE Buffer

For 6 lab groups, you will need at least 300mL of buffer for preparing the agarose and 3L of buffer for filling the gel boxes to run the gels.

1. For 1L 1x TAE, combine 20mL of 50x TAE buffer with 980mL of DI water.

Prepare 1% agarose

You will need 50mL of agarose for each gel. For 6 lab groups, you will need 300mL of agarose, but can prepare extra.

1. For 100mL of 1% agarose, add 1g of agarose to 100mL of 1x TAE Buffer in a 250mL flask.
2. Microwave until the solution is clear.
3. Pour 50mL aliquots into 125mL flasks. Cover flasks with foil. Place in 52-55C water bath with ring weight.
4. For 6 lab groups, you will need to repeat this procedure 3 times. Alternatively, you can prepare a larger volume using a hot plate and a stir bar rather than a microwave.
5. Just prior to pouring the gels, add 5uL of GelRed per gel flask (per 50mL volume).

Loading gels and running gels

1. The mastermix may already have a loading dye, so no additional dye is needed. However, if the PCR product is clear, add a small volume (1-2uL) of loading dye to each sample.
2. Load 7uL of DNA ladder to an outside lane.
3. 5uL of PCR product to each lane, remembering to record which PCR product was added to which lane.
4. Run the gel at ~135v until dye front is more than halfway through the gel. The negative electrode should be at the well end of the gel. DNA moves in the gel toward the positive electrode.
5. Visualize the gel using the UV box. Bands for successful PCR products should align close to the top of the DNA ladder.

Prepare PCR products for Sanger sequencing

1. For each successful PCR reactions, students should provide at least 10µL of reserved PCR product in a tube to be sent off for sequencing. Currently, we use GeneWiz for Sanger sequencing and they require that samples be sent in PCR tubes strips. Clearly mark the side of each tube with the sample name.

2. Prepare a spreadsheet indicating which sample went into each sequencing tube and email to Emory.
3. Tubes with PCR products should be mailed to GeneWiz along with a print out of the order that will be emailed to you in a padded envelope (room temperature), along with a completed sample spreadsheet. Ice is not necessary.
4. Results will be emailed to you in a few days.

<u>Important reminders for students</u>

- Which samples are loaded into which lanes of the gel should be recorded.
- If the negative control indicates a successful PCR, all samples should be discarded and the PCR should be repeated with new colonies.
- Unsuccessful PCRs should be repeated with new colonies.
- For each PCR reaction, students should record the following data:
    - Beetle ID #
    - Beetle life cycle stage (egg, larva, pupa, adult)
    - Beetle sex (if adult)
    - Beetle host bean type
    - Experimental Treatment Group
    - Media (nutrient agar, EMB, PEA, blood, other)
    - Colony phenotype (color, gloss, form, elevation)
    - Tube number on tube that will be sent to sequencing center