Colony-based PCR – 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

- PCR machine
- Microcentrifuge (1 per group, if possible)
- P20 micropipeters (at least 1 per group)
- P200 micropipeters (at least 1 per group)
- Racks for microfuge tubes (1 per group)
- Empty tip box to hold PCR tubes (1 per group)
- Bunsen burner (1 per group)
- Fine tip sharpies to mark tubes (1 per group)

Supplies

- Mastermix (New England Biolabs OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer)
- PCR Primers (Eurofins or IDT)
    - forward primer – 27F (5' – AGA GTT TGA TCC TGG CTC AG)
    - reverse primer – 1492R (5' – GGT TAC CTT GTT ACG ACT T)
- Molecular grade water (split into 5mL aliquots per group)
- Diluted E coli as positive control
- Sterile P20 tips (1 box per group)
- Sterile P200 tips (1 box per group)
- PCR tubes (1 strip of 8 tubes per group)
- 1.5mL sterile microfuge tubes (4 per group)
- Plates with bacterial colonies (these plates should be kept at 4C until at least the following week in case students need to repeat their PCR)

Prepare tips and microfuge tubes

At least one day prior to lab, autoclave P20 tips, P200 tips, and 1.5mL microfuge tubes on a standard dry cycle to insure that they are not cross-contaminated with bacteria that will be amplified during the PCR.

Prepare PCR Primers

1. Spin down tube that primers were delivered in to insure that the pellet is at the bottom.
2. Reconstitute primers, in molecular grade sterile water, to 100 uM in the original tube. To determine the amount of TE buffer to add, multiply the number of nmol of primer (indicated on the tube) by 10 and add that number of ul of TE buffer. Vortex to mix. Let sit 10 minutes at room temperature, and then vortex again before proceeding to the next step.
3. Further dilute primers to 10 uM with molecular-grade water by pipetting 10ul of reconstituted primer into a microfuge tube along with 90ul of molecular-grade water.
4. Freeze primers at -20C until needed.

Prepare Mastermix and Primers

1. For a single, 25ul PCR reaction, 12.5ul of mastermix should be mixed with 0.5ul of 10uM forward primer and 0.5ul of 10uM reverse primer. For a class of 24 students (6 groups), 36 PCR reactions will be done (6 per group). Groups should be given excess mastermix/primer solution. We recommend enough for 7 reactions for each group. You can prepare enough solution for 50 reactions and then aliquot it into microfuge tubes for each group.
2. For mastermix/primer solution for 50 reactions, add 625ul of mastermix, 25ul of 10uM forward primer, and 25ul of 10uM reverse primer into a sterile 1.5mL microfuge tube. Vortex to mix.
3. Aliquot 95ul of the mastermix/primer solution into sterile 1.5mL microfuge tubes for each group. There will be extra in the original tube.
4. Freeze solution at -20C until needed.

PCR program

95C, 10 min (purpose is to help disrupt bacterial cell walls/membranes to release DNA)

Then 36 cycles of:

95C, 30 sec

55C, 30 sec

72C, 1.5 min

Lastly,

72C, 4 min,

4C, hold.

PCR products can be held at 4C until the following week for electrophoresis.

Important reminders for students

- All microfuge and PCR tubes should be labeled with sample information
- When conducting the PCR, they only need to touch the colony with a pipet tip. More is NOT better.
- 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 species
    - 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