Purification of PCR reactions for sequencing using Ethanol/ Sodium Acetate Precipitation

For 50 µl PCR reaction

1. If Mineral Oil is used, remove the PCR product (~ 45 µl) from the original PCR plate to a new PCR plate.
2. Add 5 µl 3 M sodium acetate to each well.
3. Add 125 µl of 100% ethanol (stored at –20 C) to each well.
4. Seal the plate with sealing tape (Costar 6524) and mix by inverting 4 times.
5. Incubate at –20 C for 20 min.
6. Spin the plate at 2,500 x g and 4 C for 30 min (van der Knaap lab)
7. Invert the plate to remove ETOH; then, spin up to 185 x g for <1 min to remove excess ETOH.
8. Add 175 µl of 70% ethanol (stored at –20 C) to each well.
9. Spin at 2,500 x g and 4 C for 15 min.
10. Invert the plate to remove ETOH; then, spin up to 185 x g for <1 min to remove excess ETOH.
11. Dry the pellet for a few minutes in air, and re-suspend the samples in 20 µl ddH_2O.

Sample preparation and submission to MCIC

1. Prepare primer dilution. The concentration of primer working solution is 10 mM. Dilute PCR primers as follows: 10 ul primer to 15 ul ddH2O, which results in a concentration of 4 mM.
2. Sequencing reactions will be performed in duplicate, using F and R primers in order to correct for sequencing error.
3. Add 5 ul of DNA template (PCR amplification products prepared by TA and instructor) to each well, then add 1 ul of the appropriate primer (4 mM dilution). Note: samples need to be carefully tracked so that, for example, PCR amplification product generated from individual QCR 101 using CosOH44 is prepared mixed with the appropriate F and R primer (CosOH44F and CosOH44R).
4. Naming of samples will follow an established pattern: "population_individual_primer". For example, QCR101OH44F
5. Cover the plate with sealing tape.
6. Spin the plate briefly using the TJ centrifuge.
7. Send the plate to MCIC.
8. Fill the sequencing request form and send to mcic_seq@osu.edu. Print the form as your reference to paste into lab notebooks.