Cycle Sequencing Reaction Lab

Determining amount of clean PCR product for cycle sequencing reaction (lab instructor)

Remember the purposes of the electrophoretic assay of the PCR clean up: determine success of PCR clean up and *determine amount of clean PCR product to be used in cycle sequencing reaction*.

Determining the amount of clean PCR product to be used begins with estimating the concentration of DNA in the clean PCR product. Once the PCR clean up gel has been run and photographed, the DNA concentration can be estimated by comparing the intensity of the clean-PCR-product band with the intensity of the pGEM (the standard) band on the gel.

Generally, if the intensity of the clean-PCR-product band is equal to that of the pGEM, then 2.0 μl clean PCR product can be used for a good cycle sequencing reaction. However,

- if the band for the clean PCR product is stronger in intensity than the band for the pGEM, then 1.5 μl will be needed for the cycle sequencing reaction
- if clean PCR band is weaker than pGEM band, then > 2.5 μl

The amount of clean PCR product in a cycle sequencing reaction is *very important*. Too much or too little will screw it up.

If there are still primers in the product (bands below clean-PCR-product band), then it is unsuitable for cycle sequencing.

Cycle Sequencing Reaction

1. Label the collar of a strip-tube with your number. Label the tab on end of strip-lids with lab instructor's initials.
2. Add ***x* μl ddH$_2$O** to a strip-tube ($x + y$ = 17ul). Total reaction volume should be 20 μl; be sure yours adds up to 20!
3. Add **1 μl primer (ND4)**.
4. Add ***y* μl clean PCR product**.
5. Add **2 μl Quick Start DTCS Mix** (cycle sequencing mix from Beckman Coulter Inc.). Your lab instructor will distribute the Quick Start Mix.
6. Put lids on tight. Strip-tubes go in strip-tube rack. Store cycle sequencing reactions at 4°C (fridge) until ready for thermal cycling.
7. Thermal cycling is conducted (lab instructor) with the GeneAmp® PCR System 9700. The cycle sequencing profile consists of 40 cycles, each of which consists of 20 seconds at 96°C for denaturation, 20 seconds at 50°C for primer annealing, and 4 minutes at 60°C for primer extension.
8. Store cycle sequencing reactions, after thermal cycling, at 4°C until cycle sequencing reaction clean up (this is your next lab).
9. Return Quick Start Mix, primer (ND4), and ddH$_2$O to freezer after each class.