PCR Clean Up Lab

Purpose: to prepare PCR product for cycle sequencing reaction by washing PCR product of materials (salts, dNTPs, primers, and polymerase), which will inhibit the cycle sequencing reaction. We will use the Wizard SV Gel and OCR Clean-up System from Promega.

Main steps:

- bind PCR product to membrane - steps 3-6
- wash - steps 7-9
- elute - steps 10-12

PCR Clean Up Protocol

Gloves should always be worn. Chemicals in use!

1. Label the lid of a sterile 1.5 ml tube (in autoclaved beaker) with number and "clean PCR". Label the side of this tube with lab instructor's initials. The clean PCR product will be stored in this tube.
2. Label the side of a spin column with number only. Label the side of a 2 ml collection tube with number only. Place the spin column in the collection tube. Neither have lids.
3. Add 40ul of **Membrane Binding Solution** to the tube with your PCR reaction.
4. Transfer, using a pipettor, the entire contents of your PCR tube from step #3 to the minicolumn.
5. Incubate at room temperature for at least 1 minute.
6. Centrifuge at 13200 rpm for 1 minute. Discard the flow-through and re-insert the minicolumn into the collection tube.
7. Add 700 uL **Membrane Wash Solution**, and centrifuge at 16000 rpm for 1 minute. Discard the flow-through and re-insert the minicolumn into the collection tube.
8. Add 500 uL **Membrane Wash Solution**, and centrifuge at 16000 rpm for 5 minutes. Discard the flow-through and re-insert the minicolumn into the collection tube.
9. Centrifuge at 16000 rpm for 1 minute to allow evaporation of any residual ethanol.
10. Carefully transfer the minicolumn to a clean, labeled 1.5 mL tube (Number, letter, "clean", date, and IA's initials on the side).
11. Add 50uL **nuclease-free water** to the column and incubate at room temperature for 1 minute.
12. Centrifuge at 16000 rpm for 1 minute. Discard the minicolumn and store the 1.5 mL tube with the purified PCR product in the refrigerator.

Success of PCR clean up is assayed by gel electrophoresis using a 2% agarose gel. After staining, bands of DNA are visualized to determine success and to determine the amount of clean PCR product to be used in the cycle sequencing reaction. The intensity of the DNA band is compared to the intensity of a standard band to assess the amount to be used in a cycle sequencing reaction.