Cleaning Up the PCR Product

ExoSAP-IT is a product developed by the USB Corporation for cleaning up the PCR DNA product. It essentially makes use of two hydrolytic enzymes (Exonuclease I and Shrimp Alkaline Phosphatase) that respectively digest or deactivate all the unconsumed primers and dNTPs remaining in the PCR reaction tube that may interfere with the sequencing process. These enzyme are active at 37°C but get quickly deactivated at 80°C. After ExoSAP-IT treatment, the PCR product is pure enough for any sequencing or digestion work.

Laboratory Supplies

Eppendorf tubes (0.5 and 0.2 ml capacities)	1/sample
ExoSAP-IT	2 μl/sample
Microfuge	2/lab
Adapters for small Eppendorfs	8/lab
PCR machine	1/lab
PCRID1 primer, 1.1 μM	3 μl/sample
PCRID2 primer, 1.1 μM	3 μl/sample
UPSF forms	1/section

Procedures

1. Take the tubes out of the freezer, thaw and microfuge for 1 minute.

2. Transfer 5 μl from the top of the supernatant into a fresh 0.5 ml Eppendorf and label this new tube. Make sure no cell debris is transferred.

3. Add 2 μl of ExoSAP-IT to the tube, tap the tube with your fingers to mix, pulse 3 seconds in a microfuge and place back into the PCR machine. Start the ExoSAP? program. This program is just one cycle of 15 minutes at 37°C and 15 minutes at 80°C and a hold at 4°C. This should take about 30 minutes to finish.

4. Add 3 μl of EITHER 1.1 μM PCRID1 OR PCRID2 (NOT BOTH) to the tube. Tap gently to mix, microfuge 3 seconds, label clearly and give to your instructor to be sent to the UPSF.

   Note: Contrary to PCR where 2 primers are needed, material for sequencing require only one primer. Why?

5. Within a few days, the results would be back from UPSF and will be posted as individual files on the course BlackBoard Website. You need to download the file which has the DNA sequence of your sample and use BLAST to determine the name of your unknown species as described next.

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