PEG Precipitation of PCR products

Travis Glenn: Travis.Glenn@sc.edu

We use the following solutions & protocol to clean up our PCR products prior to cycle sequencing. In general, you will need about 10ng of purified PCR product per 100 bp of length (e.g., for a 500 bp PCR product, you need about 50 ng of template for sequencing). The purpose of this protocol is to remove unused primers and dNTP's from the PCR. There are many alternative protocols (e.g., Qiagen columns, Microcon filters, etc.). This is the cheapest and most reliable in my hands. Note: If your PCR works really well (i.e., 50+ ng of product per µL of PCR & no extra bands or primer dimers) then you can often simply use 0.5 to 1.0 µL of your PCR reaction as the template (I also double the sequencing primer concentration, though I doubt it makes any difference).

Stock Solutions:

1. 20% PEG, 2.5 M NaCl - Note: PEG takes 20+ min to go into solution when you make the initial solution. For 50mL, mix the following in a 50 mL conical:
10.0 g PolyEthylene Glycol 8000 (MW = 6000 - 8000 is fine)
7.3 g NaCl
dH2O up to 45 mL - shake & let PEG go into solution; I put it into a rocking incubator set to 37°C after everything is in solution, fill with dH2O up to 50 mL.
2. 80% EtOH in a 50mL conical or something similar.
3. TLE = 10 mM Tris, 0.1 mM EDTA

This protocol assumes a 50 µL PCR reaction and use of 0.2 mL thin walled tubes. If you have a different volume of PCR, then scale everything using the following proportions. If you use 0.5 mL tubes for PCR, then you don't need to use any new tubes.

A) Run out 5 µL of PCR onto an agarose gel to ensure the PCR reaction worked.

B) Add 50 µL of PEG to a 0.5 mL tube. Transfer the remainder of the PCR to the tube with PEG and mix by pipetting up & down.

C) Let the the PCR + PEG incubate at 37°C for 15 min.

D) Place the 80% EtOH on ice.

E) Centrifuge PCR + PEG at high speed (~15,000 x g) for 15 min. (at room temp.).

F) Using a P200 pipetter, pull off the supernatant & discard it.

G) Add 125 µL of cold 80% EtOH to the tube. If you shoot the EtOH into the bottom of the tube, you must spin for two minutes. If you place the EtOH onto the side of the tube, you can just let the tube sit for one minute. Using a P200 pipetter, pull off the supernatant & discard it.

H) Repeat step G.

I) Dry off the EtOH in a centrivap for 5-10 min, low heat, no vacuum. There should be no trace (visible or by smell) of EtOH when done.

J) Dissolve the PCR product in 25 µL of TLE or gdw. Pipette up & down several times to ensure the DNA has gone into solution. If you can let it sit for several minutes, that is also helpful.

K) Run out 2-4 µL onto an agarose gel for 10 min. to roughly quantify recovery. Use 20 and 100 ng of uncut lambda or plasmid DNA as a standard.