Combination PEG precipitation and gel purification of PCR products for LiCor Sequencing

This protocol is designed to give you a large quantity of purified PCR generated DNA for sequencing. The protocol can be used for both LiCor sequencing (which labels the DNA at the primer) and ABI BigDye sequencing (which tags each ddNTP with its own tag). It is estimated to save about an hour and a half from the standard gel purification while concentrating your whole PCR reaction in a manageable volume. One major advantage is that I have never lost a purified DNA pellet using this method, something that I cannot say of the old method of EtOH precipitation.

1) Do your PCR reaction as you would normally do. Run a few microliters out on a 2% agarose gel to ensure that the reaction worked.

2) PEG precipitate the PCR reaction using the following protocol.

PEG Precipitation of PCR products

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).

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.

3) 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 very well**.

4) Let the PCR + PEG incubate at 37°C for 15 min. Place bottle of 80% EtOH on ice to keep ice cold.

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

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

7) 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.

8) Repeat step 6.

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

10) Dissolve the PCR product in 25 μL of water or TLE. 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.

11) Run out 2-4 μL onto an agarose gel for 10 min. to roughly quantify recovery. Use DNA of a known concentration as a standard. **(Omit this step if you are going to gel purify the precipitated product)**

12) Load the entire concentrated product into a well of a 2% 1X **Modified** TAE gel with EtBr. The recipe for Modified TAE is listed below. Make up the gel like you would make a typical gel with TBE, just use the Modified TAE. Modified TAE contains 1/10 of the EDTA that normal TAE has and none of the boric acid of TBE, which inhibits PCR.

13) Run the gel as you would normally do.

14) Cut the band out of the gel using a EtOH-cleaned razor blade under long wavelength UV light. Place the gel slice in a labeled Spin-X column or equivalent. Spin at the manufacture-recommended RPM for 10 minutes. You should recover 30-50 µl of liquid containing your DNA.

15) Quantify the DNA you have by running 5 µl on a 2% gel (either TAE or TBE) using a DNA standard of known concentration. Once you know the concentration, you can use this directly in your sequencing reactions. If you need to concentrate the DNA again, just repeat the PEG precipitation step above and requantify the DNA.

16) Since there is some EtBr with the DNA, you will have to treat the DNA in a slightly different manner. The EtBr does not inhibit any of the sequencing reactions but it does make the DNA slightly photosensitive. Keep the DNA out of the light as much as possible to avoid thymine dimer formation.

Stock Solutions needed for PEG/Modified TAE protocol:
(final concentration in parentheses)

PEG Solution
10.0 g Polyethylene glycol 8000 (MW = 6000 - 8000 is fine) (20% PEG)
7.3 g NaCl (2.5 M NaCl)
ddH2O up to 45 mL - shake & let PEG go into solution. Note: PEG takes 20+ min to go into solution when you make the initial solution.
after everything is in solution, fill with ddH2O up to 50 mL.

TLE
10 mM Tris, 0.1 mM EDTA

80% EtOH

50X TAE Stock Solution
For each litre of solution:

242 g Tris Base (MW=121.1)
57.1 mL Glacial Acetic Acid
100 mL 0.5 M EDTA
mix Tris with stir bar to dissolve in about 600 mL of ddH2O.
add the EDTA and Acetic Acid.
bring final volume to 1 L with ddH2O.
store at room temperature.
Note: Final (1x) working concentration :
0.04 M Tris - Acetate
0.001 M EDTA

50X Modified TAE Stock Solution
For each litre of solution:
242 g Tris Base (MW=121.1)
57.1 mL Glacial Acetic Acid
10 mL 0.5 M EDTA
mix Tris with stir bar to dissolve in about 600 mL of ddH2O.
add the EDTA and Acetic acid, pH to 8.0 with additional Acetic acid
bring final volume to 1 L with ddH2O.
store at room temperature.
Note: Final (1x) working concentration :
0.04 M Tris - Acetate
0.0001 M EDTA **(this is 1/10 of the normal TAE EDTA concentration)**