HOMEMADE AGAROSE TUBES FOR DESALTING AND DIALYSIS

Adapted from BV's Cookbook, TCH 1/27/02

Regular Dialysis

1. Make an apparatus by pipetting 1ml of hot, 1% agarose (Sigma A-6013) into a 2 ml microfuge tube. Stick a beveled P200 tip (e.g., OPS H-1033-96RGN) into the tube, making sure the pipette tip is at the very bottom of the tube (in the little cone-shaped part).
2. After 1 hour of gelling, remove the tip. Add 50-100ul of water (bought from BRL) to the hole to keep the gel wet. Store at room temp.
3. To dialyze, remove the 50-100ul water, and add your solution (generally 25 ul or less) with a needle nosed pipette tip. You can dialyze more (e.g., 50 ul) if you use longer dialysis time periods [i.e., instead of dialyzing 1 hr, dialyze 1.5 hr to get approximately same results]).
4. After an appropriate time period (see below) remove the solution with a needle-nosed pipette tip and either use directly or add to a new agarose dialysis apparatus if you would like further purification.
5. For labeled, 32P-dCTP, the following results will be achieved:
   a) 5min dialysis: 80% dCTP removed.
   b) 10min dialysis: 88% dCTP removed.
   c) 0.5hr dialysis: 96% dCTP removed.
   d) 1.0hr dialysis: 96% dCTP removed.
   e) x 30 min dialyses: 96% dCTP removed.

   (After a 60 minute dialysis, only 12% of an oligo-labeled PCR product is removed).

Dialysis prior to electroporation

1. For electroporation, you can avoid ethanol precipitation in the following way:
2. Do the ligation reaction in an 0.5 ml polypropylene Eppendorf tube (Eppendorf catalog # 2236430-8). This makes the subsequent recovery of the PC8 extraction more efficient because of the tube's smaller size and the optimal surface properties of these tubes (among several tested), but is not absolutely necessary.
3. To 10 - 15 ul ligation reaction (containing enzyme + 5 X BRL Ligation Buffer, etc.),

add BRL water to a total volume of 30 ul (i.e., 20 ul water to a 10 ul ligation reaction).

4. Add 30 ul PCR, vortex 5 - 10 seconds, cf. 5 - 10 seconds in a little personal microfuge (don't need to use the Fisher high speed microfuges).
5. Remove 25 ul with a needle nosed pipette and place it in the agarose apparatus.
6. After 60 min at room temperature, remove the dialyzed reaction.
7. Place 15 ul in a new microfuge tube on ice, and electroporate with 10 competent E. Coli as usual.