PREPARING AND USING HOMEMADE SPIN-COLUMNS GEL PURIFICATION

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

1. To prepare column, snip off a blue, 1 ml pipet tip ~3 cm from the top and discard the bottom.
   Place the truncated tip in a 15 ml centrifuge tube. It comes up to about the 4 ml mark.
   Put a QS-GS column in the tube (Isolab, with plastic disc), on top of the tip.

2. Place 1 ml Sephacryl S-300 or Sephadex-G25 in the column.
   Centrifuge 5 minutes in bench-top centrifuge at maximum speed (=~1800 RPM = 500g).
   ~ 0.8 ml liquid is recovered in the bottom of the 15 ml centrifuge tube.
   ~ 2 cm of Sephacryl S-300 is left in the column,
   and ~1.9 cm of Sephadex G-25.

1. Remove column and put it into screw cap 1.5 ml tube in a rack, then put the column/screw cap tube back into the same 15 ml centrifuge tube (with the pipet tip still in it).
   Pipette 50 - 100 ul of DNA into the column, either against wall or directly on top of Sephacryl/Sephadex.
   For 0.5% agarose gels, place 100 - 1000 mg agarose containing DNA in the column
   Centrifuge 5 minutes as above.
   Volume recovered is about the same volume loaded.

4. For 0.5% agarose gels, place 100 - 1000 mg agarose containing DNA in the column after the same 5 minute pre-spin.
   Centrifuge 10 minutes as above.
   Volume liquid recovered is > 60% of the weight of agarose.

5. Recoveries:

   Sephacryl S-300: **> 150 bp**: ~ 50%
   22 base oligo: 0.3%
   ATP: < 0.02 %
   1 - 9 kb fragments: ~ 50%
   (from 0.5% agarose gels).

   Sephadex G-25: 1.6 kb PCR frag: ~50%

>150 bp:	~50%
22 base oligo	~40%
20 bp oligo:	38%
ATP:	~3%

Notes:

a. If you want DNA really pure, you can use two sequential columns (though the yields are obviously less (multiplicative, as expected).

b. You can use larger volumes of DNA-containing solutions on the Spin-columns, though the background recovery of ATP is higher (0.02% for < 100 ul, 0.3% for 150 ul, 2% for 200 ul, 7% for 250 ul), so there is less purification from low molecular weight contaminants.

c. You can also purify DNA from 1.2% agarose gels, but the yield is only about half that recovered from 0.5% gels for fragments < 4kb, and the yield is three to five fold less for larger fragments.

d. In 0.5% agarose gels, BPB migrates with 2.1kb marker.

e. Five minute spins are sufficient. Virtually identical results are obtained with centrifugation times of 2 - 10 minutes.

Materials

1. **Sephadex G-25, DNA grade, Pharmacia 17-0572-01.**
   5 g in 250 ml water in T-75.
   Overnight at 4°, change water twice.
   Remove sup, add ~10 ml TE (10 mM Tris, pH 8.0, 1 mM EDTA; Quality Bio.)
   Transfer to 50 ml tube.
   Top to 50 ml with TE.
   Settle, suck off TE, and add new TE to 35 ml.
   After settling, should be ~22 ml swelled gel + 12 ml TE on top.
   Store at 4°.

2. **Sephacryl S-300, Pharmacia 17-0599-10.**
   50 ml resin in T-75, add water to ~250 ml.
   Shake, sit at room temp. until settled, suck off sup.
   Add ~10 ml water, transfer to 50 ml tube.

Top to 50 ml with TE.
Settle, suck off sup, add new TE to 50 ml.
Resin occupies ~38 ml when settled overnight.
Aliquot into two tubes.
    Store at 4°.