DNA LABELING BY RANDOM PRIMING (OLIGOLABELLING)

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

Use screw-cap tubes for everything hot!

1) Prepare the fragment to be labeled either by PCR or by cutting it out of a plasmid. Either way, be sure to run a slow gel overnight to gel purify it - even the tiniest amount of vector contaminant can lead to a huge amount of background on your blot. Fast gels lead to contaminated bands.

2) Denaturing the DNA. Q.S. about 30ng of DNA to 6ul with LoTE. Add 1ul special HiTE (20 mM Tris, 0.2 mM EDTA, pH 8.5). Boil 4 minutes, place on ice to cool briefly, then quick spin.

3) Labeling Reaction. Add: 1ul 10mg/ml BSA, 4ul 5xOLB, 6ul α-^{32}P dCTP, 1ul Klenow. Incubate at RT 30-60 minutes. Add 30ul TE at the end of reaction.

4) Passing spin column. Save 1ul of the above reaction for CPM check. Pass the rest through a spin column. Take 1ul of the eluted solution for CPM check.

% incorporation = counts before column/counts after column.

5) Ethanol precipitate the rest of the probe or use Qiaquick or Spin-column method.

Ethanol precipitation provides highest yield and purify, but other methods are acceptable.

To labelling mix, add: 180ul LoTE, 3ul glycogen, 83ul 10M NH4Ac, 750ul EtOH. Vortex, spin 10 minutes, wash with ethanol as usual, using long round tips to remove last dregs of ethanol (don't Speed-vac).

6) Preanneal: Add 50ul LoTE, 100ul 2 mg/ml placental DNA, 50ul 20x SSC. Mix, boil 10 minutes, then preanneal at 68° for 10 minutes.

7) For Southern Blot, add entire probe (optimally 100 million CPM total) to 15 ml Blotto-10 with 400ul sssssDNA. Use for hyb.

Tips:

- Do everything in screw cap tubes, since regular eppendorfs frequently get leaky tops when closed hard at an angle.

- Can be modified for low-gel temperature (LGT) gels as follows (J. Pietenpol 5/11/88): Run DNA on LGT gel, and cut out the desired fragment and place in a screw-capped tube. Add 500 ul TE 20/0.2, pH 8.5, and boil 6 minutes. Store at -20°C until ready to label. Label as usual except a) boil probe 3 minutes, then place at 37 degrees for 5 minutes. Remove amount needed for oligo and place in labeled tube. b) After adding ^{32}P and enzyme, carefully pipette up and down because the gel has begun to solidify. c) During EtOH ppt., remember you're dealing with a gel pellet.