PROBE LABELING WITH THE PRIME-IT KIT

1. Mix DNA fragment to be labeled with QH2O, 10 µl of random primers supplied with kit to a total volume of 34 µl. DNA fragment can be purified in solution or can be a LMP gel fragment, that has been melted at 65C. Make sure the DNA is no more than 50 ng.

2. Heat in block to 95C for 5 min. Remove, popspin, and add 2.5-5.0 µl of 32P-dCTP (3000 Ci/mmole; 20 to 10 µCi/ul). Add 10 µl of 5X dCTP labeling mix and then add 1 µl of (exo-)klenow. Incubate at 37C for 10 min. Then stop reaction by adding Stop buffer

3. While labeling rxn is incubating, prepare spin column. Take a biorad P-30 column, remove in the following order1) its top, and 2) its base. Place over a microfuge tube and let drain. Flip out eluate, and then place column resting in microfuge tube into a Baxter snap cap bacterial culture tube and spin on desk top centrifuge for 1.5 min at ~1000 rpm.

4. Flip out eluate. Then add labeled probe to top of the column, and place it all into the baxter tube and spin ~1000 rpm for 5 min.

5. Check eluate and column with Geiger. A 50/50 distribution of heat means good labeling.