DNA/OLIGO LABELING WITH T4 POLYNUCLEOTIDE KINASE

TCH 1/27/02

1. Use 1.5 ml screw cap tubes and add the following:

~0.5 ug DNA (preferably a gel purified oligonucleotide)
2 ul 10x polynucleotide kinase buffer (NEB)
2 ul γ-^{32}P-rATP (6000 mCi/mmol, 150 uCi/ul)
1 ul T4 kinase (10 U/ul, EpiCentre, not NEB)
x ul H2O
Total: 20ul

37°C, 30-40'

2. Place tube in 68°C for 5' to terminate the reaction.

3. Add: 25 ul 10M NH4OAc
52 ul TE
3 ul See DNA
200ul 100% EtOH

Vortex to mix, let sit 5' @ RT

4. Spin at top speed for 10' and transfer supernatant to a fresh tube labeled "S".

5. Add 500 ul 70% EtOH to rinse the pellet and transfer sup. to "S" tube.

6. Spin the tube briefly and transfer the remaining liquid to "S" tube using 200 ul pipette.

7. Dissolve DNA at desired concentration and count 1/100 of both the sup. and the pellet.

*Efficiency of incorporation: 20-60%.
*Specific activity: >108 cpm/ug DNA.

NOTES:
1 ug 30-mer = 100 Pm, 1 uCi ^{32}P-rATP = 0.16pM