END LABELING DNA

1. Digest DNA in appropriate restriction buffer.

2. Add 1 μl shrimp alkaline phosphatase (SAP). Incubate either for 30' at 37°C if cut leaves 5' overhang or for 15' at 37°C and then 15' at 56°C if cut leaves 3' overhang.

3. Add 1 μl SAP and repeat incubation.

4. Add 2 μl 0.5 M EDTA. Incubate 10' at 68°C.

5. Extract twice with phenol/chloroform and then ethanol precipitate.

6. To 0.5 – 1.0 μg DNA add:

   5 μl **10 x kinase buffer**
   0.5 μl 10 mM spermidine
   25-50 μCi ^{32}PγATP
   up to 50 μl with dH_2O

7. Add 1 μl polynucleotide kinase and incubate for 5' at 37°C.

8. Add 2.5 μl 20μM ATP and incubate for a further 30' at 37°C.

9. Ethanol precipitate to remove free nucleotides.

10 x kinase buffer (1 ml) (or use manufacturer's)

500 mM Tris pH 7.5	250 μl 2 M
100 mM MgCl2	100 μl 1M
100 mM β-mercaptoethanol	6.9 μl