**End-labeling of DNA Fragment for DNase I Footprint Analysis**

1/28/94 TT

1. Prepare DNA fragment with 3'-recess end (for example, Hind III is OK. Kpn I is not) only at one end.
2.

| | |
|---|---|
| DNA fragment | 100 ng/x µl |
| $^{32}$P-α-dXTP (which can be incorporated into 3' recess end) | 2.5 µl/25 µCi |
| 5 mM 3 dNTP | 1 µl x 3 = 3 µl |
| 10 x Klenow buffer | 2.5 µl |
| $qH_2O$ | (17 - x) µl /25 µl |
| Klenow fragment | 0.5 µl |

   Incubate at RT for 25 min.
3. Add 1 µl of 5 mM 4 x dNTP. Incubate at RT 5 min.
4. Add 25 µl of TE and extract with 100 µl of P/C/I. Recover the interphase with 40 µl of TE.
5. Extract with 100 µl of C/I.
6. Pass the sample through spin column.
7. Add 10 µl of 3 M NaOAc (pH 5.2) and 300 µl of 100 % ethanol. Store at -70 °C for 30 min.
8. Spin at 12,000 rpm, 4 °C for 15 min.
9. Wash the pellet twice with 500 µl of ice-cold 70 % ethanol.
10. Dry the pellet and resuspend in 100 µl of $qH_2O$ (not in TE).
11. Take 1 µl and determine cpm. Usually, 1 x $10^4$ cpm/µl is expected. Store the labeled probe at 4 ° C.

    Do not freeze. It is recomended to use within 1 week.