MULTIPRIME DNA LABELLING

1. Follow the protocol specified by Amersham, Inc.
2. Circular DNA is linearized by restriction digestion then heated to inactivate restriction enzyme; DNA is diluted to 10 ng/µl in TE.
3. Linearized or gel-isolated fragment DNA is denatured by heating to 95-100°C for 2 minutes then quickly chill on ice.
4. Use 2.5 µl of DNA (25 ng) per reaction.
5. Add 15 µl multiprime buffer solution 1 (supplied by Amersham and containing random primers, nucleotides and buffer).
6. Add H_2O to 43 µl, then 5 µl of [^{32}P]dCTP (>2000 Ci/mmole).
7. Add 2 µl of multiprime solution 2 (containing Klenow fragment of E. coli DNA polymerase I). Mix by gently tapping tube.
8. Place at room temperature for 3-16 hours.
9. Check incorporation by TCA precipitation of 1 µl and store at -20°C.
10. Because incorporation is 90%, no separation of unincorporated dNTPs is necessary.