

PREPARATION OF MULTIMERS OF PLASMIDS AS MOLECULAR-WEIGHT MARKERS³

1. Digest the plasmid DNA to completion with a restriction enzyme that cleaves at only one site and generates protruding termini.
2. Extract the digestion mixture with phenol/chloroform and precipitate the DNA with ethanol.
3. Dissolve the DNA at a concentration of approximately 500 $\mu\text{g}/\text{ml}$ in TE (pH 7.5). Heat to 56°C for 5 minutes. Cool in ice.
4. Add 0.1 volume of 10 \times ligation buffer.

10 \times Ligation buffer

0.66 M Tris · Cl (pH 7.5)
50 mM MgCl₂
50 mM dithiothreitol
10 mM ATP

5. Add approximately 1 unit (Weiss unit) of T4 DNA ligase per microgram of DNA. Incubate for 5 minutes at 12°C (for *Eco*RI termini) or at 16°C (for termini generated by other enzymes).
6. Chill the reaction to 0°C and remove an aliquot. Heat the aliquot to 68°C for 5 minutes and analyze by electrophoresis through a 0.4% agarose minigel to judge the extent of ligation. If the desired set of size markers is obtained, purify the remainder of the DNA as described above. If the amount of ligation is insufficient, warm the sample to the appropriate temperature and continue the incubation.

Note

An alternative approach is to ligate the linear DNA to completion at a high concentration and purify the concatenated DNA by gentle extraction with phenol and chloroform. The concatenated DNA is then partially digested with the restriction enzyme used for the original digestion. This approach has the advantage that the restriction reaction is sometimes easier to control than the ligation reaction.

³B. Seed (unpubl.).