Preparation of Vectors Treated with Alkaline Phosphatase

Removal of the 5′-phosphate groups from the termini of bacteriophage λ arms can effectively prevent self-ligation and reduce the background of nonrecombinant bacteriophages. This method should be used when the vector contains a single site for cloning (e.g., λgt10, λgt11, λgt18–23, λZAP, or λORF8) or when a stuffer fragment cannot be removed by physical procedures. It is very important that the cohesive termini of the vector be reannealed and ligated before treatment with phosphatase; otherwise, concatemerization will be inhibited and the packaging efficiency of the DNA will be reduced significantly.

1. Ligate the cohesive termini of undigested vector DNA as described in the second note to step 2, page 2.85, and digest the ligated DNA with the appropriate restriction enzyme (steps 1–3, page 2.83). Test the efficiency of packaging of intact bacteriophage λ DNA (0.01 μg) and annealed/ligated DNA (0.1 μg) before and after digestion with the restriction enzyme (see page 2.107 for the packaging protocol). If digestion is complete, the packaging efficiency should be reduced by at least three orders of magnitude. Purify the digested DNA by extraction with phenol:chloroform and chloroform followed by precipitation with ethanol (see Appendix E).

2. Resuspend the DNA at a concentration of 100 μg/ml in 10 mM Tris · Cl (pH 8.3), and store an aliquot (0.5 μg) on ice. Treat the remainder of the DNA with an excess of calf intestinal alkaline phosphatase (CIP) for 1 hour at 37°C as follows:

   a. Add 0.1 volume of 10 × CIP dephosphorylation buffer and 0.01 unit of CIP for every 10 μg of bacteriophage λ DNA.

      10 × CIP dephosphorylation buffer

      10 mM $ZnCl_2$
      10 mM $MgCl_2$
      100 mM Tris · Cl (pH 8.3)

   b. Mix, and incubate for 30 minutes at 37°C. Then add a second aliquot of CIP and continue incubation for an additional 30 minutes at 37°C.

      If the arms carry blunt or recessed 5′ termini, the second half of the incubation should be carried out at 55°C.

      Approximately 0.01 unit of CIP is needed to remove the terminal phosphates from 1 pmole of 5′ termini of DNA (1 pmole of 5′ termini of a 40-kb linear DNA is 16 μg).

3. Add SDS and EDTA (pH 8.0) to final concentrations of 0.5% and 5 mM, respectively. Mix well, and add proteinase K to a final concentration of 100 μg/ml. Incubate for 30 minutes at 56°C.

Proteinase K is used to digest the CIP, which must be completely removed if the subsequent ligation reactions are to work efficiently. An alternative method to inactivate the CIP is to heat the reaction (at the end of step 2) to 65°C for 1 hour (or 75°C for 10 minutes) in the presence of 5 mM EDTA and then to extract once with phenol:chloroform.

4. Cool the reaction to room temperature, and purify the bacteriophage λ DNA by extracting once with phenol:chloroform and once with chloroform alone. Add 0.1 volume of 3 M sodium acetate (pH 7.0). Mix well, and add 2 volumes of ethanol. Mix well, and store at 0°C for 15 minutes. Recover the DNA by centrifugation at 12,000*g* for 10 minutes at 4°C in a microfuge. Wash the pellet with 70% ethanol at 4°C and recentrifuge.

5. Redissolve the DNA in TE (pH 7.6) at a concentration of 300–500 μg/ml. Store the dephosphorylated DNA at −20°C in aliquots containing 1–5 μg.

6. Measure the efficiency of the phosphatase treatment by ligating a portion (0.5 μg) of the digested vector before and after treatment with phosphatase (see discussion of trial ligations on page 2.94). Package the DNA into bacteriophage particles (see page 2.107 for protocol), and titrate the infectivity. Phosphatase treatment should reduce ligation and the efficiency of packaging of the arms by two to three orders of magnitude.

Notes

i. The pH of the 3 M sodium acetate solution is 7.0 rather than the usual 5.2. At acid pH, EDTA precipitates from solution if its concentration exceeds 5–10 mM.

ii. Until recently, CIP was supplied as a suspension in a slurry of ammonium sulfate. However, it may now be obtained as a stabilized solution (in 30 mM triethanolamine buffer [pH 7.6], 3 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$). It should be stored undiluted in this buffer (not frozen).