Ligation of DNA fragments by homopolymer tailing

Richard Powell
Department of Microbiology, National University of Ireland, Galway, Ireland

Frank Gannon
European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, D-69012 Heidelberg, Germany

Reagents

- 10 × tailing buffer (1 M sodium cacodylate, pH 7.0, 10 mM $CoCl_2$, 1 mM DTT)

Extreme care is required when making up the 10 × tailing buffer. Add the chemicals in the order stated to prevent precipitation.

- Stock solution (e.g. 20 mM) of the appropriate dNTP
- TdT (terminal deoxynucleotidyl transferase)
- 10 × annealing buffer (500 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1 M NaCl)

Method

1. Prepare a tailing mixture of 50 µl containing DNA, 1 × tailing buffer and a 20 µM concentration of the nucleotide to be added to the DNA.
2. Add 10 units of TdT and incubate at 37 °C for 10 min. Under these conditions the enzyme will add 20 nucleotides to the 3'-end of the DNA in 10 min if the reaction includes 4 pmol of DNA. Trial experiments can be carried out with trace amounts of a radioactive dNTP to determine the extent of addition of nucleotides on to the 3'-ends of the DNA.
3. Stop the reaction by heating at 75 °C for 10 min. Purify the DNA by phenol extraction and ethanol precipitation (see Purification of DNA by phenol extraction and ethanol precipitation). It is also useful to dialyse the DNA against sterile water for 1–2 h to remove components from the tailing mixture that may inhibit bacterial transformation (see Purification of DNA by phenol extraction and ethanol precipitation).
4. Prepare an annealing mixture containing vector DNA and target DNA with complementary homopolymer tails in 100 µl of 1 × annealing buffer. The DNA concentration and ratio of vector to target depends on the nature of the cloning experiment (see DNA ligation). Heat the sample at 70 °C for 30 min in a water bath. Turn off the heat and allow the sample to cool in the water bath to room temperature over a period of 7–12 h.
5. The sample is now ready for transformation of *E. coli* cells.

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Notes

Choosing the appropriate complementary nucleotides to "tail" the termini of the two DNA fragments to be ligated may facilitate further subsequent analysis. If the aim is to ligate DNA fragments of interest to plasmid vectors in order to produce bacterial clones, it is sensible to design the homopolymer tailing based ligation procedure so that the subsequent analysis of cloned DNA fragments (e.g. analysis of cloned DNA fragments length) is user-friendly. For example, digestion of the vector DNA with the restriction endonuclease *Pst*I (recognition site: CTGCAG) which will produce the 3′-extension TGCA at both termini. If the *Pst*I digested vector DNA is then homopolymer-tailed with dGTP, and consequently the target DNA fragments are tailed with the complementary dCTP, then successfully ligated recombinant DNA products will now contain two *Pst*I recognition sites either side of the target DNA. Therefore, simple digestion of the cloned recombinant DNA with *Pst*I will liberate the cloned DNA fragments (including homopolymer tails) from the vector DNA.

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