

DNA ligation

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Equipment and reagents

- ◆ T4 DNA ligase
- ◆ 10 × ligation buffer (0.66 M Tris-HCl, pH 7.6, 50 mM MgCl₂, 50 mM DTT, 10 mM ATP)
- ◆ Low temperature water-bath or heating-block
- ◆ Gel electrophoresis apparatus

Method

- 1 The volume of the ligation mixture and the DNA concentration depend on the type of ligation experiment. Use a 10 µl reaction with DNA at a concentration of > 100 ng/µl to promote the formation of concatamer ligation products, or a 10 µl (or larger) reaction with DNA at a concentration of <10 ng/µl to promote the formation of circular ligation products.
- 2 Add T4 DNA ligase. Check the supplier's documentation on enzyme activity, or more generally, add 0.25 units enzyme/µg of DNA for cohesive-end ligations, and 2.5 units/µg of DNA for blunt-end ligations.
- 3 Incubate the reaction mixture at 15 °C for 1–16 h. Simple cohesive-end ligations are usually complete in 1 h.
- 4 Analyse for correct and complete ligation by gel electrophoresis using unligated material as a suitable control, or also by transformation of *E. coli* cells if appropriate (i.e. the ligation includes an appropriate plasmid vector).