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Rapid ligation protocol for plasmid cloning of DNA fragments

Standard protocols for DNA ligations are carried out for 1 to 24 h depending on the temperature and type of DNA ends. For routine subcloning, these long incubations are often unnecessary (see figure 1). For library construction, we recommend the longer incubations to ensure the maximal number of clones are obtained. For routine subcloning, the rapid protocol below can be used.

The following reaction conditions are for rapid ligation of DNA inserts to vectors to produce circular recombinant molecules. A molar ratio of 3:1 insert:vector is recommended. Subsequent to restriction digestion, gel purify the insert DNA from agarose using the S.N.A.P.™ Gel Purification Kit. Following restriction endonuclease digestion, dephosphorylate the vector DNA. Dephosphorylated vector can be used without purification if Calf Intestinal Alkaline Phosphatase (CIAP) is heat inactivated prior to ligation.

Protocol

1. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

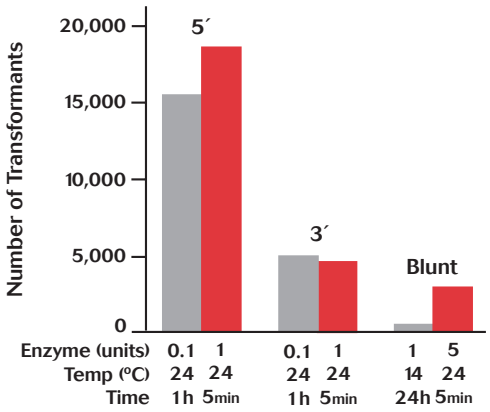
Component	Cohesive Ends	Blunt Ends
5X ligase reaction buffer	4 µl	4 µl
vector DNA	3 to 30 fmol	15 to 60 fmol
insert DNA	9 to 90 fmol	45 to 180 fmol
autoclaved distilled water	up to 19 µl	up to 19 µl
T4 DNA ligase	1 unit (in 1 µl)	5 units (in 1 µl)
Note: *Final reaction volume*	*20 µl*	*20 µl*

2. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
3. Incubate at room temperature for 5 min.
4. Use 2 µl of the ligation reaction to transform competent cells. Visit www.invitrogen.com for available competent cells.

Analysis of ligations

Ligation reactions are analyzed by transformation of bacteria. For more details, see the Troubleshooting Guide for Cloning in TECH-ONLINE on the Invitrogen web page.

Figure 1 - Ligation conditions



Comparison of standard and rapid ligation protocols. Inserts were ligated into pUC19 treated with *Hind* III (5´ overhang, 1.1-kb insert), *Kpn* I (3´ overhang, 1.0-kb insert), and *Eco*R V (blunt ends, 1.1-kb insert) using the standard protocol (■) or the rapid ligation protocol (■). Time and amount of T4 DNA Ligase were as shown, all other parameters were as describd in the rapid protocol. MAX Efficiency DH10B™ cells were transformed and incubated overnight on LB agar with ampicillin and X-gal. White colonies were counted. **Note:** For the blunt-end ligation, 1 U of ligase for 5 min at 24˚C resulted in 480 colonies.

Ordering information

Description	Concentration	Quantity	Cat. No.
T4 DNA Ligase	1 unit/µl	100 units	15224-017
	1 unit/µl	500 units	15224-025
	1 unit/µl	4 x 500 units	15224-090
	5 units/µl	250 units	15224-041
T4 DNA Ligase Buffer*	5X	2 x 1 ml	46300-018
S.N.A.P. Gel Purification Kit		25 rxns	K1999-25
CIAP (Calf Intestinal Alkaline Phosphatase)	20/µl	1,000 units	18009-019
	1 unit/µl	1,000 units	18009-027

*T4 DNA Ligase is supplied with one vial of 5X reaction buffer.
For research use only. Not for diagnostic or therapeutic use in humans or animals.

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