Ligation Protocol with T4 DNA Ligase (NEB# M0202)

Materials Required but not Supplied

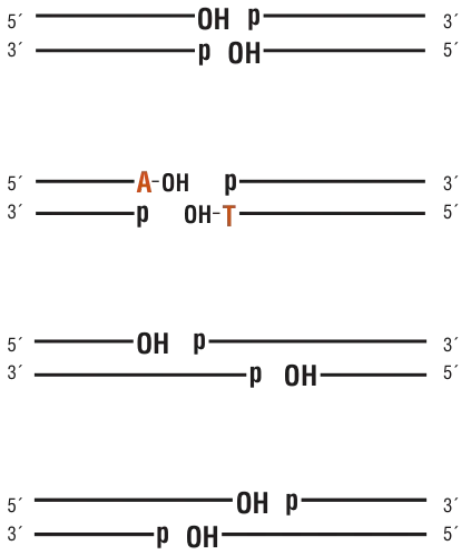
T4 DNA Ligase

- Nuclease-free Water (NEB #B1500)

Overview

This is a general protocol for using T4 DNA ligase for standard two fragment ligation experiments, such as ligating a backbone and insert into a circular plasmid or the linear ligation of two fragments. For multi-fragment assembly or high-complexity library cloning, NEBuilder® HiFi DNA Assembly Products or NEBridge® Golden Gate Assembly products are recommended.

T4 DNA Ligase will ligate these dsDNA substrates



Precautions:

Heat-inactivate restriction enzymes or purify DNA using a spin column (NEB #T1130) before ligation.

Protocol

1. Set up the following reaction in a microcentrifuge tube on ice. T4 DNA Ligase should be added last. The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.

Use NEBioCalculator to calculate molar ratios.

COMPONENT	20 µl REACTION	FINAL CONC. OR AMOUNT
T4 DNA Ligase Buffer (10X)*	2 µl	1X
Vector DNA (4 kb)	X µl	50 ng (0.020 pmol)
Insert DNA (1 kb)	X µl	37.5 ng (0.060 pmol)
Nuclease-free Water	To 20 µl	
T4 DNA Ligase (400,000 u/ml)	1 µl	400 units (standard concentration) or 2,000 units (high concentration)

** The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*

*** Recommendations are based on a standard ligation reaction in a cloning workflow. Molar ratios of the two DNA fragments being ligated (vector and insert) can be adjusted based on the relative size differential. In the example shown above, a 1:3 ratio has been used. Use NEBioCalculator to calculate molar ratios.*

2. Gently mix the reaction by pipetting up and down and microfuge briefly.

    a. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.

    b. For blunt ends or single base overhangs:

        i. Incubate at 16°C overnight or room temperature (25°C) for 2 hours.

        ii. Alternatively, high concentration (2,000,000 u/ml) T4 DNA Ligase (can be used in a 10-minute ligation at room temperature (25°C).

3. Heat-inactivate the reaction at 65°C for 10 minutes.

4. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

General Guidelines

1. **Reaction Buffer:**
   ATP is an essential cofactor for the reaction. Ligation can also be performed in any of the four restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM of ribo ATP (NEB #P0756). Deoxyribo ATP will not work.

2. **Room Temperature Ligation:**
   For convenience, ligations may be done at room temperature (20-25°C). For cohesive (sticky) ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 10 minutes. For blunt ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 2 hours or 1 µl high-concentration T4 DNA Ligase for 10 minutes. Alternatively, NEB's Quick Ligation Kit (NEB #M2200) is uniquely formulated to ligate both blunt and cohesive (sticky) ends in 5 minutes at room temperature.

3. **DNA:**
   If using a phosphatase (e.g. Quick CIP) or kinase (e.g. T4 Polynucleotide Kinase) to modify your DNA ends, you should heat-inactivate the enzyme before ligation. We recommend a vector amount of 20-30 fmol with an overall concentration of vector + insert between 1-10 ng/µl for efficient ligation. Concentration lower than 1 ng/µL may result in intramolecular ligation/circularization of the fragments. Vector: Insert molar ratios between 1:1 and 1:10 are optimal for single insertions (up to 1:20 for short adaptors). Use NEBioCalculator to calculate molar ratios. For multi-fragment assembly or high-complexity library cloning, NEBuilder® HiFi DNA Assembly Products or NEBridge® Golden Gate Assembly products are recommended. If you are unsure of your DNA concentration, perform multiple ligations with varying ratios.

Related Resources

- NEBioCalculator®
- NEBcloner®
- DNA Ligase Selection Chart
- NEB Diluent and Buffer Table
- Properties of DNA and RNA Ligases
- Substrate-based Ligase Selection Chart