Ligation with T4 DNA Ligase (M0202)

Introduction

This protocol joins a restriction-digested, column-purified insert to a linearised plasmid backbone using T4 DNA Ligase, generating a circular recombinant plasmid. The vector and insert are cut with the same enzyme pair so that their ends are compatible, and are combined at a defined vector-to-insert molar ratio — 1:3 is the usual starting point, since an excess of insert favours single-insert recombinants while suppressing vector self-ligation. The mass of each fragment required to achieve that ratio is calculated from its length, using a tool such as NEBioCalculator, because the same mass of a longer fragment contains fewer molecules. Before the ligase is added, a small aliquot may be retained as an unligated reference for gel electrophoresis. The product is a ligation mixture ready for transformation into competent *E. coli*, where recombinants are recovered by selection on the antibiotic marker carried by the vector and verified by colony PCR.

Reference

1. New England Biolabs. *Ligation Protocol with T4 DNA Ligase (NEB #M0202)*. Available at: https://www.neb.com/en/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202
2. New England Biolabs. *T4 DNA Ligase (M0202) product page and Reaction Buffer specification*. Available at: https://www.neb.com/en/products/m0202-t4-dna-ligase

Materials

- 10X T4 DNA Ligase Reaction Buffer 0.020 pmol (1 ng/µL) 5 µL
- T4 DNA Ligase (400 U/µL (8 U/µL) 1 µL
- Vector DNA (0.020 pmol (1 ng/µL) 20 µL
- Insert DNA (37.5 ng = 0.060 pmol (0.75 ng/µL), 1:3 ratio) 20 µL
- Nuclease-free water, MilliQ 4 µL

Procedure

Procedure Ligation with T4 DNA Ligase

Note: 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.

1. Prepare the work area and thaw the 10X T4 DNA Ligase Buffer and resuspend it at room temperature. Mix until the solution is completely clear, then keep it on ice.

2. Set up the following reaction in a microcentrifuge tube on ice:

Table1

	Component	Volume (µL)
1	**10X T4 DNA Ligase Buffer**	**5**
2	Vector DNA: 50 ng (0.020 pmol)	20
3	Insert DNA: 37.5 ng (0.060 pmol)	20
4	Nuclease-free water	4
5	T4 DNA Ligase	1
6	Total	50

3. Gently mix the reaction by pipetting and do not vortex vigorously.

4. Microfuge briefly 3–5 sec pulse at approximately 2,000–6,000 × g (roughly 5,000–8,000 rpm in a standard 1.5 mL fixed-angle rotor), at room temperature to collect the pellets at the bottom of the tube.

5. For cohesive (sticky) ends, incubate at 16 °C overnight or room temperature for 10 minutes. For blunt ends or single base overhangs, incubate at 16 °C overnight or room temperature for 2 hours.

6. Heat inactivate at 65 °C for 10 minutes to stop the reaction. Handle the heat block with care and use a tube rack or forceps to place and retrieve tubes.

7. Chill on ice for the following step or store it at − 20 °C.

8. Clear and clean the work area by using ethanol**.** Discard used tips and tubes into the biological waste for autoclaving, disinfect the bench surface, remove gloves and wash hands before leaving the laboratory.

9. **ENDPOINT**: The ligation product is now ready for transformation, which is documented in a separate protocol. For reference: NEB specifies 1–5 µL of the reaction into 50 µL of competent cells; our laboratory transforms 2 µL into 25 µL of chemically competent E. coli DH5α.