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Protocol status: Working
We use this protocol and it's working

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Ligation Protocol with T4 DNA Ligase (M0202) V.3

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ABSTRACT

This product can be used for the following applications:

- Cloning of restriction fragments
- Joining linkers and adapters to blunt-ended DNA

MATERIALS

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T4 DNA Ligase **New England Biolabs Catalog #M0202**

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE START INSTRUCTIONS

Thaw the T4 DNA Ligase Buffer and resuspend at Room temperature .

Keywords: Ligase, Ligation, DNA, T4

1 Set up the following reaction in a microcentrifuge tube On ice .

(T4 DNA Ligase should be added last. Note that 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.

A	B
COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

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

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

3 For **cohesive (sticky) ends**, incubate at 16 °C Overnight or Room temperature

for 00:10:00 .

4 For **blunt ends** or **single base overhangs**, incubate at 16 °C Overnight or

Room temperature for 02:00:00 (alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation).

5 Heat inactivate at 65 °C for 00:10:00

6 Chill On ice and transform 1 μL - 5 μL of the reaction into 50 μL competent cells.