Version 3

Apr 01, 2021

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

New England Biolabs[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.bcchist6

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SUBMIT TO PLOS ONE

ABSTRACT

This product can be used for the following applications:

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

EXTERNAL LINK

https://www.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202

DOI

dx.doi.org/10.17504/protocols.io.bcchist6

EXTERNAL LINK

https://www.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202

PROTOCOL CITATION

New England Biolabs 2021. Ligation Protocol with T4 DNA Ligase (M0202). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bcchist6

KEYWORDS

Ligase, Ligation, DNA, T4

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MATERIALS TEXT

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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 STARTING

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

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 

10m

For **cohesive (sticky) ends**, incubate at **16 °C** **Overnight** or **Room temperature** for **00:10:00** .

4 

2h 10m

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** .

10m

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

Citation: New England Biolabs (04/01/2021). Ligation Protocol with T4 DNA Ligase (M0202). https://dx.doi.org/10.17504/protocols.io.bcchist6