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Version 1

Ligation Protocol WITH T4 DNA Ligase (M0202) V.1

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<https://dx.doi.org/10.17504/protocols.io.cdks4v>



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Abstract

Please see the NEB website for more information.

Materials

MATERIALS

⊗ T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S**

STEP MATERIALS

⊗ T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #B0202S**

⊗ T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #B0202S**

Protocol materials

⊗ T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #B0202S**

⊗ T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S**



- 1 Thaw the T4 DNA Ligase Buffer and resuspended at room temperature.

 T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #B0202S**

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

Note

Note that these are instructions for a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. Use [NEBioCalculator](#) to calculate molar ratios.

Protocol



NAME

T4 DNA Ligase Reaction

CREATED BY

New England Biolabs

Preview

- 2.1  2 μL

 T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S**

- 2.2 Vector DNA (4 kb) 50 ng (0.020 pmol)

 50 μL

- 2.3 Insert DNA (1 kb) 37.5 ng (0.060 pmol)

 38 μL

- 2.4 Nuclease-free water to 20 μL

- 2.5 T4 DNA Ligase, 1 μL

 1 μL

 T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S**

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



- 4 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.

Note

Alternatively, high concentration of T4 DNA Ligase can be used in a 10-minute ligation for blunt ends.

- 5 Heat inactivate at 65 degrees C for 10 minutes.

 00:10:00

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