VERSION 3

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

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Quick Ligation Protocol (M2200) V.3

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ABSTRACT

This protocols is to be performed with the Quick Ligation Reaction Buffer. Please see the NEB website for more information.

MATERIALS

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⊗ Quick Ligation Kit - 30 rxns **New England Biolabs Catalog #M2200S**

SAFETY WARNINGS

⚠ Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE START INSTRUCTIONS

Thaw and resuspend Quick Ligase Reaction Buffer at 🌡 Room temperature

Keywords: ligation

1

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

Note

Quick 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.)

COMPONENT	20 μ l REACTION
Quick Ligase Reaction Buffer (2X)*	10 μ l
Vector DNA (3 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free Water	to 20 μ l
Quick Ligase	1 μ l

**The Quick Ligase Reaction Buffer should be thawed and resuspended at room temperature.*

2

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

3

Incubate at Room temperature (25 °C) for 00:05:00 .

4

Chill On ice and transform 1 μL – 5 μL of the reaction into 50 μL competent cells. Alternatively, Store at $-20\text{ }^{\circ}\text{C}$.

Note

Do not heat inactivate – heat inactivation dramatically reduces transformation efficiency.