Protocol for Salt-T4® DNA Ligase (NEB #M0467)

Overview

1. Set up the following reaction in a microcentrifuge tube on ice. *(Salt-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.

Cohesive End Ligation: **COMPONENTS**	**20 µl REACTION**		**Blunt/TA Overhang Ligation:** **COMPONENTS**	**20 µl REACTION**
T4 DNA Ligase Reaction Buffer (10X)*	2 µl		StickTogether™ DNA Ligase Buffer (2X)	10 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)		Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)		Insert DNA (1 kb)	37.5 ng (0.060 pmol)
1M NaCl**$_{(optional)}$	2-10 µl		1M NaCl**$_{(optional)}$	2 µl
Nuclease-free water	to 20 µl		Nuclease-free water	to 20 µl
Salt-T4 DNA Ligase	1 µl		Salt-T4 DNA Ligase	1 µl

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

** Addition of 1M NaCl is optional. Salt-T4 DNA retains 100% activity on cohesive ends up to 300 mM$_{[final]}$ NaCl and >50% activity up to 500 mM$_{[final]}$ NaCl. Blunt ligation is much more sensitive to salt concentration; therefore, we recommend performing blunt ligation using Salt-T4 DNA Ligase in 1X StickTogether™ DNA Ligase Buffer containing ≤100 mM NaCl.

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

3. For cohesive (sticky) ends (1X T4 DNA Ligase Buffer), incubate at room temperature (20-25°C) for 10 minutes. Heat inactivate at 65°C for 10 minutes.

4. For blunt ends or single base overhangs (1X StickTogether™ DNA Ligase Buffer), incubate at room temperature (20-25°C) for 10 minutes or 16°C overnight. Do not heat kill the reaction because heat treating the PEG in the StickTogether™ DNA Ligase Buffer will inhibit transformation.

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