Ligation Protocol with T7 DNA Ligase (M0318)

Overview

1. Set up the following reaction in a microcentrifuge tube on ice.
   (T7 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.

COMPONENT	20 µl REACTION
T7 DNA Ligase Reaction Buffer (2X)*	10 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
T7 DNA Ligase	1 µl

   ** The T7 DNA 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 15-30 minutes.
4. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells. Alternatively, store at –20°C.
5. Do not heat inactivate – Heat inactivation dramatically reduces transformation efficiency.