Ligation Protocol WITH T4 DNA Ligase (M0202)

Introduction

Please see the NEB website for supporting information on this protocol.

Materials

› 10X T4 DNA Ligase Reaction Buffer

› T4 DNA Ligase

› Vector DNA (4kb)

› Insert DNA (1kb)

› Nuclease-free water

Procedure

Set up the T4 DNA Ligase Reaction

Note: T4 DNA Ligase should be added last. The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. *Use NEB calculator to calculate molar ratios.*

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

   Tip: Alicuote the 10x buffer less concentrated so when thawing, the DTT gets soluble more easily.

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

Table1

	A	B
1	**Component**	**Volume (µl)**
2	10X T4 DNA Ligase Buffer	2
3	Vector DNA: 50 ng (0.020 pmol)	
4	Insert DNA: 37.5 ng (0.060 pmol)	
5	Nuclease-free water	17
6	T4 DNA Ligase	1
7	Total	20

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.

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.

   Use 25 uL DH5α cells, and add 2 uL of reaction mixture.