Ligation of Sticky Ends with T4

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Abstract

Adapted T4 Protocol to reflect the one used by Northeastern_Boston for ligation of sticky end DNA.

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Protocol

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

Step 2.
Set up the following reaction mixture at room temp:

10x T4 DNA Ligase Buffer	2 ul
Vector DNA	0.020 pmol
Insert DNA	0.060 pmol
Nuclease-free Water	to 20 ul
T4 DNA Ligase	1 ul

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

Step 4.
For sticky ends, incubate at 22 C for 10 minutes.

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

DURATION
00:10:00

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