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Ligation Protocol with T4 DNA Ligase

Alba Balletbó[1]

[1]Wageningen University

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Alba Balletbó

ABSTRACT

The final step in the construction of a recombinant plasmid is connecting the insert DNA (gene or fragment of interest) into a compatibly digested vector backbone. This is accomplished by covalently connecting the sugar backbone of the two DNA fragments. This reaction, called ligation, is performed by the T4 DNA ligase enzyme. The DNA ligase catalyzes the formation of covalent phosphodiester linkages, which permanently join the nucleotides together. After ligation, the insert DNA is physically attached to the backbone and the complete plasmid can be transformed into bacterial cells for propagation.

The majority of ligation reactions involve DNA fragments that have been generated by restriction enzyme ligation (See Protocols). Most restriction enzymes digest DNA asymmetrically across their recognition sequence, which results in a single stranded overhang on the digested end of the DNA fragment. The overhangs, called "sticky ends", are what allow the vector and insert to bind to each other. When the sticky ends are compatible, meaning that the overhanging base pairs on the vector and insert are complementary, the two pieces of DNA connect and ultimately are fused by the ligation reaction.

MATERIALS

NAME	CATALOG #	VENDOR
T4 DNA Ligase	M0202	New England Biolabs
T4 DNA Ligase Buffer (10X)	B69	Thermo Fisher

SAFETY WARNINGS

Wear laboratory coat, gloves and googles during the whole procedure.

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

'Pro-tip': Alicuote the 10x buffer less concentrated so when thawing the DTT gets soluble more easily.

2 Set up the reaction in a microcentrifuge tube on ice (T4 DNA Ligase should be added last.):

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 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
T4 DNA Ligase	1 µl

*T4 DNA Ligase should be added last.

'Note': the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.

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

4 For cohesive (sticky) ends, incubate at 16ºC overnight or room temperature for 10 minutes. For blunt ends or single base overhangs, incubate at 16ºC overnight or room temperature for 2 hours.

5 Heat inactivate at 65ºC for 10 minutes.

6 Chill on ice and transform 1-5 µL of the reaction into 50 µL competent cells (See Protocols for Transformation).