DNA Insert Ligation (sticky-end and blunt-end) into Vector DNA

Protocol

This protocol is for the DNA Insert Ligation (sticky-end and blunt-end) into Vector DNA.

Sticky-end Ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
10x T4 DNA Ligase buffer	2 µL
Thermo Scientific T4 DNA Ligase (Cat #EL0016)	1 U
Water, nuclease-free	to 20 µL
Total volume	**20 µL**

2. Incubate 10 minutes at 22 °C.
3. Use up to 5 µL of the mixture for transformation of 50 µL of chemically competent cells or use 1-2 µL per 50 µL electrocompetent cells.

Note

- The electrotransformation efficiency may be improved by:
  – heat inactivation of T4 DNA Ligase at 65 °C for 10 minutes or at 70 °C for 5 minutes,
  – purification of DNA, using Thermo Scientific GeneJET™ PCR Purification Kit or by chloroform extraction.
- The overall number of transformants may be increased by extending the reaction time to 1 hour.
- If more than 2 U of T4 DNA Ligase is used in 20 µL reaction mixture, it is necessary to purify DNA (by spin column or chloroform extraction) before electrotransformation.

Blunt-end Ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
Thermo Scientific 10x T4 DNA Ligase buffer	2 µL
50% PEG 4000 solution	2 µL
T4 DNA Ligase	5 U
Water, nuclease-free	to 20 µL
Total volume	**20 µL**

2. Incubate 1 hour at 22 °C.
3. Use up to 5 µL of the mixture for transformation of 50 µL of chemically competent cells. Purify DNA for electrotransformation, using the GeneJET™ PCR Purification Kit or by chloroform extraction. Use 1-2 µL of DNA solution per 50 µL of electrocompetent cells.

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