Sticky-end Ligation

Aim

Performing ligation of DNA insert (sticky-end) into vector DNA.

Materials

- Linear Vector DNA
- Insert DNA
- 10x T4 DNA Ligase buffer
- T4 DNA Ligase (EL0014/EL0016, Thermo Scientific)
- Nuclease free water

Procedure

1. Prepare the following reaction mixture:

Component	Quantity
Linear Vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio (insert:vector)
10x T4 DNA Ligase buffer	2 µl
T4 DNA Ligase	1 µl
Nuclease free water	to 20 µl
Total volume	**20 µl**

2. Incubate for at least 10 minutes at 22 °C.

3. Use 5 µl of the mixture for transformation of 50 µl of chemically competent cells (or use 1-2 µl per 50 µl electrocompetent cells).

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Notes

The molar ratio calculations can be done with:
insilico.uni-duesseldorf.de/Lig_Input.html

To increase the overall number of transformants, it is **highly** recommended to extend the reaction time to 3 - 18 h (or overnight).

The ligation efficiency may be improved by:

- purification of digested DNA, using a PCR purification kit for insert DNA and gel extraction/PCR purification kit for vector DNA.

The transformation efficiency may be improved by:

- heat inactivation of T4 DNA Ligase at 65 °C for 10 minutes or at 70 °C for 5 minutes.

References

This protocol is a modified version of the "DNA Insert Ligation (sticky-end and blunt-end) into Vector DNA" protocol distributed by Thermo Fisher Scientific Inc. (2012)