

Sticky-end DNA insert Ligation into vector DNA protocol

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 1:5 molar ratio over vector
10x T4 DNA Ligase buffer	2 μ l
T4 DNA ligase	1 μ l
dH₂O	to 20 μ l

Total volume	20 μl
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2. Incubate 1h at 22 °C or over night at room temperature.

3. Use 5 μ l of the mixture for transformation of 50 μ l of chemically competent cells.