

Ligations:

(we use T4 ligase from NEB)

- vector DNA ~ 100 ng
- insert DNA ~ 300 ng
- 2 μ l 10x ligase buffer (make small alicots to prevent freezing and thawing cycles)
- 1 μ l T4 ligase
- adjust to 20 μ l with H₂O

As an negative control, carry out a ligation reaction without the insert.

Incubate 3hrs at RT.

Transform ~3 μ l of ligation product in ~50 μ l of chemically competent cells made in house by Trevin.