

Ligations:

(we use T4 ligase from NEB)

- vector DNA ~ 100 ng
- insert DNA ~ 300 ng
- 2  $\mu$ l 10x ligase buffer (make small alicots to prevent freezing and thawing cycles)
- 1  $\mu$ l T4 ligase
- adjust to 20  $\mu$ l with H<sub>2</sub>O

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

Incubate 3hrs at RT.

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