

# Standard Ligation Protocol

- T4 ligase joins the 5' phosphate and the 3'-hydroxyl groups of DOUBLE stranded DNA molecules.
- Estimate the vector and insert concentrations.
- If insert is from PCR, assume that 50% is recovered, typically 5 µg, and resuspended in 10 µl of H<sub>2</sub>O.
- Same with vector, assume half is recovered in purification/precipitation and resuspend in 10-20 µl of H<sub>2</sub>O if it is not re-suspended already.

## Plan control reactions.

- One reaction with no insert
- One reaction with no vector (if enough insert is available)

## Setup each ligation mixture in the following way (see note below if over 2 µl of vector or over 4 µl of insert required)

- 200 ng of vector DNA
- 500 ng of insert DNA
- 10X Ligase buffer (Promega #M1801)
- 1 µl T4 Ligase (Promega #M1801, 3 U/ml)
- Bring volume to 10 (or 20 see note below) µl with nuclease-free water.
- IF you are doing a blunt-end ligation, you may need to add PEG (up to 15%) to increase the efficiency.
- Mix by pipetting. Do not vortex.
- If over 2 µl of vector is needed or over 4 µl of insert, do a 20 µl reaction. Otherwise 10 µl is sufficient.

## Incubate according to the guidelines below

- Incubate sticky end ligation reactions at room temperature for 3 hours (or at 4 to 8 deg C, overnight).
- Incubate blunt-end ligation reactions at 17 deg C for 4 to 18 hours.
- Heat inactivate the ligase by placing tube in 65C water bath for 10 minutes.
- This has been shown to increase the transformation efficiency
- Electroporate using 2 µl of the ligation mixture.
- The optimal concentration is measured using the electroporation time constant (between 3 to 4.5 µl).
- The more DNA/salt present, the lower the time constant.

Plate on selective media and incubate.