# Ligation Protocol (Promega)

**Materials:**

- Vector DNA (~100ng)
- Insert DNA
- Ligase 10x buffer
- T4 DNA ligase
- Nuclease-free water

**Protocol:**

Promega recommends using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. The ng of insert can be obtained by using the below equation.

$$\frac{vector\ (ng) \times insert\ (kb)}{vector\ (kb)} \times molar\ ratio\ of\ \frac{insert}{vector} = insert\ (ng)$$

1. Assemble the following reaction in a sterile microcentrifuge tube:

| | |
|---|---|
| Vector DNA | 100 ng |
| Insert DNA | From equation |
| Ligase 10x Buffer | 1 µl |
| T4 DNA Ligase (Weiss units) | 0.1-1 |
| Nuclease-Free Water to final volume of | 10 µl |

1. Incubate the reaction at room temperature for 3 hours, or at 4°C overnight.
2. Transform with 2 µl of product.