# DNA Ligation

## Introduction

Once we have a DNA insert from a digestion reaction, we can put it into a plasmid backbone that has been precut.

## Materials

- Digested pSB1C3 backbone
  - Plasmid backbone most commonly used, although a different one could be used. Confers chloramphenicol resistance.
- DNA insert
  - From the digestion reaction/gel extraction
- T4 DNA ligase
  - enzyme that ligates 2 or more pieces of DNA together at sticky ends.
- T4 ligase buffer
  - Allows the enzyme to function.

## Procedure

### Calculate the amount of backbone and insert needed.

1. Use this website to calculate the amount of insert needed for the reaction: : https://nebiocalculator.neb.com/#!/ligation

   NEB suggests we use around 50ng of vector (according to https://www.neb.com/protocols/1/01/01/dna-ligation-with-t4-dna-ligase-m0202)

2. The insert length should be verified by the gel and on Benchling.

3. The mass of the insert and vector can be calculated using the concentration from the nanodrop.

4. A ratio of 5:1 or 3:1 usually works best, but ask before running the reaction.

5. Calculate the amount of water needed for the reaction:

Table1

| | A | B | C |
|---|---|---|---|
| 1 | **Component** | **amount** | approx mass |
| 2 | DNA backbone | x | 25 ng |
| 3 | DNA Insert | y | use ratio |
| 4 | T4 Ligase Buffer | 2 ul | - |
| 5 | T4 enzyme | 1 ul | - |
| 6 | dH2O | z | - |
| 7 | total | 20 ul | |

## Setting up the reaction

6. Keep T4 ligase enzyme on ice until it needs to be added.

7. Add the components in the following order to a 1.5 ml microcentrifuge tube.

   Water
   T4 Buffer
   DNA backbone
   DNA insert
   T4 enzyme

8. Mix by pipetting up and down, and briefly spin to get contents to bottom of tube.

## Running the reaction

9. Incubate for 10 minutes at room temperature.

10. Heat inactivate at 65 C for 20 minutes.

11. Column purify or proceed to transformation. Reaction can also be stored at -20 C.