Ligation

Aim:

- Ligate fragments from Type IIs and BioBrick Assembly.

Timeframe:

- Preparation: 10 minutes
- Wait-time: 2 hours
- Overall: 2 hours 10 mins

Materials:

- X T4 Ligase buffer
- 1 µl T4 DNA ligase (400,000 units/ml)
- ddH2O25 ng vector DNA
- 75 ng insert DNA
- 10

Procedure:

1. In an eppendorf tube, combine the following:
    - 25 ng vector DNA
    - 75 ng insert DNA
    - Ligase buffer (1 µl/10 µl reaction for 10X buffer)
    - 1 µl T4 DNA ligase
    - ddH2O water to reach a total volume 10 µl when combined with other components
    - **Reactions can be carried out in larger volumes (adjusting water and buffer volumes) but may require subsequent DNA purification. PCR purification kits can be used to purify ligation reactions (post-ligation).*
2. Incubate at room temperature for 2 hrs or at 16°C overnight.
3. Proceed with bacterial transformation.