Ligation protocol (follows iGEM official protocol)

1. Calculate the amount of insert as \( \frac{\text{vector(ng)}}{\text{vector length}} = \frac{\text{insert(ng)}}{\text{insert length}} = 1:1 \), let the vector amount = 25 ng.

2. Add the materials as follow:
   - T4 DNA ligase: 0.5 ul
   - Ligase buffer: 1 ul
   - Vector (25 ng): \( x \) ul
   - Insert A: follow the result you calculates
   - Insert B: follow the result you calculates
   - ddH2O: to final volume 10 ul

3. Mix and centrifuge the reaction mixture.

4. Put the reaction under room temperature for 2~3 hours

5. Heat-kill in 80°C for 20 mins. (This step can be ignored.)

6. Transform 1~2 ul of the product.
Ligation protocol:

1. Calculate the amount of insert as \[ \frac{\text{vector(ng)}}{\text{vector length}} : \frac{\text{insert(ng)}}{\text{insert length}} = 1:1 \text{ (or} 1:3 \text{ or} 1:5 \text{ or} 1:7) \], let the vector amount = 50 ng.

2. Add the materials as follow:
   - T4 DNA ligase 1 ul
   - Ligase buffer 2 ul
   - Vector (25 ng) x ul
   - Insert A follow the result you calculates
   - Insert B follow the result you calculates
   - ddH2O to final volume 10 ul

3. Mix and centrifuge the reaction mixture.

4. Put the reaction under room temperature for 2~3 hours or 16 ℃ over night

5. Transform 5~10 ul of the product.