Protocol: Ligation with pET-11a

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This protocol was originally provided by ThermoFisher. We have added additional details, clarifications, and tips for practical use in the lab.

Ligation ThermoFisher T4 ligase, 5 U/µL

Note:

- Ligase buffer contains ATP → keep on ice at all times.
- If precipitate appears after thawing ligase buffer, warm gently by hand to dissolve, then place back on ice.
- Also keep T4 DNA ligase on ice.

Step 1: Measurement of DNA concentrations (If necessary)

- Measure DNA concentrations of:
  - Digested vector (pET-11a, 5677 bp)
  - Digested insert
- Use micro-volume spectrophotometer following:
  Protocol: Plasmid Concentration Measurement Using Micro-Volume Spectrophotometer
- Record concentrations and total volumes of your DNA samples.

Step 2: Calculate insert amount needed

- Calculate the insert amount (in ng) to achieve a 3:1 molar ratio using the formula below (note: the ratio can be increased to 6:1 or other if needed):

$$\text{Insert (ng)} = \frac{\text{Insert size (bp)}}{\text{Vector size (bp)}} \times \text{Vector amount (ng)} \times 3$$

- Or you can use this online calculator to automatically calculate the required amount of insert for our plasmid: https://nebiocalculator.neb.com/#!/ligation
  - vector DNA length (pET11a): 5677 bp
  - ⚠️**Important! Remember to change to bp instead of kb!**

- If needed, dilute the insert DNA to ensure you pipette at least ≥1 µL for better pipetting accuracy.
- Clearly note your calculations, DNA concentrations and if you have done any dilutions

Step 3: Set up ligation reactions

- Total ligation volume: 20 µL

Component	*Amount/Volume*	***Ligation control***
T4 DNA Ligase Reaction Buffer (10x)	2 µL	2
Digested vector	0.02 pmol = 70 ng - Calculate volume based on plasmid concentration!	0.02 pmol = 70 ng
Digested insert	Calculate! 1-5 times the molar concentration of the vector	0 µL
T4 DNA ligase (5 U/µL)	1 Unit = 0.2 µL	1 Unit = 0.2 µL
Sterile MilliQ H2O	Adjust up to 20 µL	Adjust up to 20 µL

- Prepare:
    - Test ligation: vector + insert
    - Negative control: vector only (no insert)
    - **Work sterile**. Pipette into bottom of tubes as volumes are small. Mix by pipetting up and down.

Step 4: Incubation

- Incubate ligation reactions for 2 hours at room temperature (20–25°C). Alternatively at 10-16˚C overnight, or in the fridge (+4˚C).
- After incubation, store any remaining ligation mix at −20 °C if it will not be used immediately. However, it is preferable to proceed directly with the transformation.

Step 5: Controls for transformation

- Controls:
    - Positive control: Use 1 µL of undigested pET-11a plasmid for transformation. **No ligation buffer or ligase needed. No water either!**
    - Negative control: Use 10 µL of MilliQ H2O for transformation. This control serves as a test for contamination, and no colonies (or only few) should grow.
    - Ligation control: Use 10 µL of ligation control for transformation. This control is used as a measure of plasmid self-circularization.

Step 6: Transformation

- Use 10 µL of ligation reaction per transformation.
