Protocol: *E. coli* Transformation - Heat Shock Method

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This protocol was originally provided by our PI, Johan Bonde, and was adapted by last year's team, adding additional details, clarifications, and tips for practical use in the lab.

Part 1: Ligation with pET11a (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 (pET11a, 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): 5637 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*
T4 DNA Ligase Reaction Buffer (10x)	2 µL
Digested vector	100 ng - Calculate volume based on plasmid conc.!
Digested insert	Calculated! 3-5 times the molar conc of the vector
T4 DNA ligase (5 U/µL)	0.2 µL
H2O	Adjust up to 20 µL

- NOTE: Johan gave us a different ligation mixture, take a look into it!

- 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.
- 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: Positive control for transformation

- Prepare 1 µL of undigested pET-11a plasmid for transformation.
- **No ligation buffer or ligase needed. No water either!**

Step 6: Transformation

- Use 10 µL of ligation reaction per transformation.

Part 2: Transformation into Competent *E. coli* Cells

Materials

- Competent *E. coli* TG1 cells (200 µL aliquots)
- Ligation mixtures (Plasmid + inserts)
- Positive control plasmid (undigested)
- LB + Ampicillin agar plates (pre-warmed to room temp)
- Sterile water (H_2O)
- Sterile LB medium
- Ice bucket
- Sterile plastic spreaders

Instructions

- Work aseptically near a flame at all times.

1. Thaw Competent Cells

- Remove Eppendorf tubes of competent *E. coli* TG1 cells (200 µL aliquots per tube) from the -80°C freezer.
- Label them, for example as follows:
    - **S1** = Ligation mixture with insert 1
    - **S2** = Ligation mixture with insert 2
    - **Sx** = Ligation mixture with insert x
    - **N** = Negative control (digested plasmid only, no insert)
    - **P** = Positive control (undigested plasmid)
- Place all tubes **on ice immediately** and thaw for **30 minutes** without mixing.

2. Add DNA to Cells

- Work on ice. Do not let cells warm up.
- To each sample (S) tube of competent cells, add 10–50 ng of DNA (ideally <10 µL volume).
- For controls:
    - Negative control: Use 10 µL from ligation mixture with vector only (no insert).
    - Positive control: Add 1 µL of intact plasmid from plasmid prep (not digested).
- Mix gently but thoroughly by inverting the tubes 4–5 times. Do not vortex.
- Save the rest of your ligation mixtures in the freezer in case re-transformation is needed. Label everything clearly!

3. Incubate on Ice

- Incubate the tubes on ice for **40–60 minutes** (can extend up to 2 hours if needed).
- Do not mix during this step.

- Meanwhile, preheat your heating block to 42°C. Let it equilibrate at least 10 minutes before use to ensure uniform temperature.

4. Heat Shock

- Do not mix during these steps.
- Place tubes in a 42°C heating block for **45-60 seconds**.
- Immediately return tubes to ice for **5 minutes**.

5. Recovery

- Add 400 µL of sterile LB medium to each transformation tube.
- Mix the cells gently by inverting the tube.
- Incubate at 37°C, shaking at 150 rpm, for **45 minutes**. (No more than 60 minutes.)

6. Pellet and Resuspend Cells

- Centrifuge the tubes at maximum speed (e.g., 13,000 rpm) for **1 minute**. You can use the tabletop centrifuge on our bench.
- Carefully remove the supernatant with a pipette, leaving ~50 µL.
- Gently resuspend the cell pellet in the remaining supernatant by pipetting up and down a few times. Then plate the cells.

7. Plate the Transformed Cells

1. Label LB + Ampicillin plates clearly (you can find them in the fridge) Label the agar part of the plate, NOT THE LID:
    - Sample name (S1, S2, etc.)
    - Date
    - Your initials
2. Pipette the full 50 µL of resuspended cells onto the center of the agar plate.
3. Use a sterile L-shaped spreader:
    - If metal/glass, dip in ethanol and briefly flame; let it cool before spreading.
    - Gently spread the drop across the entire surface in a circular motion.
    - Do not press hard on the agar—just glide the liquid evenly.
4. Close the lid and let the plate sit for **5–10 minutes** to allow the liquid to absorb.
5. **Invert the plates (agar side up)** and place in an incubator.

8. Incubation

- Incubate inverted plates:
    - Overnight at 37°C, or
    - 30°C for 24–36 h, or
    - 25°C for 48 h

Expected Results

Tube	***Plate Type***	***Expected Result***
S1, S2, Sx …	LB + Amp	Colonies expected if transformation successful. Each colony = a single transformed cell.
N (Negative Control)	LB + Amp	No colonies or very few. Confirms that the vector alone (no insert) has low background (i.e., vector did not re-ligate easily).
P (Positive Control)	LB + Amp	Many colonies expected. Confirms that the competent cells were viable and the transformation process worked.

Expression controls for transformation in E.coli BL21:

Tube	***Plate Type***	***Expected Result***
P (positive control): empty vector control (E coli with vector only)	LB + Amp	Many colonies expected. Confirms that the competent cells were viable and the transformation process worked. (no expression of the protein of interest expected after induction with IPTG as no gene insert present (for SDS) → useful to check if the band is our protein of interest or there are other proteins in the same size range)
N (Negative Control): No plasmid control (just E coli without any plasmid)	LB + Amp	No colonies expected, does not carry the plasmid.
Uninduced control to check for leaks for the IPTG induced promoter (regular transformation of cloned plasmid in this case, this step is	LB + Amp	Many colonies expected. Confirms that the competent cells were viable and the transformation process worked.

introduced when doing induction with IPTG)		