

Standard Operation Procedure (S.O.P.) for Bacterial Transformation of an Expression Plasmid

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This S.O.P. will direct you in the procedure of bacterial transformation of your expression plasmid to amplify the amount of your target cDNA/vector that will be employed in subsequent procedures, such as preparation of glycerol stocks of your plasmid, DNA sequencing, transfection of cells lines for protein expression, generation of the Bacmid vector to be used for baculovirus-mediated protein expression, cRNA transcription of your target cDNA to be expressed in *Xenopus laevis* oocytes, among others. Transformation must be performed in competent *Escherichia coli* (*E. coli*) such as Top10, DH5 α , DH10B, DH5 α T1, DH10B T1, DH10Bac, BL21 (DE3), BL21 Star (DE3), BL21(DE3)pLysE, and BL21(DE3)pLysS.

Different factors could affect cell transformation, including

- The age of the bacterial cell.
- The amount of plasmid DNA used.
- Duration and intensity of the heat shock.
- Temperature and duration of the recovery period. Competent cells are highly sensitive to changes in temperature.

Important:

- **If the incubators and bath have not been recently used, check that the temperature of the water bath (42 °C) and the incubators (the orbital shaker and non-shaker incubator; 37 °C) are correctly set using a calibrated thermometer.**
- **This SOP is designed to be used in a volume of 50 μ L of chemically competent *E. coli* cells**
- **Chemical transformation procedure should be employed when trying to maximize the number of transformants obtained, when utilizing an antibiotic other than ampicillin to select your plasmid, or when transforming a plasmid containing a large insert (>1000 bp)**
- **Be sure you are using the Luria–Bertani (LB) recipe for the agar plates for your bacterial cell line and plasmid is correct. LB is also known as Lysogeny Broth. See the appendix for more information about the recipe.**
- **Be sure you are using the correct antibiotic and concentration in your LB-agar plates. See the appendix for more information about the concentrations.**
- **If the plasmid to be transformed comes from a ligation reaction, use 5 μ L of reaction. If the plasmid comes from a purified stock, use 5 ng of plasmid.**
- **If you are using One Shot TOP10 cells, IPTG is not required to induce expression from the lac promoter.**
- **All tips and microtubes must be autoclaved.**
- **All volumes and amounts are given on a per 50- μ L bacterial cells tube basis.**

Materials:

- 70% Alcohol
- Waste Container with Concentrated Bleach (2% Sodium Hypochlorite)
- Inoculation Loops
- DNA to be Transformed (purified plasmid at 1 ng/ μ L or ligation reaction)
- Micropipettes and Tips for 10, 100, and 1000 μ L
- Chemically Competent *E. coli* Cells (50 μ L per Tube, stored at –80 °C)
- 1.5, 2.0, or 5 mL Microtubes

- S.O.C. Media (stored at 4 °C)
- LB-Agar Plates with Appropriate Antibiotics (usually 10-cm diameter, stored at 4 °C)
- X-Gal Solution (optional, for blue/white selection)
- pUC19 Control Vector (optional, for control transformation)
- Ice Bucket
- Water Bath at 42 °C
- Incubator with Orbital Shaker at 37°C and 225 r.p.m
- Non-shaker Incubator at 37°C

Procedure:

Standard Transformation:

- a) Equilibrate the water bath to 42 °C and both incubators to 37 °C for at least 30 minutes before warming the **S.O.C Media** and **LB-Agar Plates**.
- b) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench.
- c) Prepare the waste container with 2% bleach for decontamination. **Any material that is in contact with intact bacteria must be decontaminated in this container for at least 30 minutes.**
- d) Determine the volume of **S.O.C. Media** that required, transfer the S.O.C. to an appropriate microtube, and warm the microtube in the shaker incubator (37 °C, 225 r.p.m.) until it is required.
 - A volume of 250 µL of **S.O.C. Media** is required for each 50-µL bacterial cells tube. Multiply 250 by the number of tubes to be transformed to determine the required S.O.C. volume.
Add 50 µL to the required volume to compensate for pipetting errors.
- e) Determine the number of **LB-Agar Plates** with antibiotics to be used and label the plates accordingly. Spread **X-Gal Solution** onto the plates, if desired for blue/white selection. Warm the plates upside up at 37°C in the non-shaker incubator until they are required.
 - For transformation of a ligation reaction use 3 plates per bacterial cells tube. For a plasmid stock use 2 plates per tube.
- f) Place the vial containing the **DNA** (ligation reaction or the purified plasmid) on ice.
- g) Determine the number of tubes containing 50 µL of bacterial cells to be use and thaw them on ice.
- h) Briefly centrifuge the tubes containing the **DNA** and the bacterial cells and place them on ice. Vortex the tube with **DNA** and transfer 5µL directly into the tube of bacterial cells. **Mix by gently tapping or brief vortex, not by pipetting.**
 - Plasmid at 1 ng/µL or the ligation reaction.
 - Pipetting may induce mechanical lysis.
 - The remaining **DNA** (tube) could be stored at -20 °C.
- i) Incubate the bacterial cells tube on ice for 30 minutes.
- j) Transfer the bacterial cells tube to the water bath (42°C) and incubate for exactly 30 seconds. **Do not centrifuge, mix, or shake the tube before or after incubation.**
- k) Remove the tube from bath and place them on ice.

- l) Add 250 μL of pre-warmed **S.O.C. Media** (37 $^{\circ}\text{C}$) to the bacterial cells tube, close the cap firmly, place the tube on its side on the shaker platform of the incubator (37 $^{\circ}\text{C}$), and secure the tube with tape. Shake the tube for exactly 1 hour at 225 r.p.m.
- m) Spread the appropriate volume of the transformation on separate, labeled **LB–Agar Plates**.
 - o For transformation of a ligation reaction, spread 40, 75, and 150 μL in three different plates.
 - o For transformation of a plasmid stock, spread 75 and 40 μL in two different plates.
 - o The remaining transformation mix may be stored at 4 $^{\circ}\text{C}$ and plated out the next day if desired.
- n) Place the **LB–Agar Plates** upside down in the non-shaker incubator (37 $^{\circ}\text{C}$) and incubate overnight.
- o) Decontaminate the work area with 70% ethanol. Carefully discard the bleach solution in the waste container into the proper waste container and discard the materials in a biological waste (trash can with red bag).
- p) Be sure all equipment are shut down: shaker incubator, water bath, pipettes, ect. Return any material and equipment to its proper place. The pipettes must be returned to their maximal volume.
- q) The next day the plates could be sealed with paraffin and stored at 4 $^{\circ}\text{C}$ for further processing, or colonies could be selected for preparing glycerol stocks (for long-term storage, if required) and plasmid amplification.

Control Transformation (in case it is necessary):

- a) Use **LB–Agar Plates** containing ampicillin.
- b) Transform 10 pg of the **pUC19 Control Vector** according to the standard transformation protocol (above).
- c) Add 20 μL of **S.O.C. Media** at 37 $^{\circ}\text{C}$ and 10 μL of the transformation onto a **LB–Agar Plate** and spread the solution around the plate.
- d) Incubate overnight at 37 $^{\circ}\text{C}$ and the next day count colonies. Calculate transformation efficiency using the formula below:

$$\frac{\# \text{ of colonies}}{10\text{pgtransformedDNA}} \times \frac{10^6\text{pg}}{\mu\text{g}} \times \frac{300\mu\text{L total transformation volume}}{X \mu\text{L plated}} = \frac{\# \text{ transformants}}{\mu\text{g plasmid DNA}}$$

Appendix:

LB Recipes (in g/L)

Ingredient	Luria	Lennox	Miller
Tryptone	10	10	10
Yeast Extract	5	5	5
NaCl	0.5	5	10

Antibiotics

Antibiotic	Concentrations ($\mu\text{g}/\text{mL}$)
Ampicillin	50–100
Kanamycin	50–100
Gentamycin	7
Zeocin	75–400
Neomycin	50
Streptomycin	50–100
Tetracycline	10