Protein Expression with T7 Express Strains

E. coli strains encoding the T7 RNA polymerase gene are especially useful for robust over-expression of recombinant protein. The NEB T7 Express strain is a BL21 derivative with several unique features. Importantly, the T7 RNA polymerase gene is expressed from the wild type *lac* promoter, resulting in a lower basal expression of the target protein than strains carrying the lambda DE3 prophage where T7 RNA polymerase expression is under *lacUV5* control.

However, T7 expression of recombinant protein is often improved by the co-expression of T7 lysozyme that binds to and inhibits T7 RNA polymerase function until the point of induction (1). To enable the expression of more difficult proteins, T7 Express derivatives were constructed to carry a single copy of either a T7 lysozyme gene (*lysY*), *lacI*^{q} gene or both (*lysY/I*^{q}) on a mini-F plasmid. The mini-F plasmids are stably maintained without antibiotic selection. The *lysY* gene encodes the T7 lysozyme variant K128Y, which lacks amidase activity yet retains T7 RNA polymerase inhibition (2). The T7 Express *lysY/I*^{q} strain is less susceptible to lysis during the over-expression of an inner-membrane protein than pLysS and pLysE strains. Strains encoding the *lysY* gene provide complete repression of T7 expression in the absence of an inducer molecule. Yet, a timecourse analysis indicates that T7 expression is rapidly activated (within 30 minutes) after induction. Therefore, the T7 Express *lysY* strains express an optimal level of lysozyme for maximal control of T7-mediated toxic protein expression.

Recommended Protocols

T7 Protein Expression

1. Transform expression plasmid into a T7 strain. Plate out on antibiotic selection plates and incubate overnight at 37°C.
2. Resuspend a single colony in 10 ml liquid culture with antibiotic.
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6.
4. Induce with 40 µl of a 100 mM stock of IPTG (final conc. = 0.4 mM) and induce for 2 hours at 37°C.
5. Check expression by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight.

T7 Express High Efficiency Sampler

Find the optimal level of expression for your experiments by purchasing the sampler. It contains 2 (200 µl ea.) tubes of each of our superior high efficiency T7 Expression strains. This is an exceptional value when compared to purchasing each strain separately. Each strain of chemically competent *E. coli* cells is suitable for high efficiency transformation and protein expression.

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Troubleshooting Tips

No colonies or no growth in liquid culture.

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expression protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In I^q strains, over-expression of the LacI^q repressor reduces basal expression of the T7 RNA polymerase.
  - In *lysY* strains, mutant T7 lysozyme is produced which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA ploymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues.
- Check antibiotic concentration (test with control plasmid)

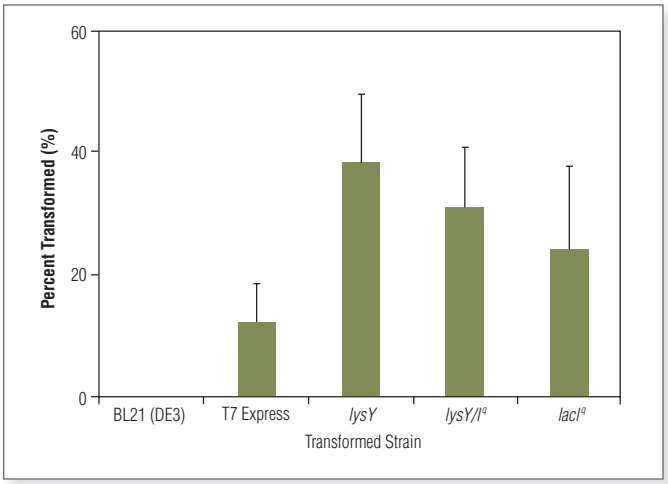
No protein visible on gel or no activity.

- Check for toxicity – the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test I^q and/or *lysY* strains to reduce basal level expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced protein is insoluble.

- T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:
  - Induce at lower temperatures (12–15°C overnight).
  - Reduce IPTG concentration to 0.01 mM – 0.1 mM.
  - Induce for less time (as little as 15 minutes).
  - Induce earlier in growth (OD600 = 0.3 or 0.4).

T7 Express Strains Allow Transformation and Expression of Toxic Clones



Each T7 expression strain was transformed with a plasmid containing a gene encoding a toxic mammalian protein. Comparison of the relative transformation efficiencies demonstrates that the T7 Express hosts provide the levels of control necessary for transformation of potentially toxic clones. BL21(DE3) could not be transformed with the toxic clone.

References

1. Studier, F.W. (1991) *J. Mol. Biol.* 219, 37–44.
2. Cheng, X. et al. (1994) *Proc. Natl. Acad. Sci. USA,* 91, 4034–4038.
3. Samuelson, J. et al. (unpublished results).

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Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, in practice, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocols

1. Thaw cells on ice for 10 minutes.
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing.
3. Place on ice for 30 minutes.
4. Heat shock at 42°C for 10–30 seconds or according to recommendations.
5. Place on ice for 5 minutes.
6. Add 950 µl of room temperature SOC.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle™ strains) or according to product recommendations.

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand.
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing.
3. Place on ice for 2 minutes.
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes.
6. Add 950 µl of room temperature SOC. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37–42°C. NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

DNA Contaminants to Avoid

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitation
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspension
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

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Transformation Tips

Thawing

- Cells are best thawed on ice.
- DNA should be added as soon as the last trace of ice in the tube disappears.
- Cells can be thawed by hand, but warming above 0°C decreases efficiency.

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium.
- Incubation while shaking or rotating the tube results in a 2-fold higher TE.

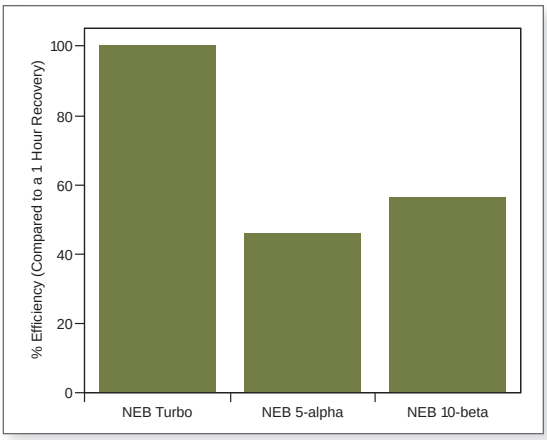
Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE.
- Warm, dry plates are easier to spread and allow for the most rapid colony formation.

DNA

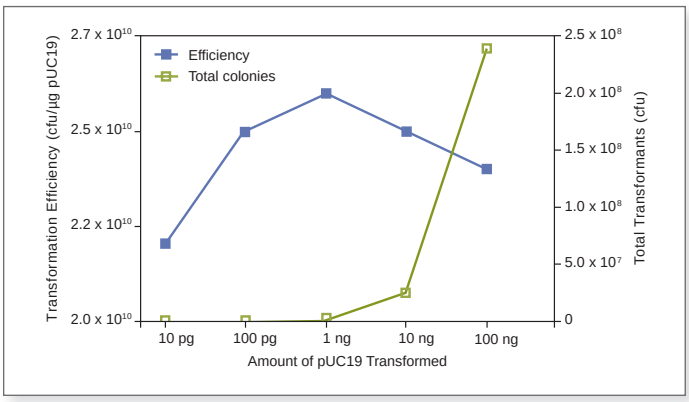
- DNA for transformation should be purified and resuspended in water or "TE" Buffer.
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency.
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation should be performed.
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total number of colonies that can be obtained from a single transformation reaction increases up to approximately 100 ng.

Effect of Outgrowth Period on Electroporation Efficiency of Electrocompetent Cells



Strains were electroporated with 10 pg of pUC19 DNA suspended in deionized water. Immediately after electroporation, SOC (pre-warmed to 37°C) was added to the cuvette to a final volume of 1 ml. The cells were diluted 1:1000 in SOC and plated on LB-ampicillin plates pre-warmed to 37°C. The electroporation efficiency of NEB Turbo is not dependent on the outgrowth period. Elimination of the 1 hour outgrowth period results in an 56% and 46% reduction in electroporation efficiency for NEB 5-alpha and NEB 10-beta, respectively.

DNA Effects on Transformation Efficiency and Colony Output



The optimal amount of DNA to use in a transformation reaction is lower than commonly recognized. Using clean, supercoiled pUC19 and NEB 5-alpha electrocompetent cells, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

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