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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.

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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)

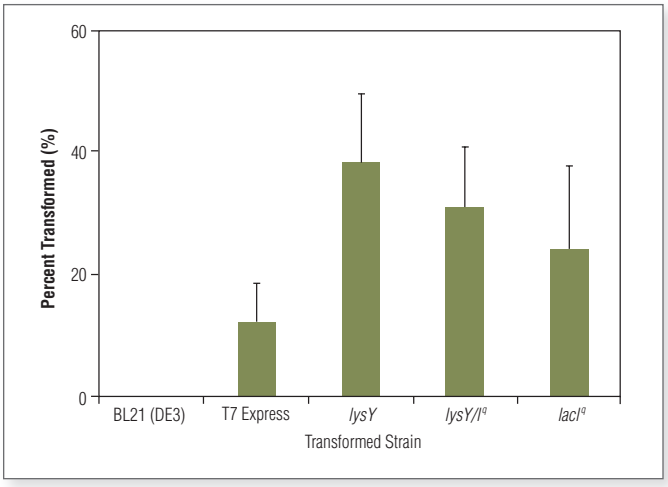
No protein visible on gel or no activity.

- 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 (OD_{600} = 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

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New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054
Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com