E. coli Lemo21(DE3)

A T7 RNA Polymerase-based protein overexpression platform for routine and difficult targets.

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Introduction

In almost all *E. coli* protein production strains, the overexpression of proteins is driven by T7 RNA polymerase. Finding the optimal conditions for the overexpression of a protein is usually based upon time consuming and laborious screens involving many different strains and culture/induction regimes. Lemo21(DE3) is a T7 strain designed for the expression of challenging proteins, including membrane proteins, toxic proteins and proteins with solubility issues. In Lemo21(DE3), T7 RNA polymerase activity can be modulated precisely by its natural inhibitor T7 lysozyme, which is expressed from the extremely well titratable rhamnose promoter (Figure 1)(1). However, Lemo21(DE3) is also well-suited for routine protein expression. The versatility of this strain makes it possible to identify the optimal conditions for the overexpression of any protein using only one strain and a limited number of culture/induction conditions, which is both time-saving and cost-effective.

General Protocol

1. Transform the T7 promoter based expression construct into Lemo21(DE3). Always use fresh transformants (not older than 4-5 days) for overexpression experiments. The Lemo21(DE3) strain contains pLemo, a pACYC184 derivative carrying the *lysY* gene. Accordingly, chloramphenicol (30 µg/ml) is required to maintain pLemo. In most cases, the T7 promoter-based expression vector will be compatible with pLemo.

2. Inoculate a single colony into 1 or 2 ml culture medium with the antibiotics required to maintain both pLemo and the overexpression vector, and grow overnight to produce a starter culture. Use medium without glucose for optimal strain performance.

3. To sample different expression levels, set up 10 ml expression cultures at the beginning of day 2 with various levels of L-rhamnose: for example 0, 100, 250, 500, 750, 1,000 and 2,000 µM. Inoculate each 10 ml expression culture with 0.2 ml of starter culture.

4. Culture at 30°C until OD_{600} reaches 0.4–0.8.

5. Induce with 40 µl of a 100 mM stock of IPTG (final concentration of 400 µM). IPTG should not be varied, only L-rhamnose concentration is varied. Induce for 4 hours to overnight at 30°C.

6. Check for expression after different induction times either by Coomassie stained protein gel, Western Blot or activity assay (Figure 2). Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction only. In the case of over-expressed membrane protein, most of the target should be in the low-speed spin supernatant after cell breakage by French Press, cell disruption or sonication (in combination with EDTA-lysozyme treatment).

   If a significant fraction of the target protein is insoluble (low speed pellet), repeat expression at a temperature lower than 30°C. Membrane protein expression may be improved by early induction (OD_{600} = 0.4) at 20 to 25°C.

7. For large scale, prepare liquid medium with antibiotics and the optimal level of L-rhamnose determined in a small scale trial. Both shake flasks and fermenters can be used for scaling up expression using Lemo21(DE3). For shake flasks, use 10 ml of freshly grown culture per liter for inoculation. Incubate at 30°C until OD_{600} reaches 0.4–0.8. Add 400 µM IPTG and express protein using optimal time/temperature determined in a small scale trial.

(see other side)

Materials

- Lemo21(DE3) Competent *E. coli* (NEB #C2528)

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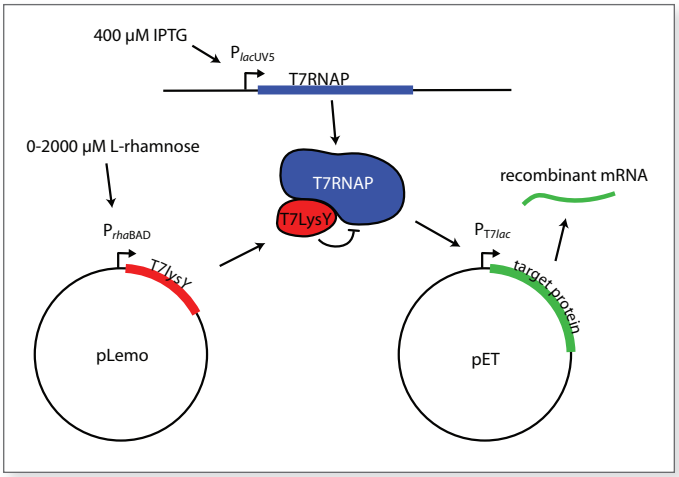
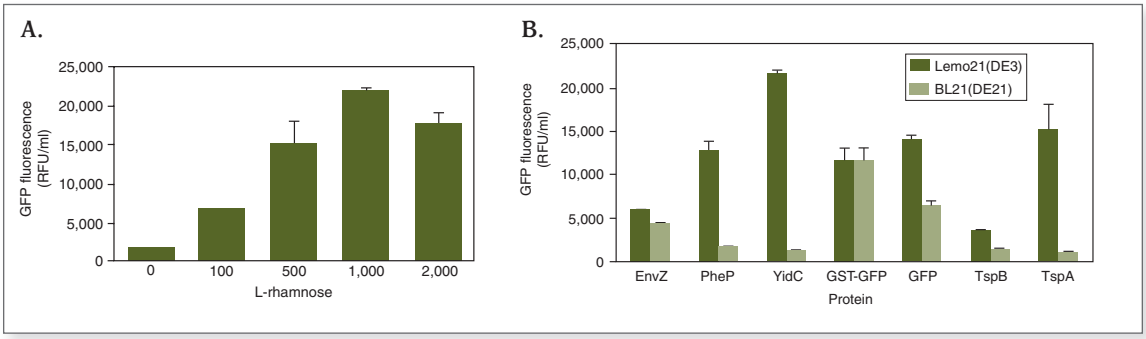
Results

Figure 1. Tuning protein expression in Lemo21(DE3)



In Lemo21(DE3) T7 RNA polymerase activity can be modulated precisely by its natural inhibitor T7 lysozyme, which is expressed from the extremely well titratable rhaBAD promoter. The combination of PlacUV5 expression of T7 RNA polymerase from the chromosome and rhamnose inducible expression of T7 lysozyme from pLemo guarantees the greatest possible range of target protein expression.

Figure 2. Optimizing protein overexpression in Lemo21(DE3)



A. Optimizing expression of the membrane protein YidC in Lemo21(DE3). To monitor expression levels of the membrane protein YidC, green fluorescent protein (GFP) was fused to its C-terminus. Thus, GFP fluorescence may be used as a direct measure of protein overexpression yields in whole cells (2,3,4). Cells were cultured in Luria Bertani medium in the presence of different concentrations of L-rhamnose. B. Comparison of protein overexpression in BL21(DE3) and Lemo21(DE3). All proteins were C-terminally fused to GFP. Cells were cultured in Luria Bertani medium and whole cell fluorescence was measured eight hours after induction with IPTG. For expression of target proteins in Lemo21(DE3) the optimal rhamnose concentration was used. The target proteins are: bacterial membrane proteins EnvZ, PheP and YidC; human membrane proteins Tetraspanin A and B (TspA and TspB); soluble proteins Glutathione S-transferase and GFP alone. For graphical reasons, fluorescence values of TspA and TspB were multiplied by 10, and fluorescence values of GST-GFP and GFP were divided by 10 and 50, respectively.

Summary

The T7 expression strain Lemo21(DE3) allows researchers to sample a wide range of expression levels to find the optimal conditions for each unique target protein. More specifically, Lemo21(DE3) can be used for the expression of routine and difficult targets, eliminating the testing of multiple strains to achieve desired expression levels. This can result in both time and cost savings.

References

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