Protocol for Lemo21(DE3) Competent *E. coli*

5 Minute Transformation Protocol (C2528)

Introduction

A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable.

Follow the Transformation Protocol with the following changes:

Protocol

- Steps 3 and 5 are reduced to 2 minutes.
- Omit outgrowth (step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Protocol for Protein Expression Using Lemo21(DE3) (C2528)

Protocol

1. Transform expression plasmid into Lemo21(DE3). Transformation plates must contain antibiotic to select the expression plasmid and 30µg/ml chloramphenicol to maintain pLemo, a pACYC184 derivative with a p15A origin of replication. Incubate overnight at 37°C.
2. Resuspend a single colony in a 1 or 2 ml liquid culture with both antibiotics and grow overnight to produce a starter culture. Use media 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 for difficult targets. Inoculate each expression culture with 0.2 ml of starter culture.
4. Incubate at 37°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 5 hours to overnight at 30 or 37°C.
6. Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. 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 lower temperature (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 1 Liter of liquid medium with antibiotics and the optimal level of L-rhamnose determined in a small scale trial. Inoculate with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°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.

Transformation Protocol (C2528)

Introduction

Perform steps 1–7 in the tube provided.

Protocol

. Thaw a tube of Lemo21(DE3) Competent *E. coli* cells on ice for 10 minutes.
. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex.**
. Place the mixture on ice for 30 minutes. Do not mix.
. Heat shock at exactly 42°C for exactly 10 seconds. Do not mix.
. Place on ice for 5 minutes. Do not mix.
. Pipette 950 µl of room temperature LB or SOB into the mixture.
. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
. Warm selection plates to 37°C.
. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in LB or SOB.
0. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or at 25°C for 48 hours.