Protocol for BL21(DE3) Competent *E. coli*

5 Minute Transformation Protocol (C2527)

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 BL21(DE3) (C2527)

Protocol

1. Transform expression plasmid into BL21(DE3). Plate 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 0.4–0.8.
4. Induce with 4 or 40 µl of a 100 mM stock of IPTG (final concentration of 40 or 400 µM) and induce for 3 to 5 hours at 37°C.
5. 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.
   *If a fraction of the target protein is insoluble, repeat expression at a lower temperature (15 to 30°C) or test expression in Lemo21(DE3) (NEB #C2528).
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 0.4–0.8. Add 40 or 400 µM IPTG and express protein using optimal time/temperature determined in a small scale trial.

Transformation Protocol (C2527)

Introduction

Perform steps 1–7 in the tube provided.

Protocol

1. Thaw a tube of BL21(DE3) Competent *E. coli* cells on ice for 10 minutes.
2. 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.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 10 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. 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.