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Transformation Protocol for BL21(DE3) Competent Cells (C2527I) V.2

Forked from a private protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This transformation protocol is for the C2527I cells. (For the C2527H protocol, see **here**.)

Guidelines

Transformation Protocol Variables

Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes you shorten this step.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 10 seconds at 42°C is optimal.

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 transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.

Chemically competent *E. coli* cells suitable for transformation and protein expression.

Highlights

- Transformation efficiency: 1–5 × 10^7cfu/µg pUC19 DNA
- T7 Expression Strain
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhu*A2)
- B Strain
- Free of animal products

Genotype

fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS
λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5

Materials

MATERIALS

BL21(DE3) Competent E.coli - 6×0.2 ml **New England Biolabs Catalog #**C2527I

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Before start

Perform steps 2–9 in the tube provided.

1 Thaw a tube of BL21(DE3) Competent *E. coli* cells On ice until the last ice crystals disappear.

2 Mix gently and carefully pipette 50 µL cells into a transformation tube On ice .

3 Add 1 µL – 5 µL containing 1 pg – 100 ng plasmid DNA to the cell mixture.

4 Carefully flick the tube **4–5 times** to mix cells and DNA. **Do not vortex.**

5 Place the mixture On ice for 00:30:00 . Do not mix.

6 Heat shock at exactly 42 °C for exactly 00:00:10 . Do not mix.

7 Place On ice for 00:05:00 . Do not mix.

8 Pipette 950 µL of Room temperature SOC into the mixture.

9 Place at 37 °C for 01:00:00 . Shake vigorously (250 rpm) or rotate.

10 Warm selection plates to 37 °C .

11 Mix the cells thoroughly by flicking the tube and inverting.

12 Perform several 10-fold serial dilutions in SOC.

13 Spread 50 µL – 100 µL of each dilution onto a selection plate and incubate Overnight at 37 °C .

Note

Alternatively, incubate at 30 °C for 24:00:00 – 36:00:00 or at 25 °C for 48:00:00 .