Transformation Protocol for BL21(DE3) Competent Cells (C2527H)

New England Biolabs

Abstract

This transformation protocol is to be performed directly in the C2527H tubes. (For the C2527I protocol, see here.)

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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.

Materials

BL21(DE3) Competent E.coli - 20x0.05 ml C2527H by New England Biolabs

Protocol

Step 1.
Thaw a tube of BL21(DE3) Competent E. coli cells on ice for 10 minutes

DURATION
00:10:00

Step 2.
Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture

Step 3.
Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex**

Step 4.
Place the mixture on ice for 30 minutes. Do not mix

DURATION
00:30:00

Step 5.
Heat shock at exactly 42°C for exactly 10 seconds. Do not mix

DURATION
00:00:10

Step 6.
Place on ice for 5 minutes. Do not mix

DURATION
00:05:00

Step 7.
Pipette **950 µl** of room temperature SOC into the mixture

AMOUNT
950 µl Additional info:

Step 8.
Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate

DURATION
01:00:00

Step 9.
Warm selection plates to 37°C

Step 10.
Mix the cells thoroughly by flicking the tube and inverting

Step 11.
Perform several 10-fold serial dilutions in SOC

Step 12.
Spread **50–100 µl** of each dilution onto a selection plate and incubate overnight at 37°C

DURATION
15:00:00

NOTES
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Alternatively, incubate at 30°C for 24–36 hours or at 25°C for 48 hours.