

Transformation Protocol for BL21(DE3) Competent Cells (C2527I)

New England Biolabs

Abstract

This transformation protocol is for the C2527I cells. (For the C2527H 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 - 6x0.2 ml [C2527I](#) by [New England Biolabs](#)

Protocol

Step 1.

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



REAGENTS



BL21(DE3) Competent E.coli - 6x0.2 ml [C2527I](#) by [New England Biolabs](#)



ANNOTATIONS

Phillip Venter 14 Feb 2018

Does it make a difference if you use ice in water, "wet ice"?

Step 2.

Mix gently and carefully pipette **50 µl** of cells into a transformation tube on ice



ANNOTATIONS

Phillip Venter 14 Feb 2018

Hi. When using the C2527I kit you receive 0.2 ml in 6, 2 ml reaction tubes. You therefor have to transfer 50 **µl into another tube not supplied. It is said in your FAQ that using Eppendorf 1.5 ml tubes** makes no difference in the transformation efficiency - but is the heat shock time (10 seconds) still the same? Should it be longer in the other tubes?

Step 3.

Add 1-5 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture

Step 4.

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

Step 5.

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



DURATION

00:30:00

Step 6.

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



DURATION

00:00:10



ANNOTATIONS

Phillip Venter 14 Feb 2018

Is the heat shock time the same if 1.5 ml Eppendorf tubes are used?

Step 7.

Place on ice for 5 minutes. Do not mix



DURATION

00:05:00

Step 8.

Pipette **950 µl** of room temperature SOC into the mixture

AMOUNT

950 µl Additional info:

Step 9.

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

DURATION

01:00:00

Step 10.

Warm selection plates to 37°C

Step 11.

Mix the cells thoroughly by flicking the tube and inverting

Step 12.

Perform several 10-fold serial dilutions in SOC

Step 13.

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.