High Efficiency Transformation Protocol using NEB 10-beta Competent E. coli (C3019I) Version 5

New England Biolabs

Abstract

This is the correct protocol if you are using the C3019I cells. If you are using the C3019H cells, please refer to this protocol.

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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 this step is shortened.

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, 30 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 this step is shortened. 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.

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate

Ethanol or Isopropanol	Dry pellet before resuspending
PEG*	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g. Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

* Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Materials

NEB 10-beta Competent E.coli (High Efficiency) - 6x0.2 ml C3019I by New England Biolabs

Protocol

Step 1.

C3019I: Thaw a tube of NEB 10-beta Competent *E. coli* cells on ice until the last ice crystals disappear.

REAGENTS
NEB 10-beta Competent E.coli (High Efficiency) - 6x0.2 ml C3019I by New England Biolabs

NOTES
New England Biolabs 17 Sep 2014

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.

Step 2.
Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.

AMOUNT
50 µl Additional info:

REAGENTS
NEB 10-beta Competent E.coli (High Efficiency) - 6x0.2 ml C3019I by New England Biolabs

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

NOTES

New England Biolabs 17 Sep 2014

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 this step is shortened.

Step 6.
Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.

DURATION
00:00:30

NOTES

New England Biolabs 17 Sep 2014

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, 30 seconds at 42°C is optimal.

Step 7.
Place on ice for 5 minutes. Do not mix.

DURATION
00:05:00

Step 8.

Pipette 950 µl of room temperature NEB 10-beta/Stable Outgrowth Medium into the mixture.

AMOUNT
950 µl Additional info:

Step 9.
Place at 37°C for 60 minutes., shaking vigorously (250 rpm) or rotating.

DURATION
01:00:00

NOTES

New England Biolabs 17 Sep 2014

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 this step is shortened. 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.

Step 10.
Warm selection plates to 37°C.

NOTES

New England Biolabs 17 Sep 2014

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.

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

Step 12.

Perform several 10-fold serial dilutions in NEB 10-beta/Stable Outgrowth Medium.

NOTES
New England Biolabs 22 Jun 2017

Please note that NEB no longer recommends SOC for use in this protocol. NEB 10-beta/Stable Outgrowth Medium is now provided with NEB 10-beta Competent E.coli and will be available also as a stand-alone product in the fall of 2017.

Step 13.
Spread 50-100 µl of each dilution onto a selection plate

Step 14.
Incubate overnight at 37°C

DURATION
15:00:00

NOTES
New England Biolabs 02 Oct 2014
Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.