High Efficiency Transformation Protocol (modified)

Harold Bien

Abstract

This is a modified protocol from the original NEB splitting the 50uL aliquot into two separate tubes for dual reactions from a single tube.

Citation: Harold Bien High Efficiency Transformation Protocol (modified). **protocols.io** dx.doi.org/10.17504/protocols.io.dq45yv
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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 5-alpha Competent E.coli (High Efficiency) - 20x0.05 ml C2987H by New England Biolabs

Protocol

Step 1.
Warm SOC media to room temperature

REAGENTS
SOC Outgrowth Medium - 100 ml B9020S by New England Biolabs

Step 2.
Thaw a tube of NEB 5-alpha Competent E. coli cells on ice for 10 minutes.

AMOUNT
50 µl Additional info:

REAGENTS
NEB 5-alpha Competent E.coli (High Efficiency) - 20x0.05 ml C2987H by New England Biolabs

DURATION
00:10:00

Step 3.
Aliquot 25uL of the cells into a new tube

AMOUNT
25 µl Additional info:

REAGENTS
NEB 5-alpha Competent E.coli (High Efficiency) - 20x0.05 ml C2987H by New England Biolabs

Step 4.
Add 0.5-2.5 µl containing 0.5 pg-50 ng of plasmid DNA to each cell mixture.

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

Step 6.
Place the mixture on ice for 30 minutes. Do not mix.

DURATION
00:30:00

Step 7.
Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.

DURATION
00:00:30

Step 8.
Place on ice for 5 minutes. Do not mix.

DURATION
00:05:00

Step 9.
Pipette 475 µl of room temperature SOC into the mixture in each tube

AMOUNT
475 µl Additional info:

REAGENTS
SOC Outgrowth Medium - 100 ml B9020S by New England Biolabs

Step 10.
Place at 37°C for 60 minutes, shaking vigorously (250 rpm) or rotating.

DURATION
01:00:00

Step 11.
Warm selection plates to 37°C.

Step 12.
Mix the cells thoroughly by flicking the tube and inverting.

REAGENTS
SOC Outgrowth Medium - 100 ml B9020S by New England Biolabs

Step 13.
Perform several 10-fold serial dilutions in SOC.

Step 14.
Spread 50-100 µl of each dilution onto a selection plate

Step 15.
Incubate overnight at 37°C

DURATION
15:00:00