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High Efficiency Transformation Protocol using NEB 10-beta Competent *E. coli* (C3019) V.6

New England Biolabs[1]

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This is the High Efficiency Transformation Protocol for C3019H and C3019I cells.

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Transformation , Bacterial, C3019I, C3019, C3019H

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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.

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MATERIALS

NEB 10-beta Competent E.coli (High Efficiency) - 20x0.05 ml **New England Biolabs Catalog #C3019H**

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

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

1 Please select whether you have C3019H or C3019I cells.
Step 1 includes a Step case.
C3019H
C3019I

step case

C3019H

2 Thaw a tube of NEB 10-beta Competent *E. coli* cells **On ice** for **00:10:00** .

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.

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.

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.

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6 Heat shock at exactly **42 °C** for exactly **00:00:30** . Do not mix.

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.

7 

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

8 

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

NEB 10-beta/Stable Outgrowth Medium gives 2-fold higher transformation efficiency than LB medium.

9 

Place at **37 °C** for **01:00:00** , shaking vigorously (**250 rpm**) or rotating.

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. Incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

10 Warm selection plates to **37 °C** .

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.

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Mix the cells thoroughly by flicking the tube and inverting.

12

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

> 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 available also as a stand-alone product.

13

Spread **50 µL** - **100 µL** of each dilution onto a selection plate.

14

Incubate **Overnight** at **37 °C** .

> Alternatively, incubate at **30 °C** for 24-36 hours or **25 °C** for **48:00:00** .

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