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High Efficiency Transformation Protocol (C2987H) V.3

New England Biolabs[1]

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New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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This is the protocol for C2987H cells. If you are using the C2987I cells, please refer to this protocol.

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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

A	B
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 (NEB #T1030) or phenol/chloroform extraction and ethanol precipitation should be added.

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MATERIALS

SOC Outgrowth Medium - 100 ml **New England Biolabs Catalog #B9020S**

NEB 5-alpha Competent E. coli cells **New England Biolabs Catalog #C2987H**

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

For this protocol, perform steps 1-8 in the tube provided.

1 Thaw a tube of NEB 5-alpha 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.

2 

Add **1 µL** - **5 µL** containing **1 pg** - **100 ng plasmid DNA** to the cell mixture.

3 

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

4 

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.

5 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

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the transformation volume and vessel. Using the transformation tube provided, 30 seconds at 42°C is optimal.

6 

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

7 

Pipette **950 µL room temperature SOC** into the mixture.

8 

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. 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.

9 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.

10 

Mix the cells thoroughly by flicking the tube and inverting.

11 Perform several 10-fold serial dilutions in SOC.

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12 

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

13 

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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