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Electroporation Protocol (C2986) V.2

New England Biolabs[1]

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NEB Turbo Electrocompetent *E. coli* cells are suitable for high efficiency electroporation and rapid colony growth. These cells are ideal for DNA library constructions and all cloning purposes.

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Features

Electroporation Tips

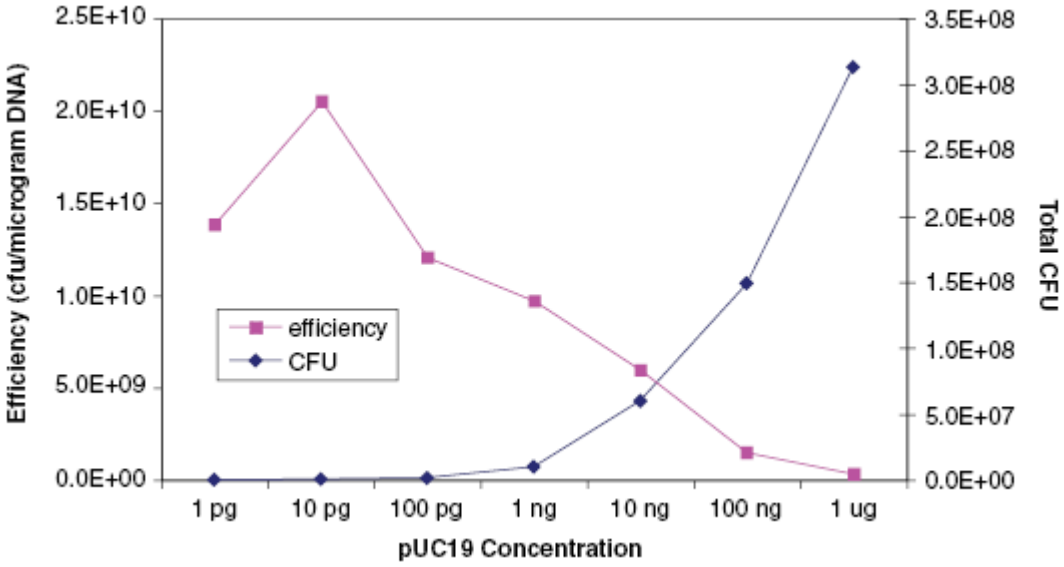
1. Electroporation cuvettes and microcentrifuge tubes should be pre-chilled on ice.
2. Electrocompetent cells should be thawed on ice and suspended well by carefully flicking the tubes.
3. Once DNA is added to the cells, electroporation can be carried out immediately. It is

not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.

4. Contaminants such as salts and proteins can lower electroporation efficiency. Ideally, DNA for transformation should be purified and suspended in water or TE. Transformation efficiency is more than10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns (NEB #T1030) are recommended for cleanup of ligation reactions.
5. Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators or cuvettes not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1mm gap are recommended (e.g. BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage is required for cuvettes with 2 mm gap.
6. Arcing may occur due to high concentration of salts or air bubbles.
7. It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.
8. Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
9. Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

Application Features

DNA Effects of Transformation Efficiency and Colony Output:



Electroporation efficiency remains extremely high up to about 1 ng of input DNA, then decreases at higher DNA concentrations.

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MATERIALS

NEB Turbo Electrocompetent E.coli - 6x0.1 ml **New England Biolabs Catalog #C2986K**

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

Preparation

1 Prepare 17 mm x 100 mm round-bottom culture tubes (e.g. VWR #60818-667) at **Room temperature** .

2 Place SOC recovery medium in a **37 °C** water bath.

3 Pre-warm selective plates at **37 °C** for **01:00:00** .

Electroporation

4 Place electroporation cuvettes (1 mm) and microcentrifuge tubes **On ice** .

5 As a positive control for transformation, dilute the control pUC19 by 1:5 to a final concentration of **10 pg/µl** using sterile water.

> Heat-denatured ligation reactions can be used for electroporation directly; however, column purification is recommended.

6

Thaw NEB Turbo Electrocompetent cells **On ice** (about **00:10:00**) and mix cells by flicking gently.

7 Transfer **25 µL cells** (or the amount specified for the cuvettes) to a chilled microcentrifuge tube.

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Add **1 µL DNA solution** .

9 Carefully transfer the cell/DNA mix into a chilled cuvette without introducing bubbles and make sure that the cells deposit across the bottom of the cuvette.

10 Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators:
2.1 kV, 100 Ω, and 25 µF

The typical time constant is ~2.6 milliseconds.

11 

Immediately add **975 µL 37°C SOC** to the cuvette.

12 

Gently mix up and down **twice**.

13 Transfer to the 17 mm x 100 mm round-bottom culture tube.

14 Shake vigorously (**250 rpm**) or rotate at **37 °C** for **01:00:00** .

15 Dilute the cells as appropriate, then spread **100 µL** - **200 µL cells** onto a pre-warmed selective plate.

16 

Incubate plates **08:00:00** to **Overnight** at **37 °C** .

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