Electroporation Protocol (C2986)

New England Biolabs

Abstract

This electroporation protocol is for use with the NEB Turbo Electrocompetent E. coli cells (C2986). These cells are suitable for high efficiency electroporation and rapid colony growth; they are ideal for DNA library constructions and all cloning purposes.

Citation: New England Biolabs Electroporation Protocol (C2986). **protocols.io**
dx.doi.org/10.17504/protocols.io.crgv3v
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Materials

NEB Turbo Electrocompetent E.coli - 6x0.1 ml C2986K by New England Biolabs

Protocol

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

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

Step 3.
Pre-warm selective plates at 37°C for 1 hour

DURATION
01:00:00

Step 4.
Place electroporation cuvettes (1mm) and microcentrifuge tubes on ice

Step 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

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

Step 6.
Thaw NEB Turbo Electrocompetent cells on ice (about 10 min) and mix cells by flicking gently

DURATION
00:10:00

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

AMOUNT

25 µl Additional info:

Step 8.

Add **1 µl** of the DNA solution

AMOUNT

1 µl Additional info:

Step 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

AMOUNT

1 µl Additional info:

Step 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

Step 11.

Immediately add **975 µl** of 37°C SOC to the cuvette

AMOUNT

975 µl Additional info:

Step 12.

Gently mix up and down twice

Step 13.

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

Step 14.

Shake vigorously (250 rpm) or rotate at 37°C for 1 hour

DURATION

01:00:00

Step 15.

Dilute the cells as appropriate then spread 100-200 µl cells onto a pre-warmed selective plate

Step 16.

Incubate plates 8 hours to overnight at 37°C.

DURATION

08:00:00