Electroporation Protocol

New England Biolabs

Abstract

This protocol may be used with electrocompetent cells prepared by you according to this protocol.

Citation: New England Biolabs Electroporation Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.cruv6v
Published: 03 Feb 2015

Guidelines

Appropriate Antibiotics for Your Application

Antibiotics for Plasmid selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Electroporation Protocol

The electroporation protocol will vary depending on the strain so this protocol may need to be optimized. For control electroporation dilute pUC19 to 10 pg/µl with Milli-Q water.

Calculation:

If the culture was diluted 1000-fold when plated, the total cfu per ml is 1000 times the number of colonies counted. The cfu is divided by the amount of pUC19 (10 pg per ml)

cfu/ µg = (colonies counted*1000) / (0.00001 µg pUC19)

Before start

For control electroporation dilute pUC19 to 10 pg/µl with Milli-Q water.

Protocol

Step 1.
Turn on electroporator and set to 1.7-2.5 kv (optimize for strain), 200 ohms and 25 µF

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

PROTOCOL

. SOC Media

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Step 2.1.
SOB Media

PROTOCOL

. SOB Media

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Step 1.1.
2% tryptone

Step 1.2.
0.5% yeast extract

Step 1.3.
10 mM NaCl

Step 1.4.
2.5 mM KCl

Step 1.5.
10 mM MgCl2

Step 1.6.
10 mM MgSO4

Step 2.2.
20 mM glucose

Step 3.
Pre-warm LB-antibiotic plates at 37°C

Step 4.
Thaw cells on ice for 10 min or use freshly made cells

DURATION
00:10:00
Step 5.
Place appropriate number of microcentrifuge tubes and 1 mm-electroporation cuvettes on ice

Step 6.
Flick the tube containing cells a few times to mix and add **25 µl** to the microcentrifuge tubes

AMOUNT
25 µl Additional info:
Step 7.

Add **1 µl** of a 10 pg/µl DNA solution (in DI water) to the cells in the microcentrifuge tube

AMOUNT

1 µl Additional info:

Step 8.

Transfer the DNA-cell mixture to the cold cuvette, tap on countertop 2X, wipe water from exterior of cuvette and place in the electroporation module and press pulse (**don't hold the button down**)

Step 9.

Immediately add **975 µl** of 37°C SOC, mix by pipetting up and down once and transfer to a 15 ml-falcon tube

AMOUNT

975 µl Additional info:

Step 10.

Rotate in the 37°C incubator for 1 h

DURATION

01:00:00

Step 11.

Make appropriate dilutions

NOTES

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When using 10 pg of DNA, make two dilutions:

Dilute 10 µl cells into 990 µl SOC and plate 100 µl. (1000-fold dilution)

Dilute 100 µl cells into 900 µl SOC and plate 100 µl. (100-fold dilution)

Step 12.

Incubate overnight at 37°C

DURATION

15:00:00

Warnings

The electroporation protocol will vary depending on the strain so this protocol may need to be optimized.