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Electroporation Protocol

Forked from Electroporation Protocol

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In Development dx.doi.org/10.17504/protocols.io.bkpukvnw

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ABSTRACT

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

EXTERNAL LINK

https://www.neb.com/protocols/2012/06/21/making-your-own-electrocompetent-cells

DOI

dx.doi.org/10.17504/protocols.io.bkpukvnw

PROTOCOL CITATION

Ken Christensen 2020. Electroporation Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkpukvnw

EXTERNAL LINK

https://www.neb.com/protocols/2012/06/21/making-your-own-electrocompetent-cells

FORK FROM

Forked from Electroporation Protocol, New England Biolabs

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41428

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)

SAFETY WARNINGS

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

BEFORE STARTING

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

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

2 Place recovery SOC in 37°C water bath

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

4 Thaw cells on ice for 10 min or use freshly made cells
00:10:00

5 Place appropriate number of microcentrifuge tubes and 1 mm-electroporation cuvettes on ice

6 Flick the tube containing cells a few times to mix and add **25 µl** of competent cells to the microcentrifuge tube
25 µl

7 Add **1 µl** of a 100 pg/µl to 1 ng/µl DNA solution (in DI water) to the cells in the microcentrifuge tube
1 µl

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 (**you don't hold the button down**)

9 Immediately add **975 µl** of 37°C SOC, mix by pipetting up and down once and transfer to a microcentrifuge tube, 5 ml

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culture tube, or 15 ml centrifuge tube.

975 µl

10 Place in the shaker/incubator at 37°C incubator for 1 h

01:00:00

11 Make appropriate dilutions

When using 100 pg - 1 ng of DNA, make three dilutions:
Dilute 1 µl of cells into 990 µl SOC and plate 100 µl. (10000-fold dilution)
Dilute 10 µl of cells into 990 µl SOC and plate 100 µl. (1000-fold dilution)
Dilute 100 µl of cells into 900 µl SOC and plate 100 µl. (100-fold dilution)

12 Incubate overnight at 37°C

16:00:00

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