2

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Electroporation Protocol V.2

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

DOI

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

New England Biolabs 2022. Electroporation Protocol. **protocols.io**
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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)

MATERIALS

Magnesium sulfate heptahydrate **Sigma Aldrich Catalog #M2773**

NaCl **Sigma Aldrich Catalog #53014**

Tryptone **Fisher Scientific Catalog #BP1421-500**

Glucose **Sigma Aldrich Catalog #G8270**

Magnesium chloride hexahydrate **Sigma Aldrich Catalog #M2670**

Potassium chloride **Sigma Aldrich Catalog #P9333**

Glycerol **Thermo Fisher Scientific Catalog #17904**

Yeast Extract **Thermo Fisher Catalog #211930**

Media

SOB:
2% tryptone
0.5% yeast extract
10 mM NaCl
2.5 mM KCl
10 mM MgCl2
10 mM MgSO4

SOC:
SOB + 20 mM glucose

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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

Sterile 10% glycerol (can be autoclaved) is needed for the washes. The volume of 10% glycerol needed is 2X the culture volume (for example, a 500 ml culture requires 1L of 10% glycerol).

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

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

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

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 **00:10:00** or use freshly made cells.

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

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5 **On ice** .

6 

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

7 

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

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**).

9 

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

10 

Rotate in the **37 °C** incubator for **01:00:00** .

11 

Make appropriate dilutions.

When using **10 pg 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)

12 

Incubate **Overnight** at **37 °C** .

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