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# Electroporation Protocol

Forked from Electroporation Protocol

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**Protocol status:** In development
**We are still developing and optimizing this protocol**

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## Abstract

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

## 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 start

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 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

Note

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