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

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Electroporation Protocol

Forked from [Electroporation 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 μ g/ml
Kanamycin	30 μ g/ml
Streptomycin	25 μ g/ml
Tetracycline	15 μ g/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/ μ l 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)

$$\text{cfu} / \mu\text{g} = (\text{colonies counted} \times 1000) / (0.00001 \mu\text{g pUC19})$$

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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