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

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medium

Preparation and transformation of electrocompetent cells

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ABSTRACT

A protocol for electroporation of E. coli. Other bacteria may work, with optimization of transformation buffer and settings.

MATERIALS

Centrifuge

LAF

Vortex

Incubator

Electroporator

Preparation of GYT medium

1 In a sterile flask, add 🗸 400 mL distilled water

2	Measure and add $\ \ \ \ \ \ \ \ \ \ \ \ \ $
	Materials:
	Select Yeast Extract Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0875
	⊠ Glycerol MP Biomedicals Catalog #194680

Filter sterilize through a 0.22-μm filter, and store in aliquots at 4 °C

Preparation of cells

Inoculate 500 mL of prewarmed LB medium from 25 mL overnight E. coli culture. Incubate at and 300 rpm. Measure OD every 20 minutes, until OD $_{600}$ =0.4

Note

The density is usually archived after ${\sim}2.5$ hours of incubation with DH5 α

- Transfer the culture to appropriate centrifugation containers, and cool on ice for © 00:30:00
- 6 Centrifuge culture at

 ⊕ 1000 rcf, 4°C, 00:15:00 , and resuspend pellet in
 water

 200 mL ice-cold

 15m

Note

Combine the culture into a smaller number of centrifugation containers

7 Centrifuge culture at 1000 rcf, 4°C, 00:20:00, and resuspend pellet in 100 mL ice-cold 10% glycerol

20m

30m

- 8 Centrifuge culture at 1000 rcf, 4°C, 00:20:00, and resuspend pellet in 10% glycerol
- 9 Centrifuge culture at

 1000 rcf, 4°C, 00:20:00 , and resuspend pellet in
 2 mL ice-cold GYT 20m medium

Note

This is best done by gentle swirling rather than pipetting or vortexing

Measure OD_{600} of a 1:100 dilution of the cell suspension, and dilute the cells to between 2-3 × 10^{10} cells/mL

Note

 $1.0 \text{ OD}_{600} = \sim 2.5 \text{ X } 10^8 \text{ cells/ml}$

- Transfer 40 µl of the suspension to an ice-chilled electroporation cuvette and test whether arching occurs when an electrical discharge is applied. If so, wash the remainder of the cell suspension once more with ice-cold GYT medium to ensure that the conductivity of the bacterial suspension is sufficiently low

Transformation

1h 5m

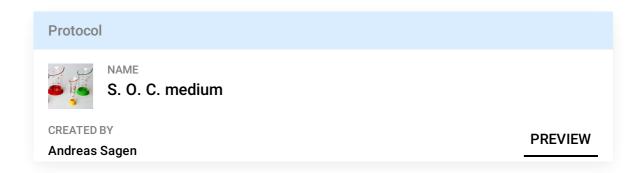
20m

- Pre-chill cuvettes on ice for 00:05:00, and pre-heat LB plates with an appropriate selection agent and SOC medium at 37 °C for 01:00:00
- Mix an appropriate amount plasmid to an aliquot of electrocompetent cells and transfer to a prechilled cuvette

1h

Note

100 pg pUC19 plasmid is appropriate in most cases, but a specific amount plasmid depend on many different factors and have to be optimized



- 15 Incubate plasmid-suspension mix for 1 minute, then perform electroporation with optimized settings
- Flush L 1 mL pre-heated S. O. C. medium, then transfer to a culture tube and recover at a 37 °C and 200 rpm shaking for 01:00:00
- 17 Plate an appropriate amount culture on selection agar plates