Preparation and transformation of electrocompetent cells

Andreas Sagen[1]

[1]University of Oslo

Andreas Sagen
University of Oslo, The National Institute of Occupational H...

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

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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 0.6 g yeast extract, 1.25 g tryptone, and 50 mL glycerol

Materials:
Select Yeast Extract **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0875**
Tryptone **Merck Millipore (EMD Millipore) Catalog #T9410**
Glycerol **MP Biomedicals Catalog #194680**

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

Preparation of cells

4 Inoculate 500 mL of prewarmed LB medium from 25 mL overnight E. coli culture. Incubate at 37 °C and 300 rpm. Measure OD every 20 minutes, until OD_{600}=0.4

> **Note**
>
> The density is usually archived after ~2.5 hours of incubation with DH5α

5 Transfer the culture to appropriate centrifugation containers, and cool on ice for 00:30:00 30m

6 Centrifuge culture at 1000 rcf, 4°C, 00:15:00, and resuspend pellet in 200 mL ice-cold water 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

8 Centrifuge culture at 1000 rcf, 4°C, 00:20:00, and resuspend pellet in 25 mL ice-cold 10% glycerol 20m

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

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

10 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 OD_{600}=~2.5 X 10^{8} cells/ml

11 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

12 Dispense 40 µL aliquots of the cell suspension into sterile, ice-cold microfuge , drop into a bath of liquid nitrogen, and transfer to a -80 °C freezer

Transformation 1h 5m

13 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 1h 5m

14 Mix an appropriate amount plasmid to an aliquot of electrocompetent cells and transfer to a pre-chilled cuvette

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

Protocol

NAME

S. O. C. medium

CREATED BY

Andreas Sagen

PREVIEW

15 Incubate plasmid-suspension mix for 1 minute, then perform electroporation with optimized settings

16 Flush 1 mL pre-heated S. O. C. medium, then transfer to a culture tube and recover at 37 °C and 200 rpm shaking for 01:00:00 1h

17 Plate an appropriate amount culture on selection agar plates