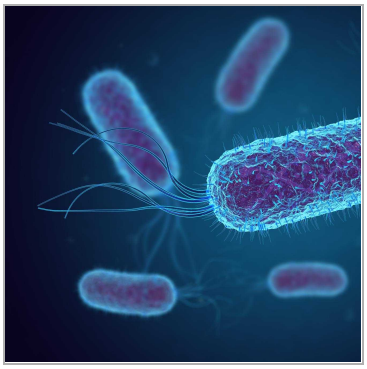

FEB 20, 2023

OPEN ACCESS

Protocol Citation: Andreas Sagen 2023. Preparation and transformation of chemically competent Escherichia coli. **protocols.io** https://protocols.io/view/preparation-and-transformation-of-chemically-compe-cn5vvg66

Protocol status: Working
We use this protocol and it's working

Created: Feb 08, 2023

Last Modified: Feb 20, 2023

PROTOCOL integer ID: 76693

Keywords: Transformation, cacl2, calcium chloride method, E. coli

Preparation and transformation of chemically competent Escherichia coli

Andreas Sagen[1]

[1]University of Oslo

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

ABSTRACT

Calcium chloride ($CaCl_2$) transformation is a laboratory technique in prokaryotic (bacterial) cell biology. The addition of calcium chloride to a cell suspension promotes the binding of plasmid DNA to lipopolysaccharides (LPS).

MATERIALS

LAF
Scale
Ice
Incubator
Centrifuge

Preparation of Calcium chloride transformation buffer

1 In a sterile flask, add 200 mL distilled water

2 Measure 25 mL ([M] 1 Molarity (M)) Calcium chloride

Materials:
Calcium chloride **Sigma-aldrich Catalog #C3881**

3 Add measured reagents and mix for 00:05:00 5m

4 Fill flask with distilled water to 250 mL

5 Filter sterilize solution with a filter (0.2 µm) and store refrigerated (4 °C)

Preparation of chemically competent cells

6 Prepare a culture of *E. coli* on an LB agar plate. Pick a single colony, and inoculate in 500 mL LB broth in a 1000 mL flask.

Incubate at 18 °C with shaking at 100 rpm overnight, until OD_{600} reaches 0.6

7 Aliquot entire culture volume into 50 mL canonical tubes

8 Place tubes on ice for 00:20:00 20m

9 Centrifuge tubes with 5000 rcf, 4°C, 00:10:00 10m

10 Discard supernatant and resuspend with 16 mL Calcium chloride transformation buffer

11 Incubate cells on ice for 00:10:00 10m

12 Centrifuge tubes with 5000 rcf, 4°C, 00:10:00 10m

13 Centrifuge tubes with 5000 rcf, 4°C, 00:10:00. Meanwhile, prepare 50 mL glycerol-Calcium chloride transformation buffer by diluting 7.5 mL Glycerol in 42.5 mL Calcium chloride transformation buffer 10m

Materials:
Glycerol **MP Biomedicals Catalog #194680**

14 Discard supernatant and resuspend with 8 mL Inoue transformation buffer. Pool into two tubes

15 Discard supernatant and resuspend with 10 mL DMSO-Inoue per tube

16 Incubate cells on ice for 00:30:00 30m

17 Aliquot 100 µL cell suspension into sterile 500 µL screw cap reaction tubes (Sarstedt #72.704.200).

Note

While creating aliquots, keep original tubes, and aliquots on ice, until snap-freeze take place

18 Snap freeze tubes in liquid nitrogen using a floating foam tube rack (Southern labware #HS2166)

19 Transfer aliquots storage box, and place in an ultra-low temperature freezer or vapor-phase nitrogen tank

Note

Store tubes in 50 mL canonical tubes, or similar containers

Transformation

1h 32m 30s

20 Quickly thaw a single reaction tube with 100 µL hyper-competent cells in Inoue-DMSO

21 Mix 1-5 µL plasmid (ligation product)

Note

Do not exceed 5% of the volume competent cells

Note

Use up-to 25 ng per 50 µL of competent cells

22 Incubate cells on ice for 00:30:00 30m

23 Heat-shock cells at 42 °C for 00:00:30, followed by 00:02:00 at 4 °C immediatly 2m 30s

24 Add 500 µL prewarmed S. O. C. medium and incubate for 37 °C at 200 rpm for 01:00:00 1h

25 Add desired amount of suspension on LB plates with ampicillin (100 µg/mL) and incubate overnight