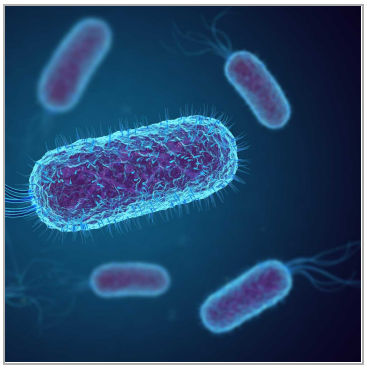

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Preparation and transformation of chemically super-competent Escherichia coli

Andreas Sagen[1]

[1]University of Oslo

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

ABSTRACT

Based on a method produced by Inoue, et al.

Inoue, Nojima, H., & Okayama, H. (1990). High efficiency transformation of Escherichia coli with plasmids. *Gene*, *96*(1), 23–28. https://doi.org/10.1016/0378-1119(90)90336-P

MATERIALS

LAF
Scale
Ice
Centrifuge
Incubator

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

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PROTOCOL integer ID: 76692

Keywords: Transformation, Inoue method, E. coli

Preparation of Inoue transformation buffer

1 In a sterile flask, add 200 mL distilled water

2 Measure 2.5 mL (1 Molarity (M)) PIPES, 2.177 g Manganese(II) chloride tetrahydrate, 3.75 mL (1 Molarity (M)) Calcium chloride and 4.660 g Potassium chloride.

Materials:
- PIPES **Sigma-aldrich Catalog #P1851**
- Manganese(II) chloride tetrahydrate **Sigma-aldrich Catalog #M3634**
- Calcium chloride **Sigma-aldrich Catalog #C3881**
- Potassium chloride **Sigma-aldrich Catalog #P4504**

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

4 Adjust pH to 6.7 with Potassium hydroxide solution

Materials:
- Potassium hydroxide solution **Supelco Catalog #P4494**

5 Fill flask with distilled water to 250 mL

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

Preparation of chemically super-competent cells

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

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

8 Aliquot entire culture volume into 50 mL canonical tubes

9 Place tubes on ice for 00:10:00 10m

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

11 Discard supernatant and resuspend with 16 mL CRM transformation buffer

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

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

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

15 Centrifuge tubes with 5000 rcf, 4°C, 00:10:00. Meanwhile, prepare 50 mL DMSO-Inoue transformation buffer by diluting 3.5 mL DMSO in 46.5 mL Inoue transformation buffer 10m

Materials:
DMSO **MP Biomedicals Catalog #196055**

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

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

18 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

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

20 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

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

22 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

23 Incubate cells on ice for 00:30:00 — 30m

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

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

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