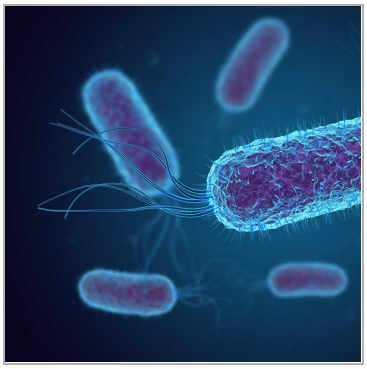

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We are still developing and optimizing this protocol

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Preparation and transformation of chemically hyper-competent Escherichia coli

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ABSTRACT

Based on a protocol produced by Liu, et al.

Liu, Chang, W., Pan, L., Liu, X., Su, L., Zhang, W., Li, Q., & Zheng, Y. (2018). An Improved Method of Preparing High Efficiency Transformation Escherichia coli with Both Plasmids and Larger DNA Fragments. *Indian Journal of Microbiology*, *58*(4), 448–456. https://doi.org/10.1007/s12088-018-0743-z

MATERIALS

LAF
Scale
Incubator
Heat cycler / Heat-bath
Ice

Preparation of CRM transformation buffer

1 In a sterile flask, add 200 mL distilled water

2 Measure 2.5 mL ([M] 1 Molarity (M)) PIPES, 2.177 g Manganese(II) chloride tetrahydrate, 7.5 mL ([M] 1 Molarity (M)) Calcium chloride, 4.660 g Potassium chloride and 110 µL (1 µg/mL) Lactoferrin B.

Materials:

- PIPES **Sigma-aldrich Catalog #P1851**
- Manganese(II) chloride tetrahydrate **Sigma-aldrich Catalog #M3634** c
- Calcium chloride **Sigma-aldrich Catalog #C3881**
- Potassium chloride **Sigma-aldrich Catalog #P4504**
- Lactoferrin B **Sigma-aldrich Catalog #L9507**

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

4 Adjust pH to pH 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 hyper-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 CRM transformation buffer. Pool into two tubes

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

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

16 Discard supernatant and resuspend with 10 mL DMSO-CRM 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 CRM-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