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

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# Preparation of Competent Cells (10β E. coli Strain)

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## ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to make competent cells that would be used for transformation.

## GUIDELINES

This protocol demonstrates the process of making 5 tubes of competent cells from a 5 mL cell culture. Generally, within our protocol, every 5 mL of cultured cells can be transformed into 5 tubes of competent cells, with each tube containing 50 µL of cells.

## MATERIALS

1. NEB 10-beta Competent E.coli (High Efficiency) - 6x0.2 ml **New England Biolabs Catalog #C3019I**
2. LB Media
3. $MgCl_2$ Solution
4. $CaCl_2$ Solution
5. 100% Glycerol Solution

## SAFETY WARNINGS

- Proper lab PPE must be worn at all times.
- Thermal gloves shall be worn when handling cell stock from the -80°C fridge.

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**Keywords:** Competent Cells,
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## Cell Culture from Cell Stock

1 Prepare a Falcon tube with 5 mL of LB media.

2 Prepare an ice box.

3 Take out a tube of Sample cell stock from the -80 °C fridge and put it into the ice box.

4 In the biosafety cabinet (BSC), use an inoculation loop to inoculate some competent cells into the Falcon tube with 5 mL of LB media.

5 Incubate the cells in an incubator at 37 °C for Overnight

## Refresh Cell Culture

6 Prepare a new Falcon tube and add 10 mL of LB media into the tube.

7 Add 100 µL of the pre-cultured cells into this new Falcon tube to refresh the cells.

8 Incubate the cells at 37 °C for 02:00:00. 2h

## Pre-Cell Washing

9 Pre-cool the centrifuge machine to 4 °C.

10 Take out the Falcon tube from the incubator, ensuring that the optical density (OD) of the cultured cells is 0.6OD to 0.8OD.

11 Place the Falcon tube in ice for 00:30:00. 30m

12 Prepare 10 mL of 0.1M $MgCl_2$ and 10 mL of 0.1M $CaCl_2$, put both tubes in ice for 00:30:00. 30m

## Cell Washing

13 Centrifuge the Falcon tube with cultured cells in the pre-cooled centrifuge machine at 5000 rpm for 00:05:00. The temperature in the centrifuge machine must be kept at 4 °C the whole time in the "Cell Washing" section. 5m

14 Discard the supernatant and keep the cell pellet.

15 Add a small amount of $MgCl_2$ solution prepared in the earlier step into the Falcon tube to resuspend the cell pellet. Then, pour the rest of the $MgCl_2$ solution into the Falcon tube.

16 Centrifuge the Falcon tube again at 5000 rpm for 00:05:00. 5m

17 Discard the supernatant and keep the cell pellet.

18 Add a small amount of $CaCl_2$ solution prepared in the earlier step into the Falcon tube to resuspend the cell pellet. Then, pour the rest of the $CaCl_2$ solution into the Falcon tube.

19 Place the Falcon tube in ice for 01:00:00. 1h

20 Centrifuge the Falcon tube with the cells at 5000 rpm for 00:05:00. 5m

21 Discard the supernatant and keep the cell pellet.

## Storage

22 Prepare a 1 mL mixed solution composed of 20% glycerol and 80% 0.1M $CaCl_2$ solution, and put it into the ice box to cool its temperature down.

23 Add 250 µL of glycerol-$CaCl_2$ solution into the Falcon tube with the cell pellet and resuspend the cell pellet.

24 Split the cells in the Falcon tube into 5 new Eppendorf tubes with each tube containing 50 µL of cells.

25 The competent cells can be stored in a -80°C fridge or used immediately.