Competent Cells using Calcium Chloride

Materials

- 1 LB agar plate
- 100 mL of liquid LB
- 2 centrifuge tubes of 250 mL
- Glycerol stock cell sample, *E. coli* DH5α in our case
- Inoculation loop
- Liquid nitrogen
- $CaCl_2$ solution: (60mM $CaCl_2$, 10mM HEPES, 15% glycerol, water to 100ml)

Apparatus

- Laminar flow hood
- Shaker
- Centrifuge
- Autoclave
- Incubator

Method

Day 1

1. Streak the LB agar plate with the cell stock.
2. Incubate the plate overnight at 37°C.

Day 2

3. Sterilize by autoclaving:
   - 100 ml of liquid LB medium
   - 100 ml of $CaCl_2$ solution
   - 2 centrifuge tubes of 250 mL
4. Prepare a 6 ml LB inoculum with a cell colony, incubate overnight at 37°C, and shake at 250 rpm.

Day 2

5. Add 2ml of the inoculum in 100 ml of liquid LB medium. Incubate at 37°C/ 250 rpm until the absorbance at 600 nm reaches 0.375.

6. Centrifuge the culture using a pre chilled centrifuge tube at 10000 rpm/ 7 min/ 4°C.
7. Discard the supernatant and resuspend the cell pellet in 10 mL of $CaCl_2$ solution.
8. Centrifuge the tubes at 7000 rpm/ 7 min/ 4°C.
9. Discard the supernatant and resuspend the cell pellet in 10 mL of $CaCl_2$ solution.
10. Incubate on ice for 30 min.
11. Centrifuge at 7000 rpm/ 7 min/ 4°C.
12. Discard the supernatant and resuspend the cell pellet in 2ml of the $CaCl_2$ solution.
13. Aliquot 50 µL of total volume in 0.5 mL microtubes.
14. Immediately freeze the samples in liquid nitrogen.
15. Store at -80°C.