Chem competent cells

Introduction
Get started by giving your protocol a name and editing this introduction.

Materials

› E. coli strain
› LB medium
› 0.1 M CaCl2 solution (ice cold)
› LB plates with proper antibiotic
› 0.1 M CaCl2 solution containing 15% glycerol (ice cold)

Procedure

The day before

1. Note: Make 1/2 portion (amounts bolded)

2. Put the 0.1 M CaCl2 solution and 0.1 M CaCl2 solution containing 15% glycerol at 4 °C.

3. Inoculate one single colony of E. coli strain in 5 (2,5) mL LB medium. Shake at 37°C overnight.

4. Put at least thirty 1.5 mL Eppendorf tubes at -80°C.

5. Inoculate 1 (0,5) mL overnight culture in 100 (50) mL LB medium within a 500 mL flask.

6. Subculture at 37°C with shaking till OD600 reaches ~ 0.25-0.3 (about 2 hours subculture time). First check 1.5 h after the inoculation.

7. Chill the culture on ice for 15 minutes.

8. Separate 100 mL chilled bacterial culture into two 50 mL Falcon tubes and centrifuge at 4°C at 4000 rpm for 10 minutes.

9. Discard the supernatant and resuspend the pellet with 40 (20) mL ice-cold 0.1 M CaCl2 solution.

10. Keep cells on ice again for 30 minutes.

11. Centrifuge cells at 4000 rpm at 4°C for 10 minutes.

12. Discard the supernatant and resuspend pellet with 5 (2,5) mL ice-cold 0.1 M CaCl2 solution containing 15% glycerol.

13. Pipet 50 µL of cell suspension into -80°C frozen eppendorfs and directly transfer them to -80°C freezer.