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.
14. http://www.genomearchitecture.com/protocols/Ecoli-heat-shock-transformation.html