E. coli competent cells

Introduction

Purpose to prepare the cells to be able to take up naked DNA from the environment efficiently.

Materials

Equipment

- Autoclave
- Centrifuge
- Spectrophotometer

Consumables

- PCR tubes
- 50 mL Falcon tubes

Chemicals

- $MgCl_2$
- $CaCl_2$
- SOC/SOB
- Glycerol

Procedure

Day 1

1. **Autoclave the following:**
   - Minimum 200 mL 0.1 M $MgCl_2$
   - Minimum 150 mL 0.1 M $CaCl_2$
   - Minimum 100 mL 85 mM $CaCl_2$ + 15% glycerol (v/v)
   - Minimum 700 mL SOC
   - 3 x shakeflasks (500 mL)
2. **Freeze at -20** °C **(after autoclaving)**
   - 0.1 M Mg Cl_2
   - 0.1 M $CaCl_2$
   - 85 mM $CaCl_2$ + 15% glycerol (v/v)
   - Minimum 10 x 50 mL Falcon tubes
   - PCR tubes

Day 2

Culture growth

3. Early in the morning, start cooling the Sorval centrifuge to 4 °C
4. Pour 200 mL SOB media into each of the shake flask (one shake flask per starter culture).
5. Mark the shake flasks to match the starter cultures

6. Measure the OD 600 of each starter culture and inoculate the shake flask with a volume so the final OD 600 value in the shake flask culture becomes 0.01.
7. Grow the shake flask culture at 37 C with shaking. Measure OD values of the sample every 20 minutes once the OD 600 value is above 0.2.
8. When OD 600 is between 0.3 and 0.4, put the cultures into an ice bath immediately, and swirl the shake flask around in the cold water to cool the culture. Chill the culture in the ice water for 20-30 minutes, occasionally swirling the cultures.

From this stage on, keep the cells at ice (4 °C) at all times.

9. For each shake flask culture, pour the culture into 3 x 50 mL frosted falcon tubes from the freezer.
   Keep the tubes on ice.
10. Centrifuge falcon tubes at 3000 x g for 15 minutes at 4 °C (Spin #1 of 4)
11. Discard supernatant, and resuspend cells in 15 mL ice-cold 0.1 M $MgCl_2$
    Keep tubes with cells on ice.
12. Pool the resuspended cells into one of their matching falcon tubes, so you now have 3 different 50 mL falcon tubes, one with cells corresponding to each of the starter cultures you had.
    Keep tubes on ice.
13. Centrifuge Falcon tubes at 2000 x g for 15 minutes at 4 °C (Spin #2 of 4)
14. Discard the supernatant and resuspend pellet in 40 mL ice-cold 0.1 M $CaCl_2$.
    Keep tubes on ice.
15. Let cell suspensions stand in ice for 20-30 minutes
16. Centrifuge Falcon tubes at 2000 x g for 15 minutes at 4 °C (Spin #3 of 4)
17. Discard supernatant, and resuspend pellet in 10 mL ice-cold 85 mM $CaCl_2$ + 15 % glycerol
    Keep tubes on ice
18. Centrifuge falcon tubes at 1000 x g for 15 minutes at 4 °C (Spin #4 of 4)
    Pellet might look small and will be a bit fragile. Handle tubes with care when taking them out of centrifuge.

Attention: The next few steps are best done on ice inside a LAF bench

19. Resuspend pellet in 800 µL ice-cold 85 mM $CaCl_2$ + 15 % glycerol.
    Put falcon tubes on ice.
20. Immediately after cells are confirmed resuspended, aliquot 30 µL of the competent cell. culture into the chilled PCR tubes.
21. Put tubes into a -80 °C freezer as fast as possible.