E.coli Competent Cell Protocol

Introduction

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Materials

- Equipment
  - Autoclave
  - Freezer
  - Centrifuge
  - Spektrophotometer
- 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 degC (after autoclaving)**
   - 0.1 M $MgCl_2$
   - 0.1 M $CaCl_2$
   - 85 mM $CaCl_2$ + 15% glycerol (v/v)
   - Minimum 10 x 50 mL falcon tubes
   - PCR tubes

Day 2:

3. **Culture growth**

4. Early in the morning, start cooling the Sorval centrifuge to 4 C

5. Pour 200 mL SOB media into each of the shakeflask (one shakeflask per starter culture).

   Mark the shakeflasks to match the starterculture

6. Measure the OD 600 of each starterculture, and inoculate the shakeflask with a volume so the final OD 600 value in the shakeflask culture becomes 0.01

7. Grow the shakeflask 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 shakeflash around in the cold water to cool culture. Chill the culture in the icewater for 20-30 minutes, occasionally swirling the cultures.

9. **FROM THIS STAGE ON, KEEP CELLS AT ICE/4 °C AT ALL TIMES**

10. For each shakeflask culture, pour the culture into 3 x 50 mL frosted falcon tubes from the freezer.

    Keep the tubes on ice

11. Centrifuge falcon tubes at 3000 x g for 15 min at 4 C (Spin #1 of 4)

12. Discard supernatant, and resuspend cells in 15 mL **icecold** 0.1 M $MgCl_2$

    Keep tubes with cells on ice

13. 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

14. Centrifuge falcon tubes at 2000 x g for 15 min at 4 o C (Spin #2 of 4)

15. Discard the supernatant, and resuspend pellet in 40 mL icecold 0.1 M $CaCl_2$

    Keep tubes on ice

16. Let cell suspensions stand in ice for 20-30 minutes

17. Centrifuge falcon tubes at 2000 x g for 15 min at 4 C (Spin #3 of 4)

18. Discard supernatant, and resuspend pellet in 10 mL **icecold** 85 mM CaCl 2 + 15 % glycerol

    Keep tubes on ice

19. 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

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

21. Resuspend pellet in 800 uL ice-cold 85 mM $CaCl_2$ + 15 % glycerol

    Put falcon tubes on ice

22. Immediately after cells are confirmed resuspended, aliquot 30 uL of the competent cell culture into the chilled PCR tubes

23. Put tubes into **-80** °C **freezer** as fast as possible