
MOLECULAR WORK

Competent Cell preparation

1. Do mini-culture of *E. coli* (eg. DH5 α) at 37°C for overnight (O/N)
2. Scale up to 50 to 100 ml culture until the OD600 is 0.5~0.6
3. Put it on ice for 30 min (optional)
4. Harvest cells at 3000 rpm for 20 min at 4°C
5. Remove supernatant completely
6. Add 25 ml of ice-cold 0.1M MgCl₂ and re-suspend gently
7. Harvest cells again at 3000 rpm for 20 min at 4°C
8. Remove supernatant completely
9. Add 2 ml of ice-cold 0.1M CaCl₂ and re-suspend carefully
10. Incubate it at 4°C for 24 hr
11. Add 300 ul of glycerol (15% v/v) to the cell solution and gently suspend them
12. Aliquot 25 to 200 ul in each sterile e-tube
13. Snap-freeze them in liquid nitrogen (LN₂)
14. Store them at -70°C