CHEMICALLY COMPETENT CELLS CALCIUM CHLORIDE (CACl2) METHOD

Protocol

1. Inoculate a single colony of appropriate cells into 10ml LB. Add antibiotic if needed and culture o/n at 37°C, 200rpm
2. Inoculate 100ml LB with 1 ml o/n culture
3. Incubate at 37 °C, 200rpm until OD600 = 0.3-0.6 (approx. 2 hrs)
4. Transfer to 2 x 50 ml Falcon and leave on ice for 30 mins
5. Centrifuge at 400 x g, 5mins, 4 °C
6. Resuspend pellet GENTLY in 25 ml ice cold 0.1 M MgCl2
7. Incubate on ice for 30 min
8. Centrifuge at 4000 x g, 5 min, 4 °C
9. Resuspend pellet GENTLY in 25 ml ice cold 0.1 M CaCl2
10. Incubate on ice for 30 min
11. Centrifuge at 4000 x g, 5 min, 4 °C
12. Resuspend pellet GENTLY in 1.25 ml ice cold CaCl2/Glycerol solution (1.7 ml 0.1 M CaCl2, 0.3 ml 100 % glycerol)
13. Aliquot 100ul and flash freeze using LN2/Ethanol dry ice bath.
14. Store at – 80 °C