Bacteria competent cells (CaCl2 Method)

Strain: BL21 DE3, E. coli

1. Start two 5 ml precultures from the desired cells and incubate overnight at 37 ° C

2. The next morning, leave two cultures of 500 ml of 2YT with the 5 ml of preculture

3. Allow to reach a D.O.600 of 1 / ml (do not exceed)

4. Centrifuge cells at 5000 rpm for 20 min with F10 rotor at 4 ° C

Do the following steps as gently as possible:

5. Resuspend the cells in 0.1M CaCl2 (100ml / pellet)

6. Leave 3 h on ice (Start resuspending after 1:30)

7. Centrifuge at 5000 rpm for 20 min

8. Resuspend pellet in 0.1M CaCl2 + 15% glycerol (25ml / pellet)

9. Aliquot in 2 ml tubes which screw and store at -80 ° C

Test: Transform 0.2ng, 0.02ng and 0.002ng of small, high-copy plasmid DNA and plate on appropriate media. Count colonies to determine transformation efficiency.