CaCl2 chemical transformation

This protocol aims at inserting plasmids notably into NM522 strains, or any other E.Coli strain

It has been found less efficient than the TSS transformation protocol, thus we do not recommend using it.

Procedure

1. Monitor the OD600 of a 50mL culture of NM522 cells (or the strain you want to transform) in LB medium at 37°C. Proceed to the next step when it reaches 0.2-0.4 (not higher than 0.6), which takes approximately 3 hours.

2. Aliquot the culture into fractions of the desired volume V (10 or 15 mL usually).

3. Incubate on ice for 10mn

4. Centrifuge at 4°C, 10mn, 5000rpm

5. Empty the supernatant

6. Add V/2 mL cold and sterile CaCl2 100mM

7. Incubate for 20mn on ice

8. Centrifuge at 4°C, 5mn, 5000rpm

9. Empty the supernatant and resuspend (do not vortex) in V/15 mL CaCl2 and V/30 mL glycerol 40%

10. Aliquot the bacteria in 200 µL fractions and keep on ice. These bacteria should be used within the day.

11. Add the desired quantity of DNA to the fractions. For positive control, we used Puc18 plasmid. For negative control, we used water.

12. Incubate for 30mn on ice

13. Heat shock at 42°C for 2mn

14. Incubate for 5mn on ice

15. Add 800µL of LB medium and incubate for 1h at 37°C

16.Optional Concentrate 10X by centrifuging for 2mn at 11000 rpm, eliminate the supernatant and resuspend in 100µL LB.

17. Plate 100µL of bacteria on LB medium with the appropriate antibiotic resistance and incubate at 37°C.