

TSS chemical transformation

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

It has been found more efficient than the CaCl₂ transformation protocol, thus we recommend using it.

Procedure

1. Monitor the OD600 of a 5mL 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 0.3-0.4 (not higher than 0.6), which takes approximately 3 hours.

2. Take 1 mL from the previous culture and centrifuge at 6000rpm for 2mn .

3. Resuspend into 100µL of TSS. Starting from this step, keep the bacteria on ice.

4. Incubate on ice for 5-10mn.

5. Add 5µL of the DNA to be transformed.

6. Incubate on ice for 10mn.

7. Heat shock the bacteria by heating them at 42°C for 50s.

8. Incubate on ice for 2mn.

9. Add 900µL LB and mix by inverting the tube.

10. Incubate for 1h at 37°C.

11. Plate 200µL on LB medium with the appropriate antibiotic.

