Heat shock Transformation for TOP10 E.coli

Materials

· Chemically competent cells

· Purified plasmid

· Petri dish- with relevant selective antibiotic

· 5X KCM

- 0.5M KCl
- 0.15M $CaCl_2$
- 0.25M $MgCl_2$

Procedure

1. Thaw Top10 *E.coli* cells on ice for 15 minutes

2. Add 2 µL KCM in a new 1.5 mL tube

3. Add 15-30 ng of plasmid (or 1-8 µL DNA mix or the ligation sample)

4. Add distilled water or ligation mix to a total volume of 10 µL

5. Incubate on ice for 2-5 minutes

6. Add 10 µL of the thawed competent cells.

7. Incubate on ice for 20 minutes

8. Heat shock at 42^{o}C for 1 minute

9. Put on ice for 3 minutes

10. Add 180 μL of prewarmed TSB/LB or any nutritionally rich medium

11. Phenotype with shaking for 1 hour at 37°C

12. Plate on petri dish (160 μL on one half, 20 μL on the other half)

13. Incubate overnight at 37°C.