Protocol: Transformation of DH5α Escherichia coli Bacteria Cells

Application:
Transforming of recombinant DNA using DH5α E. coli competent cells.

Procedure:
1. Thaw competent cells on ice (source: Invitrogen MAX Efficiency DH5α, cat# 18258012)

2. Aliquot 20 µl of cells per 1.5 mL tube (negative control, positive control, experiments)
   • Refreeze unused cells on dry ice then into -80 °C.

3. Set up control tubes:
   a) Negative control => no DNA
   b) Positive control => 1 µL of pUC19 DNA (0.01 µg/ml stock)

4. Add 1 µL of each new ligation (experiment tube) per DH5α cell tube.

5. Finger flick tube bottom to mix

6. Ice for 30 min.


8. Ice for 2 min.

9. Add 900 µL Luria-Bertani (LB) media w/o Ampicillin.

10. Shake at 225 rpm at 37 °C for 1 hour.

11. For plating:
   • Positive and Negative controls: use 100 µL of undiluted samples.
   • Each experimental tube/ligation, use:
     a) 100 µL undiluted (from above)
     b) 100 µL of 10x dilution (100 µL undiluted + 900 µL LB media)

12. Spread all conditions of DH5α containing tubes onto LB + Amp (50 µg/mL) plates.
    (negative control, positive control, 2x each experimental tube/ligation)
    ***Performed near hot flame for sterility***

13. Allow plates to dry on benchtop at room temperature.

14. Invert plates (agar side up) and incubate at 37 °C overnight.