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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.

7. Heat shock 45 seconds at 42 °C in H_2O bath.

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)
    <u>*Performed near hot flame for sterility*</u>

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

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