Transformation Protocol

1. Preparation
    a. Fill ice box
    b. Prepare 2 labelled agar + antibiotic plates
    c. Heat shaking incubator and regular incubator to 37^0C.

2. Put plasmid solution and competent cell solution (from glycerol stock) on ice for 20 minutes, to thaw.

3. Pipette 5uL plasmid solution into competent cell Eppendorf. Mix **gently** by slowly swirling the pipette tip.

4. Immediately put back on ice for 30 minutes. In the meantime, heat water bath to 42 ^{0}C.

5. Place Eppendorf in water bath for 70 seconds. No mixing.

6. Place Eppendorf back on ice for 2 minutes. No mixing.

7. Pipette 1mL LB broth to Eppendorf.

8. Put Eppendorf in Erlenmeyer flask (ensure it is tightly closed, can use parafilm to seal) and then put the Erlenmeyer flask in shaking incubator for 90 minutes at 240rpm.

9. Remove Eppendorf from shaking incubator and pipette 50uL of the solution onto the centre of agar plate.

10. Sterilize hockey stick by dipping in ethanol and flaming. Once cooled, spread the solution evenly around the plate.

11. Place agar plate in incubator.

12. Centrifuge Eppendorf in microcentrifuge at 15000 rpm for 30 seconds.

13. Dispose of supernatant.

14. Resuspend pellet with remaining ~50 ul LB broth.

15. Pipette the resuspended solution (all 50uL) onto the centre of the second agar plate.

16. Repeat steps 10-11 for the second agar plate.

17. Let incubate overnight (16-24 hours) at 37 ^{0}C