Transformation Protocol

***Remember any steps being done on ice need to be kept cold

1. Re-suspend ligation products using 10 µL of nuclease free H_2O (only from distribution kits)
2. Pre-chill a microcentrifuge tube (1.5 mL) in ice box, you will need 1 tube per transformation ***don't forget control
3. DH5α competent cells are needed thaw on ice for 10-15 minutes
4. Pipette 50 µL of competent cells into all chilled microcentrifuge tubes while keeping them on ice
5. Use 5 µL of DNA (re-suspended or ligated) and combine with 50µL of competent cells
6. Incubate tubes for 30 minutes on ice, gently agitating them intermittently
7. Heat shock for 45 seconds at 42° C
8. Return to ice for 5 minutes
9. Add 950 µL of liquid LB to each transformation microcentriftuge tube and incubate at 37° C while shaking at 200-300 rpm
10. Pipette 100 µL of each transformation onto LB CM-25 agar plates and spread with sterile glass spreader
11. Centrifuge cells for 1 minute and discard 900 µL of supernatant
12. Resuspend cells in remaining 100 µL and pipette onto LB CM-25 agar plates, again spread with sterile glass spreader
13. Incubate transformations overnight 14-18 hours at 37° C