Making Competent Cells

1. Pick frozen cells and streak out on an LB Plate.

2. Pick a single colony from the plate and grow overnight in 7 ml of LB.

3. Dilute the preparation 1:100 in LB (NO AMP) (5 ml overnight culture into 500 ml LB)

4. Prepare Fresh Solutions:
   Filter Sterilize All Solutions (Make sure bottles are sterile)
   - 0.1 M MgCl$_2$ = 10.16 g MgCl$_2$ in 500 ml FW: 203.31 g
   - 0.05 M CaCl$_2$ = 3.67 g CaCl$_2$ in 500 ml FW: 147.02 g
   - 0.1 M CaCl$_2$ = 2.94 g CaCl$_2$ + 28 ml Glycerol + 172 ml ddH$_2$O
   KEEP SOLUTIONS ON ICE !!!!!!

5. Grow the cells to Optical Density $\text{OD}_{600} = 0.6$
   (should take approx. 3 to 4 hrs. after you inoculate with 2.5 ml of cells. You should sample the OD frequently, taking out 1 ml)

6. Split the preparation into 2 large centrifuge bottles (Make sure the bottles are sterile!!!)

7. Centrifuge at 4K at 4°C for 10 minutes

8. Pour off Supernatant and redisolve each pellet in 125 ml 0.1 M MgCl$_2$. (On ice)

9. Incubate the cells on ice for 7-10 minutes (On Ice)

10. Centrifuge again at 4K for 10 minutes

11. Dissolve each pellet in 125 ml of .05 M CaCl$_2$ (Done in Cold Room)

12. Incubate on ice for 20 minutes (In Cold Room)

13. Centrifuge at 4K for 10 minutes @ 4°C.

14. Dissolve in 50 ml0.1 M CaCl$_2$ w/ glycerol. (In cold room)

15. Once dissolved, dispense 500 microliter aliquots into 1.5ml eppendorf tubes, THAT HAVE BEEN PRE-CHILLED ON ICE. Then, put on dry ice for 1.5 minutes before throwing them in the -70 freezer.

16. Store at -70°C.

17. Test the cells by performing a mock transformation with a plasmid of KNOWN concentration. Use specific dilutions of the vector (1ng, 10 ng, 100ng, 500ng, 1 microgram) to determine the transformation efficiency.