

## 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)  
(5ml overnight culture into 500 ml LB)
4. Prepare Fresh Solutions:  
Filter Sterilize All Solutions (Make sure bottles are sterile)  
0.1 M  $\text{MgCl}_2$  = 10.16 g  $\text{MgCl}_2$  in 500 ml  
FW: 203.31 g  
0.05 M  $\text{CaCl}_2$  = 3.67 g  $\text{CaCl}_2$  in 500 ml  
FW: 147.02 g  
0.1 M  $\text{CaCl}_2$  = 2.94 g  $\text{CaCl}_2$  + 28 ml Glycerol + 172 ml ddH<sub>2</sub>O  
**KEEP SOLUTIONS ON ICE !!!!!**
5. Grow the cells to Optical Density<sub>600</sub> = 0.6  
(should take approx. 3 to 4 hrs. after you innoculate 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 redissolve each pellet in 125 ml 0.1 M  $\text{MgCl}_2$ .  
**(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  $\text{CaCl}_2$  **(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 ml 0.1 M  $\text{CaCl}_2$  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°.
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.