Day 1:

1. Make 7 mL culture of E.coli cells from glycerol stock in plain LB
2. Let grow at 37C overnight

Day 2:

1. Inoculate 1 mL of overnight culture into 300mL plain LB
2. Let grow for 6 hours shaking at 37C
3. Turn on large centrifuge, shut lid, and turn temperature to 4 degrees Celsius.
4. Once centrifuge is to temperature, take cells out of incubator and put immediately on ice
5. Separate cells into 50 mL centrifuge tubes and spin down cells at 3000 RPM for 20 minutes.
6. Pour off supernatant immediately after spinning ends and put cells back in ice
7. Resuspend cells in 15mL 50mM $CaCl_2$ solution by pipetting up and down gently. And consolidate to 3 50mL tubes
8. Let resuspended cells sit on ice for 10 minutes
9. Spin down cells at 7000 RPM for 20 minutes.
10. Make sure a pellet forms (will probably be a long smear on one side) and pour off liquid quickly. Some cells will be lost (that is OK)
11. Resuspend cells in 15mL 50mM $CaCl_2$ solution by pipetting up and down gently and consolidate to 1 tube
12. Let cells sit for 10 minutes on ice
13. Spin down cells at 7000 RPM for 20 minutes
14. Make sure a pellet forms (will probably be a long smear on one side) and pour off liquid quickly. Some cells will be lost (that is OK). **Note: not all of the liquid will come out, that is OK**
15. Resuspend cells in 1 mL of final suspension solution (in the black fridge)
16. Aliquot 50 uL of resuspension into microcentrifuge tube on ice and take to -80 freezer.