Culturing Competent Cells: (3 Day Process)

>Day1:

1. Take the desired bacterial strain and streak on a plate
2. Incubate at 37 C overnight

>Day2:

1. Use 15mL centrifuge tube to measure out ~15mL of LB broth
2. Add desired antibiotic in a 1:1000 ratio (~15uL)
3. Move 15mL mixture (LB+antibiotic) to a 125 mL or 250 mL erlenmeyer flask
4. Isolate one colony using a sterile toothpick and drop into flask (ensuring the colony touches the LB, not glass)
5. Incubate at 37 C while shaking at 250 rpm overnight

>Day3:

1. Prechill 20 PCR tubes, 1 mL LB broth, and TSS Buffer
2. Put 125 mL of LB+appropriate antibiotics into a 250 mL erlenmeyer flask
3. Add 1 mL of overnight cell broth into the 125 mL of LB broth
4. Incubate at 37 C while shaking at 250 rpm for 3-6 hours
5. After 3 hours, remove 2 mL of cell broth every 30 minutes and run through a spectrophotometer (with LB as the blank)
6. Continue to do so until the optical density has reached .5
7. At optical density of .5, distribute broth across eight 15 mL centrifuge tubes
8. Centrifuge at max (3500 rpm) in blood centrifuge for 10 minutes
9. Place tubes on ice for 15 minutes
10. While on ice, decant entire supernatant and discard
11. Pipet 1 mL of prechilled LB broth into one of the 8 centrifuge tubes
12. While on ice, resuspend pellet with pipet
13. Transfer entire resuspended contents into next 15 mL tube
14. Repeat until the contents of all eight tubes are only in one tube (all eight pellets suspended in 1 mL of LB)
15. Measure total volume by pipetting back into an empty 15 mL tube in known increments
16. While on ice, add equal volume of chilled TSS buffer and resuspend
17. Pipet 100 uL of cells+TSS buffer into each of the 20 PCR tubes
18. Cap tubes, label and utilize/store in the freezer