**Preparing Electro-competent Cells**

* **Required Items (autoclaved)**
  + LB
  + dH2O
  + 15% Glycerol
  + 1 L Erlenmeyer flask
  + 2 x 250 mL Centrifuge Tubes
  + 2 x 50 mL Centrifuge Tubes
  + Centrifuge plus F14 & SH-3000 rotors

1. Inoculate 10 mL of LB with a colony of the desired cell type and grow overnight at 37 °C in a shaking incubator.
2. The next day, add 3 mL of the culture to a sterile 1 L Erlenmeyer flask filled with 300 mL of LB and take an initial OD600 reading.
3. Incubate the new culture at 37 °C in a shaking incubator at 200 RPM while periodically taking OD600 readings until an OD600 = 0.792 is reached. Place on ice (all subsequent steps should be performed on ice or at 4 °C).
4. Transfer 150 mL to each of two sterile 250 mL centrifuge tubes and centrifuge at 8,000 x g for 10 minutes at 4 °C.
5. Pour off the supernatant and resuspend the cells of each tube in 100 mL dH2O (the resuspension may be performed by pipetting up and down near the pellet).
6. Combine the volumes in the two 250 mL centrifuge tubes (200 mL total).
7. Centrifuge at 8,000 x g for 10 minutes at 4 °C.
8. Pour off the supernatant and resuspend the cells in 200 mL of 15% glycerol.
9. Centrifuge at 8,000 x g for 10 minutes at 4 °C.
10. Pour off the supernatant and resuspend the cells in 25 mL of 15% glycerol.
11. Transfer the resuspended cells to a sterile 50 mL conical centrifuge tube.
12. Centrifuge at 8,000 x g for 10 minutes at 4 °C.
13. Thoroughly remove the supernatant, and resuspend the cells in 0.5 mL of 15% glycerol.
14. Aliquot 50 μL into several 1.5 mL microcentrifuge tubes, and freeze the aliquots at -80 °C.