Making Electrocompetent Cells
Cornell iGEM 2012 Protocol

Adapted from protocol draft by Didi Waraho. Based on…
Wu et al. (2010). Enhancing DNA electrotransformation efficiency in *Escherichia coli* DH10B electrocompetent cells. *Electronic Journal of Biotechnology* **13**(5)

Day 1:

1. Grow a fresh overnight culture in 20mL LB in a 250ml flask. (Either pipette up and down to get cells into the flask from the freezer stock, or pick a single colony from a fresh (less than two weeks old) LB agar plate.) Grow at 37˚C in a shake incubator.
2. Prepare/Autoclave LB and flasks/bottles for subculturing the next day (see Day 2, Step 1).
3. Autoclave at least 200 mL of 10% Glycerol per batch of cells and leave in the fridge overnight. Alternatively, dilute a sterile 80% or 100% glycerol stock with autoclaved water in a sterile field.

Day 2:

1. Inoculate 1 L of fresh LB medium in a 2 L flask with 5 mL of fresh overnight culture and grow cells at 37˚C, shaking at 200 rpm. Grow culture to an OD_{600} of 0.15. (For DH5a, it takes about 3 hrs.)
   Subculture can be split into two 1 L or four 500 mL flasks (or bottles, if flasks are unavailable), scaling down inoculant volume accordingly. If a smaller quantity of freezer stocks is desired, the entire procedure may also be scaled down without complication if headspace ratio is maintained.
2. When cells reach desired OD_{600}, place flasks on ice **IMMEDITELY**. Incubate cells on ice for at least 1 hour. In the meantime, turn the centrifuge on so that it will be cold (4˚C) when spinning down the cells and chill more than 20 50 mL centrifuge tubes on ice (per 1 L subculture volume).
3. Transfer entire volume of subculture to chilled 50 mL centrifuge tubes, and centrifuge @ 3200rpm for 20 minutes at 4˚C.
   ***Do not** centrifuge at a higher g value than needed. Make sure to keep everything on ice and balance tubes before centrifuging. If a*
4. Dump about ¾ of the supernatant. Remove the rest of the supernatant using a 25 mL pipette tip, being careful not to suck up the cells.
   Note: Dumping out all of the supernatant may result in a great loss in cells
5. Once as much supernatant as possible has been removed, gently pipette up and down media to resuspend the pellets in 10ml of cold sterile 10% glycerol and move the solution to a 50-mL conical tube. Add more 10% glycerol. Use 30 mL of 10% glycerol per 1 L cell culture. Centrifuge @ 3200 rpm for 15 minutes at 4˚C. Note: Water has already been chilled with ice.
6. Repeat steps 4 and 5 to complete two more washes with the 10% glycerol
7. Remove the supernatant from the final wash as detailed above.
8. Chill eppendorf tubes on ice.
9. Resuspend pellet in cold 10% glycerol such that the amount of cell in each tube is $5x10^9$ cells/100 µL. Mix up and down gently to resuspend.
   Calculation of volume of 10% glycerol required to resuspend cells
   Use OD_{600} of 1 = $5x10^8$ cells for calculation of how many fold concentration is required
   ($5x10^9$ cells/100 µL)x(1mL/$5x10^8$ cells)x(1000 uL/1 mL) = 100 OD_{600}
   If the OD_{600} that you harvest is 0.15 then you need to concentrate 100/0.15 = 667 fold
   For 1 L of cell culture, you need to resuspend cells in 1000/667 = 1.5 mL
10. Transfer 50 mL aliquots of cells into eppendorf tubes and place in -80˚C