**ELECTRO-COMPETENT CELLS**

1. **(Day 1)** Streak LB plate with scraping from cell stock and grow overnight.
2. **(Day 2)** Pick single colonies and grow 3 ml overnights in 2XYT.
3. **(Day 3)** Grow 25 ml overnights in 2XYT (inoculate with 50 µl of 3 ml culture).
4. Make 1 L 10% glycerol (100 ml glycerol + 900 ml water).
5. Make 1 L SOB media:

20 g tryptone

5 g yeast extract

0.5 g NaCl

833 µl 3M KCl (2.5 mM final conc.)

Split between two baffled flasks

1. Autoclave glycerol and SOB.
2. **(Day 4)** Add 50 ml of 200 mM MgCl2 to each flask (20 mM final conc.).
3. Inoculate large cultures with 12.5 ml of 25 ml culture.
4. Grow to OD600 = 0.8
5. Set up dry ice-ethanol bath in Styrofoam container with tube rack (in cold room).
6. Chill glycerol in ice water bath in autoclave tray (in cold room).
7. Split cells into 4 JA-10 bottles and chill on ice.
8. Centrifuge at 3000 rpm for 10 minutes at 4ºC.
9. Resuspend each pellet in ~125 ml chilled 10% glycerol in cold room.
10. Combine resuspended cells into 2 JA-10 bottles.
11. Centrifuge at 5000 rpm for 10 minutes at 4ºC.
12. Carefully pour off supernatant.
13. Resuspend cell pellets in remaining glycerol.
14. Centrifuge at 5000 rpm for 10 minutes at 4ºC.
15. Pour off supernatant, leaving ~2 ml.
16. Resuspend pellets in remaining supernatant and aliquot 210 µl into labeled eppendorf tubes.
17. Snap-freeze cells by immersing tubes in dry ice-ethanol bath (or liquid nitrogen).
18. Store tubes at -80ºC.
19. Perform transformation to calculate transfer efficiency of cells.