ELECTROCOMPETENT *E.COLI* CELLS

(Competent cells for electroporation)

Day 1

1. Start an overnight culture of the appropriate strain (*e.g.* XL1-Blue, DH5α) in LB or other rich medium; grow at 37°C
2. Autoclave some large centrifuge bottles (250-500ml) for spinning down cells tomorrow
3. Place bottles of sterile water (total = ~1.5 liters) in the cold room for resuspending cells tomorrow

1. Day 2
4. Transfer 0.2 to 1 ml of the overnight culture to 500 ml aliquots of LB (or other rich medium) in 1-2 liter baffled flask
5. Incubate at 37°C with vigorous agitation for 2-6 hours
6. Monitor $O.D._{600}$ periodically (every 30 min. after the first hour of incubation)
7. When the $O.D._{600}$ reaches 0.5 to 1.0, remove flasks from the incubator and chill on ice for at least 15 minutes (cultures may be left this way for several hours if necessary)
8. Centrifuge cells at 5000g for 15 min., 4°C; pour off supernatant
2. (pellet may be stored at 4°C in 10% glycerol for a day or two if necessary at this point)
9. Resuspend cells in ice cold water (sterile). First resuspend in a small volume (a few milliliters) with vortexing or pipetting, then dilute with more water to bring centrifuge bottle to about two-thirds full.
10. Centrifuge as above; carefully pour off supernatant
11. Resuspend in cold sterile water as before
12. Centrifuge, pour off supernatant
13. Resuspend pellet in 20 ml ice cold, sterile 10% glycerol
14. Centrifuge as before; carefully remove supernatant (pellet is likely to be very loose)
15. Resuspend cells to a final volume fo 2-3 ml with 10% glycerol
16. Divide the cells into 150 µl aliquots in eppendorf tubes and store at -80°C

Electroporation of DNA into electrocompetent cells

1. Thaw an aliquot of electrocompetent cells on ice
2. Add 1-3µl DNA to the cells; incubate on ice for ~5 min.
3. Transfer to chilled 2mm electroporation cuvette (no bubbles!)
4. Have P1000 with 300µl LB or 2xYT ready to dispense
5. Pulse cuvette @ 200 ohms, 25µFd, 2.5 kV (check time constant, should be somewhere above 3)
6. IMMEDIATELY add the 300µl LB or 2xYT to the cuvette
7. incubate the cells at 37°C for 40 min to 1 hour to recover
8. plate cells onto selective medium