Preparation of Electrocompetent *E. coli* (i.e. DH5α)

revised 2/24/96

Before starting procedure, prepare/chill the following:

1 L	2XYT media (no antibiotics!), store at RT
1 L	chilled autoclaved dH_20, stored in 4° cold room.
100ml	chilled 10% glycerol / dH_20 solution, store at 4°C.
4	Corning 250ml pointed bottles (w/ orange caps) with white adapters chilled at 4°C. (used in Beckman tabletop GPR with GH-3.7 rotor, Church 64) Rinse bottles first with 95% EtOH, then really well with dH_20. No soap!

Step 1. Preferably, select single colony of *E. coli* from fresh LB plate for inoculating a 10 ml 2XYT overnight (O/N) starter culture. Alternatively, streak out frozen glycerol stock of bacterial cells onto LB plate, grow plate O/N, and then select single colony for starter culture. Grow 10 ml starter culture O/N in 37°C shaker (250rpm).

Step 2. Inoculate 1L of 2XYT media and place culture in 37° shaker. Grow cells and measure OD_{600} every 45min-1hr. When the OD_{600} equals 0.6-0.9 (log phase growth), remove the cells from the shaker and place on ice.

NOTE: It very important to keep the cells at 4°C (or on ice) for the remainder of the procedure.

Step 3. Split the 1L culture into four equal parts by pouring ~250ml of culture into each chilled 250ml Corning pointed bottle.

Step 4. Spin (#1) in GPR centrifuge at 4000rpm, 25min at 4°C.
(if you chose to use the J6/ JS-4.2 rotor (E. Davidson Lab), use 1L bottles , fill half full, spin 4000rpm, 20min, at 4°C.)

Step 5. Place bottles on ice. Remove supernate immediately as cell pellet begins to lift off quickly. Gently resuspend each pellet in 200ml ice-cold dH_20.

Step 6. Spin (#2) in GPR centrifuge at 4000rpm, 25min at 4°C.

Step 7. Place bottles on ice. Remove supernate. Gently resuspend each pellet in 100ml of ice-cold dH_20.

Step 8. Spin (#3) in GPR centrifuge at 4000rpm, 25min at 4°C.

Step 9. Place bottles on ice. Remove supernate. Gently resuspend each pellet in 20ml ice-cold 10% glycerol. For each pair of 250ml Corning bottles, transfer both 20ml cell suspension into one chilled 50ml conical tube- therefore you should end up with two 50ml conical tubes on ice where each tube contains ~40ml of cells in 10% glycerol.

Step 10. Spin (#4) in GPR centrifuge at 4000rpm, 10min at 4°C.

Step 11. Place tubes on ice. Remove supernate. Gently resuspend each cell pellet in 1ml of ice-cold 10% glycerol. Final OD_{600} of resuspended cells ≈ 200-250.

Step 12. With cell suspensions on ice, prepared 70λ aliquots of cells in pre-chilled 1.5ml eppendorf tubes. Snap freeze tubes containing cells in liquid N_2. Store frozen cells at -80°C.

NOTE: liquid N_2 very hazardous- use caution and don't contact N_2 directly!

Electroporation of Electrocompetent *E. coli* (i.e. DH5α)

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Step 1. Locate Electroporator power source and cuvette holder. (Bio-Rad). Set the conditions for transformation according to strain.
For DH5α cells, use 25 μFD, 200 Ω, and 1.8 kV. The time constant (tau value) should be 3-4 msec.)
For either TG2 or JE2 strains of *E. coli*, use 25 μFD, 200 Ω, and 2.5 kV (time constant = 4.6-4.8 msec).

Step 2. Thaw required number of frozen cell aliquots (each tube 70λ = two transformations) on ice.
Thaw plasmid DNA in TE/H_20 on ice.
Place 15ml conical tube containing 10ml of 2XYT media without antibiotics on ice.

Step 3. Place 3λ of DNA along wall of 0.2cm cuvette. Pipet 35λ of thawed electrocompetent cells onto DNA drop. Flick cuvette to settle DNA + cells mixture into bottom of cuvette.

Step 4. Have 1ml pipette containing 1ml of 2XYT media ready. Dry off any moisture from cuvette outside and immediately place cuvette in white plastic holder. Slide holder into position and zap cells. If you hear a high constant tone, immediately add the 1ml of 2XYT to cells! Transfer cells from cuvette into 1.5ml eppendorf tube and store on ice until step 5.

The tone indicates that you have successfully electroporated your cells. Record the time constant value. Repeat procedure for remaining samples.

If you see or hear sparking coming from your cuvette of cells, then the cells are dead! Repeat that sample again. Things that can cause sparking: excess water on cuvette outside, human skin oil on cuvette outside, too high salt conc. in DNA sample (try diluting DNA 10-fold), and poorly made electrocompetent cells.

Step 5. Outgrow transformed cells in eppendorf tubes by incubating the tubes in 37°C water bath for 1-1.5hrs.

Step 6. Hi/Lo plate transformed cells onto LB + Amp plates. Expect ~10^8 transformants per 1μg of DNA.

Hi plate: Plate 200λ of outgrown transformed cell suspension.

Lo plate: Spin remaining 800λ of cells in microfuge for 20-30sec to pellet cells. Gently shake open tube over sink. The volume of liquid that clings to tube is roughly 150-200λ. Resuspend cells in this volume of retained media and plate cells (using all the volume) on to LB + Amp plates.

Step 7. Place LB + Amp transformant plates in 37°C bacterial incubator for 16-24 hrs until colonies appear.