Preparation of Electro-competent DH5α Cells

**DAY 1**
1. Prepare fresh patch of cells on LB from permanent (not from plate).
2. Grow in 5 ml 2X YT at 37°C overnight. Can use YENB (0.75% YE + 0.75% Nutrient Broth).

**DAY 2**
1. Inoculate 500 ml of 2X YT with 5 ml of fresh overnight culture. Grow at 37°C in a Fernbach flask with vigorous shaking (225 rpm). Check $OD_{600}$ until it reaches 0.5 - 0.75 (about 2-3 hours).
2. Transfer cells to two 250 ml-bottles. Chill cells on ice for 15 minutes or more.
3. Harvest cells by centrifugation in GSA rotor at 5,000 rpm at 4°C for 10 minutes.
4. Pour off all supernatant. Resuspend each pellet in 40 mls ice cold, sterile ddH$_2$O. Transfer to sterile 50 ml red cap tube. Spin at 2500 rpm in IEC at 4°C for 15 minutes.
5. Aspirate off all supernatant. Wash cell pellet once again in 50 mls ice cold, sterile ddH$_2$O.
6. Aspirate off all supernatant. Resuspend each pellet in 20 mls ice cold, sterile 10% glycerol. Pool into one 50 ml red cap tube. Spin at 2500 rpm in IEC at 4°C for 15 minutes.
7. Resuspend cells with 1 ml ice cold, sterile 10% glycerol (about 2 mls of competent cells).
8. Aliquot 75 µl cells per 0.5 ml microfuge tube. Freeze by placing tubes in powdered dry ice in ice bucket. After frozen, store aliquots at -80°C. Thaw only once and use.

Transformation of DH5α by Electroporation

1. Precool on ice: 1. Sterile 0.2 cm gap electroporation cuvettes (or use 0.1 cm gap cuvette)  
2. 75 µl (or 30 µl) aliquots of DH5α cells  
3. Sterile 15 ml tubes with 0.9 ml (or 0.45 ml) LB and a Pasteur pipette in each.
2. Add up to 3 µl (or 1µl) transforming DNA directly to microfuge tube containing DH5α cells. Mix by repipetting, and add to cuvette. Keep on ice for 1 minute.
3. **BioRad Gene Pulser**: Turn on power. Set to 2.5 kV (or 1.8 kV). Set capacitance to 25 µFD.  
**BioRad Pulse Controller**: set to 200 ohms.  
**BioRad Capacitance Extender**: not used, left set on 125 µFD.
4. Take cuvette holder from refrigerator. Carefully wipe condensation off cuvettes (especially metal plates). Tap down cells so they lay evenly across the bottom before placing cuvette in holder (plastic button on side goes in first). Insert cuvette all the way into holder.
5. Press both red pulse buttons at once and hold them until buzzer sounds. At buzzer, quickly add the ice-cold LB directly to the cuvette with the pipet. Remove LB and cells and return to tube on ice.
7. Incubate cells for 1 hour at 37°C. **No shaking!** Plate transformation mixture on LB plates + antibiotic and grow overnight at 37°C. Store mixture at 4°C and plate more later, if needed.