Preparation of electrocompetent bacteria

1. Autoclave 1 L SOB and 3 L ddH2O, and autoclave or filter sterilize 10 ml 10% glycerol. Chill the water and 10% glycerol.
2. Dilute a 10 ml overnight LB or 2xYT culture into 1 L SOB (without Mg or glucose) and growing until OD550 of 0.7-0.8
3. Spin down (5000 rpm in GSA or GS-3) and resuspend in 500-1000 ml cold ddH20. Repeat twice, keeping rotors, buckets, and solutions cold by working in the cold room.
4. Resuspend final pellet in 2 ml 10% glycerol. Make 50 ul aliquots and store at -70˚C. It is not necessary to flash freeze in dry ice/ethanol, but you may if you want.

Electroporation of bacteria

1. For each ligation, chill a 1mm electroporation cuvette and an eppendorf.
2. Aliquot 20-25 ul of frozen electrocompetent cells (thawed on ice) into each eppendorf.
3. Dilute ligation 1:2 with water. Add 0.26 ul (smallest that can be measured with an eppendorf p10 pipetteman) to cells.
4. Using a normal 200 ul pipette tip, mix the cells and ligation by pipetting up and down twice, then transfer to the bottom of the cuvette. It is best to pipette out the cells at one end of the channel in the cuvette, i.e., with the pipette tip wedged along one of the clear walls of the cuvette. Do not pipette in any air bubbles! Don't use a gel-loading tip — it will slow you down and introduce tiny bubbles.
5. Tap the cuvette to bring the cells down into the channel. Look down into the cuvette to make sure that the cells cover the whole floor of the cuvette without any air spaces. Try to keep cuvette on ice.
6. Zap at 1.6 kV, resistance at 2.5 kV and timing at R5 on a BTX electroporation. The time constant should read 4.9 to 5.1.
7. When light goes from constant to blinking, add 200-1000 ul broth, preferably SOC, to cuvette. Squirt it right on the cells or mix with the cells.
8. For amp plasmids, you don't need to let the cells grow up before plating, so you can just keep the cells in the cuvette. Otherwise, transfer to an eppie and shake at 37˚ for 1 hour.
9. To do more electroporations, hit the reset button next to the voltage knob and zap again.
10. Plate at most 1/5 of the cell suspension per plate, or you will get overgrowth. You can save the rest of the bugs in the cuvette at room temp or 4˚.
11. When finished with the cuvette, aspirate out remaining bugs and store in 70% ethanol. Wash with warm water followed by deionized water. Rinse with 95% ethanol, remove excess ethanol, then air dry. You won't get any cross-contamination, and you will save lots of $$$!

If the cuvette arcs: Suspect excessive salt (did you not dilute your ligation or put in more than 0.26 ul?) or an air bubble in your cuvette, although even under proper conditions you will still get arcing occasionally. Arcing decreases tranformation efficiency by about 10-fold. If this is not a problem, e.g., if you are just transferring prepped DNA into another strain, go ahead and rescue. Otherwise, thaw out another aliquot and repeat. If you are doing a ligation, it is best to repeat both the vector control and the vector+insert ligation.