Transforming plasmid DNA into electrocompetent cells

1. Clean and dry electroporation cuvettes throroughly on the cuvette washer. Chill on ice and allow to air dry. Use one cuvette for each DNA sample you are transforming.
2. Dialyze your DNA sample(s) using a nitrocellulose filter and DI water.
   - Fill a Petri dish with DI water.
   - Place a single nitrocellulose filter paper on the surface of the water – **shiny side up.** If you are dialyzing more than one sample, it helps to cut a small notch in the paper to serve as a "key". This helps to identify/keep track of your samples.
   - Spot DNA samples onto the surface of the paper. Space out the samples a little bit because they will expand in volume as they dialyze.
   - Dialyze your sample for 10-15 minutes.

*This step is particularly important if the DNA has undergone any previous manipulations that have introduced salts (adding buffers for ligation, restriction enzyme digestions, etc.). Too much salt in the DNA will cause your sample to arc when electroporating. This will kill the cells and significantly reduce the efficiency of transformation. If your sample arcs, it may still be worth while to plate your cells. However, it is likely that you will get very few (if any) colonies. You should repeat this sample. Main factors that can cause arcing - too much salt in your DNA, water on the outside of the cuvette, oil on the outside of the cuvette from handling it too much without gloves, too much salt in the cells.

3. Thaw electrocompetent cells on ice. Our cells are generally 50 µL aliquots. (Freshly prepared electrocompetent cells may be used immediately.)
4. Turn on electroporator and set voltage – we use 2500 V for 2 mm cuvettes. (1250 V if you have 1 mm cuvettes.)
5. Add dialyzed DNA to thawed cells. Mix gently by flicking tube. Do not pipet up and down! The cells are fragile and this could kill them.
6. Add cell/DNA mixture to the electroporation cuvette. Tap the cuvette gently on the counter to move cells to the bottom.
7. Wipe off excess moisture from outside of cuvette using a kimwipe.
8. Place cuvette in electroporator. Close lid.
9. Have 250-1000 µL of SOC ready to add to your cells.
10. Press the "Pulse" button on the electroporator to shock cells.
11. Remove cuvette from the chamber and immediately add SOC. This step should be done as quickly as possible to prevent cells from dying off.
12. Transfer SOC-cell mixture to an eppendorf tube.
13. Incubate tube in 37°C shaker for at least 1 hr to permit expression of antibiotic resistance gene.
14. Place LB-agar plate(s) supplemented with appropriate antibiotic in 37 °C incubator to warm.
15. Plate transformation onto prewarmed LB-agar plate. Generally, we plate 200µL of cells, but the appropriate volume depends on efficiency of the transformation.

You can also make a “low” plate (using 50 µL of cells) if you think you may get many colonies.

16. Incubate plate overnight at 37°C.
17. Leave remaining SOC-cell mixture on the benchtop overnight. If you don't have any transformants, you can plate the rest of the transformation in the morning.

SOC Medium

Add 20 ml of sterile 1 M glucose per liter of SOB medium immediately before use. Or make frozen aliquots and store at -20 ºC.

SOB Medium

1. Measure ~900ml of distilled H_2O
2. Add 20g Bacto Tryptone
3. Add 5g Bacto Yeast Extract
4. Add 2ml of 5M NaCl
5. Add 2.5ml of 1M KCl
6. Add 10ml of 1M $MgCl_2$
7. Add 10ml of 1M $MgSO_4$
8. Adjust pH to 7.0 with 10N NaOH and adjust volume to 1 L with distilled H_2O.
9. Autoclave to sterilize on LIQUID cycle 15 minutes.