Electroporation

1. Thaw cells on ice for ~30mins. (Tubes in the -80 contain 50uL; enough volume to do 2 electroporations.)
2. Chill cuvettes on ice. (The brown topped cuvettes are 1mm gap. These are the ones I usually use.)
3. Place a p1000 pipette and tips, SOC (or LB) and Eppendorfs next to a flame for use after electroporation as it is important to get the cells in to media very quickly after.
4. For the steps below it is important to be gentle; **do not** vortex or vigorously pipette up and down. Mix by flicking the tube gently.
    a. Split thawed cells in to two tubes, 25uL in each, using a 200uL pipette.
    b. Add 0.5-2uL of plasmid DNA and mix by flicking the tube. (I tend to use 1uL for ligations and 0.5uL for miniprepped plasmid.)
    c. Transfer 20-25uL in to the chilled cuvette using a 200uL pipette.
    d. Wipe the metal contacts of the cuvette with paper towel to ensure there is no water on them.
    e. Tap the cuvette down on the bench to ensure the cells have settled at the bottom and there are no bubbles.
5. Set the electroporator to setting Ec1 and then change the display so that you can record the time constant.
6. Place the cuvette in the electroporator and press pulse. (Record the time constant)
7. **Quickly** remove the cuvette and pipette in 500-750uL of SOC or LB media and transfer to an Eppendorf.
8. Incubate for 1-2 hours.
9. **Make sure you wash the cuvettes thoroughly**. Half fill with 70% ethanol and shake with the lid on. Pour out the waste. Then fill with 70% ethanol, place the lid on and leave to sit for at least 1 hour. Empty the cuvettes and place upside down on paper towel. (If bacteria start growing in them they must be thrown away so this is really important.)
10. Spread 50-500uL on a plate with appropriate antibiotics and incubate over night.

The time constant should be ~5ms (but anywhere between 4 and 6 is usually ok).

- Too long means the pores on the cells have stayed open too long and the cells probably died. Try using a higher volume of cells to reduce the resistance.
- Too short and the cells probably fried. Try using less plasmid or lower volume of cells.