Electroporation of electrocompetent cells with plasmids or ligation mixtures

1. Retrieve the appropriate number of cell aliquots from the -80°C freezer and allow to thaw. You can do this by putting them directly on ice and waiting a long time (~20 min?) or you can put them in room-temperature water in a floatie, then put on ice as soon as they are fully thawed (~5 min?). In the latter case, don't leave them too long at room temperature, keep an eye on them ! Occasional mixing by flicking helps to speed the thawing process.

<<<note ! don't try to electroporate chemically-competent cells, these will spark! >>>

2. Divide up the cells into multiple 50 µl aliquots in sterile 1.5 ml Eppi tubes, on ice. Also put on ice the same number of sterile electroporation cuvettes (2 mm gap size). These need to chill for at last 10 min before use. Label the cuvettes on the cap before use.

3. Add your plasmid DNA or ligation mixture to the cells. For purified plasmid, 1 µl is heaps ! (and may even be too much). For ligation mixture, it would be typical to use 2-3 µl. In both cases more is not better, because the more DNA you add, the more salt you are also adding, and it doesn't take very much salt to make the electroporation procedure fail (short circuit = sparks!). Make sure you change pipette tips for each DNA sample.

4. For all electroporation experiments, you should include a negative control (no DNA added) – this will let you know firstly whether your competent cells are OK (not too salty), and secondly whether your aseptic technique is good. If your negative control makes a spark, your cells are no good (need more wash steps!) or the electroporation machine is not set up correctly. If the electroporation procedure works (no spark), but you get colonies on the plates from the negative control, this means you have contamination, either in your competent cells, or getting in somewhere in the electroporation procedure.

5. In the case of electroporation experiments with ligation mixtures, make sure you also include a positive control, which is 1 µl of a purified plasmid of known good quality with the correct antibiotic resistance. This should give you thousands of colonies or a confluent lawn of growth on the appropriate antibiotic agar plate – if it doesn't, this could mean your cells are not competent, or the plates are bad, or the electroporator is not set up properly, or your 'good quality' plasmid stock is no longer good quality.

6. Once all your cell aliquots have the appropriate DNA samples added, transfer each mixture individually to the corresponding electroporation cuvette (again make sure everything is labelled before starting!). Give each cuvette a few taps on the bench to make sure the cell mix is at the bottom of the cuvette.

7. Take your esky, a p1000 pipette, box of blue tips, some sterile LB broth, and some paper towel over to the electroporation machine (You need ~1 ml LB per sample). Turn on the electroporator, and choose "Exponential" protocol. Adjust the settings on the electroporator to 2500 V, 25 µF, and 200 Ω. These settings will work with a wide variety of Gram-negative bacteria, but note that for Gram-positives you need to increase the ohms to 800 or 1000 Ω. Also note that if you use cuvettes with a different gap size you need to adjust the electroporator settings.

8. Take your first cuvete off ice, and wipe down the outside and underneath briefly with paper towel. Place this firmly into the cuvette holder on the machine, then close the lid.

9. Press the “Pulse” button. After a few seconds, the machine will beep to let you know the pulse has been delivered. Note down the time constant displayed on the screen (ms= milliseconds). This tells you how long the pulse lasted, and a higher number here is better. Retrieve your cuvette and immediately add 1 ml of sterile LB to it. Return to ice.

10. Repeat steps 8 and 9 for all your samples. When finished, turn off electroporation machine, and return to your bench.

11. As the sample becomes more salty with increasing amounts of DNA, the time constant will decrease until eventually you will get a spark (short circuit). If any of your samples make a spark, this means either your competent cells are not prepared properly (the negative control will spark too), or that you have added too much DNA and/or the DNA is of low quality. It is possible to sometimes get transformants from a cuvette which as sparked, but don’t count on it! Its better to repeat the experiment using less DNA (or a dilution of the DNA in sterile MQ water) and/or a different batch of electrocompetent cells.

12. Aseptically pour each transformed cell mixture into a sterile 1.5 ml Eppi tube, as follows. First, label all the Eppi tubes, then open them all, positioned in a rack.(they don’t need to be in ice). Take the cap off the first cuvette and briefly pass the top of the cuvette through the Bunsen flame a few times (if you flame it too much it will melt!), then pour into the first Eppi tube. Tap the cuvette on the tube to ensure you get all the sample out. Repeat for other tubes.

13. Ensure all the Eppi tubes are tightly closed and labelled, and then incubate with shaking for 1 hour at 37°C (E.coli) or 1.5 hours at 30°C (Pseudomonas). Put the tubes lying on their side in a beaker or other container in the shaker, or you can attach them to the shaker with elastic bands or masking tape. Lying horizontal is important for good shaking action in these small tubes.

14. Retrieve tubes from shaker. Prepare 2x the number of appropriate agar plates as you have samples (double check you are using the correct antibiotic(s)!!!), and label all these plates before going any further. In addition to the usual information (antibiotic, plasmid, strain, date), label half of the plates “100 µl” and the other half “pellet”

15. Spread plate 100 µl of each sample onto the appropriate antibiotic agar. Centrifuge the remaining culture (2 min at 10,000 g in Eppi centrifuge), pour off most of supernatant, and resuspend the pellets by vortexing in the drop of liquid that remains. Plate these resuspended cells on a second set of plates of the same antibiotic type. The reason for doing two plates for each sample is to give us the best chance of getting countable and well-isolated colonies on at least one of these plates.

16. Don’t throw out the used electroporation cuvettes. These can be recycled. (repeated rinses with water then 80% ethanol, see elsewhere for this protocol). Don’t let the cuvettes dry out with cell mix still in them, you should give these a couple of rinses with WATER as soon as you finish the experimental part of the work. (discard rinsate into culture waste).