Transformation of Bacteria (Electrocompetents)

NOTE: Electrocompetent *E.coli* generally have a higher transformation efficiency (more colonies per μg / DNA) than chemically competent cells. On the minus side the protocol is more fiddly than heat-shocking.

SAFETY: *Ensure the machine is discharged before you switch it off – the capacitors can store a charge for quite a while (see **Step #7**).*

1. Thaw a vial of electrocompetent cells (usually stored at –80°C) on ice. Aliquot 40 μl of cells per transformation into microfuge tubes. Keep on ice.

2. Add DNA (≤3μl). Mix gently (and minimally). The DNA should be in TE, water, or a low salt solution. If DNA is in a high salt solution (eg. a restriction digest), precipitate and resuspend in TE (this is not a recommendation to be skirted lightly – see **Step #7**).

3. For each transformation, assemble on ice: a sterile electroporation cuvette and a sterile test tube containing 350μl of media (SOC or LB with NO selective agent).

4. Set the Gene-Pulser to 25 μFaradays, 200W, and 2.45 kEv.

5. Ensure there are a clean pipette bulb and sterile, pre-chilled long pasteur pipettes to hand.

6. Transfer the bacteria/DNA into the bottom of the cuvette. Insert into the cuvette holder and push holder all the way in to connect the cuvette to the electrodes and complete a circuit.

7. Hold the two red buttons down until the electroporator beeps (will take a few seconds – the capacitors have to charge up first). If you hear a loud pop, the sample vaporized due to too much salt in the DNA : dump the cuvette (the majority of failures are due to crappy cuvettes, unless of course you got all brave at **step #2** and used a high salt buffer) and the transformation (they're all dead).

8. Immediately add the media to the cuvette. Gently mix and transfer all to the sterile test tube.

9. Allow bacteria to grow for 30m – 1hr with shaking at 37°C.

10. Plate the desired amount of cells on a selective plate (**what plasmid did you just transform ? Don't just assume it was Amp^r**). Incubate plates inverted overnight at 37°C.

NOTE: If you incubate LB^{Amp} plates too long with well growing *E.coli* strains, satellites will develop around the true transformants. These are caused by the secretion of β-lactamase which diffuses away from the Amp^R colony. In addition to being very annoying, they make selecting the true transformant difficult if the cells were plated at a high density.