Preparation of electrocompetent cells of *Pseudomonas* or *E.coli*

1. Inoculate a 50 ml LB broth with several colonies from a freshly-grown LB agar plate culture (1-7 days old), grow overnight with shaking at 30°C (for *Pseudomonas*) or 37°C (for *E.coli*)

2. In the morning, add the entire 50 ml culture to 500 ml LB broth in a large Erlenmeyer flask. Measure the optical density (OD_{600}) – this should be ~0.1 - 0.2. Record the value.

3. Incubate the 500 ml culture with shaking at 30°C. Check the OD600 approximately every 30 minutes. When the value reaches 0.4-0.6 (*E.coli*) or 0.8-1.0 (*Pseudomonas*), take it off the shaker, and pour culture aseptically into 2 x 500 ml sterile Nalgene centrifuge bottles which have been chilled on ice. (note that these centrifuge bottles should only be half full so they don't leak in the centrifuge. Resist the temptation to just use 1 x 500 ml bottle!).

4. Ensure tubes are balanced (weigh them, and top up the lighter one with the appropriate amount of buffer). Centrifuge in large floor centrifuge for 15 minutes at 7500 g (=6700 RPM for Sorvall GS3 or equivalent rotor) at 4°C.

5. Pour off the supernatant liquids into culture waste, and resuspend each pellet in 30 ml of cold electroporation buffer *. Put caps on tight and shake vigorously to resuspend the cells. (approx 10 seconds), then pour into 2 x 50 ml Falcon tubes.

6. Ensure tubes are balanced (matching volumes by eye is OK this time). Centrifuge at top speed (~4500 rpm, or ~ 3000 g) for 15 minutes in Centaur/Centurion type centrifuge at 4°C.

7. *Gently* pour off supernatants into culture waste –the pellets may be quite soft, and are easily lost if you are not gentle! You need to retrieve the tubes immediately from the centrifuge when the spin is finished, and also handle the tubes gently in order to maintain the pellet at the bottom of the tube.

8. Resuspend each pellet in 30 ml of cold electroporation buffer, and spin again.

9. Pour off supernatant, resuspend each pellet in 30 ml cold electroporation buffer, and spin again.

10. Resuspend one pellet in 5 ml of cold electroporation buffer, resuspend cells by vortexing (~10 sec). Pour this cell suspension aseptically into the other tube, and vortex again. Place on ice.

11. Pipette the cell suspension into multiple small aliquots in sterile 1.5 ml Eppendorf tubes. For example, 25 x 200 µl aliquots would be typical. Each of these single 200 µl aliquots has enough cells for four electroporation cuvettes. Label the tubes with the strain name before you start. Although 'correct practice' here would involve setting up your 25 Eppi tubes opened, and on ice, I woudn't do this due to the potential for contamination (melted ice getting in the tubes). But you should aim to do this step quickly, and get the cell aliquots into the -80C freezer ASAP.

12. Put the cell aliquots into a plastic bag or box and freeze at -80 C. Label the bag or box in addition to the individual tubes. Don't label the bag surface directly with permanent marker – this will come off–better to write with marker in large clear text on a piece of paper, and put this in the bag.

* Electroporation buffer: For *E.coli* and *Ps.putida*, you can use 10% glycerol, but for *Ps.stutzeri*, you need a more complex buffer containing 10% sucrose, 1 mM HEPES (pH 7), 1 mM MgSO4. This is because the cells are more fragile. Both types of buffer need to be sterilised by autoclaving, and should be chilled on ice before use (at least 30 min on ice; or keep this buffer in the cold room).