Preparation of electrocompetent *Mycobacterium smegmatis* & electroporation

Protocol for making electrocompetent cells

1. Inoculate a 5 mL culture of *M. smegmatis* mc^2-155 in LB broth containing 0.05% Tween 80 (add after autoclaving from filter-sterile stock) and grow until saturation (~2 days). If there are plasmids in the *M.smeg* cells (e.g. pJV53), add antibiotic too (e.g. Km at 25 µg/ml). You can grow *M.smeg* at either 30°C or 37°C; it grows a bit faster at 37°C.

2. Measure the OD_{600} of the saturated broth, then subculture into 50 mL broths*, adding sufficient pre-culture to give starting OD_{600}'s of 0.02, 0.05, and 0.1. (we do this to maximise the chance that at least one broth will reach the desired OD_{600} at a convenient time).

   * The broth you use will depend on the cells you want to prepare:

   i. For cells with pJV53, use MSM minimal medium in a 250 ml flask (see elsewhere in lab protocols for MSM recipe) containing 1% succinate, 20 µg/mL Km, and 0.05% Tween 80.

   ii. For cells with no plasmid, subculture into 50 mL LB + 0.05% Tween 80.

3. On the following day, measure the OD_{600} of the three broths at intervals. When one culture is at late log phase (OD_{600} between 0.8-1.0), transfer this culture to ice, and incubate for 1.5 hours. Discard the other cultures that are at the 'wrong' stage of growth.

4. Transfer chilled culture to 50 mL Falcon tube(s), centrifuge at ~ 4000 g for 10 min at 4ºC, discard the supernatants. For cultures containing pJV53, first add acetamide (0.2% w/v) and grow a further 3 hours to induce the recombination enzymes before centrifuging the cells.

5. Add 20 ml ice cold 10% glycerol + 0.05% Tween 80 solution, centrifuge, discard supernatant. Repeat this step two more times. This removes salt, which is bad for electroporation. If you have multiple cell pellets, resuspend the first one in 20 ml, then add that cell suspension to the next tube, and repeat, so that all the pellets go into the same 20 ml of buffer.

6. Resuspend cells in 3 mL of the same glycerol-Tween solution, divide into 160* µl aliquots, freeze at -80°C. *This will give 3 x 50 µl aliquots with a little bit left over.

Protocol for electroporation

1. Thaw aliquots of electrocompetent cells (from above) equivalent to half of your number of DNA samples, divide into 100 µl aliquots in Eppi tubes and keep on ice.

2. Add plasmid DNA to the cells. Depending how clean and how concentrated your plasmid is, and what the aim of the experiment is, you may be adding 1-10 µl of plasmid (could be 10-1000 ng, depending on the experiment). If unsure, start with less plasmid and see how you go. Too much plasmid will cause sparking (bad).

3. Allow 10 min on ice for the DNA to adsorb to the cells. While you are waiting, add the electroporation cuvettes to the ice to allow them to chill. We usually use 2 mm gap cuvettes for this. Careful not to get ice inside the cuvettes (ice is not sterile!).

4. Transfer each cell+DNA mix into an electro-cuvette, tap gently to get liquid to bottom. Take samples on ice to the electroporation machine. Set the machine for 2.5 kV, 800 Ω, 25 µF.

5. Wipe down the outside of the cuvette with paper towel, and put it into the holder. Ensure that the metal sides of the cuvette are contacting the electrodes. For some combinations of machine+cuvette, you need to put a spacer in the bottom of the holder to ensure good contact (we use a 5 mm glass bead for this).

6. Hit the button to initiate the electric pulse. Note the time constant – this should be between 15-25 milliseconds. If it sparks, you can try adding 100 µl of 10% glycerol and re-pulsing, but usually this means you need to add less DNA and/or better-quality DNA.

7. Immediately add 1 ml LB-Tween to each cuvette, mix with p1000, transfer to Eppi tube, recover for 2 h with shaking at 30°C or 37°C.

8. Spread-plate 100 µl on the appropriate antibiotic agar plate. Pellet remaining cells, resuspend in a drop of the residual liquid, then spread on a second plate. This gives a mini-dilution series, so hopefully one of the plates will have nice well-separated pickable colonies (this may not matter for some experiments, but does matter for others).