ELECTRO-TRANSFORMATION OF *E. COLI*

Reagents:
1 liter LB (2 x 500 ml flasks)
2 liter sterile dH_2O
30 ml 10% glycerol
4 x 250 ml centrifuge bottles

Day 1

1. Inoculate 2 x 5 ml LB + tetracycline (12.5 $\mu g ml^{-1}$) with single colonies of XL1-BlueMRF' and incubate shaking at 37°C overnight.

2. Chill bottles, water, glycerol and rotor in cold room overnight.

Day 2

1. Inoculate 2 x 500 ml of LB + tet with 2 x 5 ml of overnight culture.

2. Grow cells at 37ºC with vigorous shaking to an OD_{600} of 0.5 (the best results are obtained with cells that are harvested at early to mid log phase; the appropriate cell density therefore depends on the strain & growth conditions).

3. To harvest, chill the flask on ice for 30' & centrifuge in 4 x 250 ml bottles in a cold rotor at 5K for 15'.

4. In the cold room, remove as much of the supernate as possible. It is better to sacrifice the yield by pouring off a few cells than to leave any supernate behind. Spin 5K 1' & pipet off any remaining supernate.

5. Resuspend the pellets in a total of 250 ml (i.e. 4 x 62.5 ml) ice cold water taking care not to lyse them. Centrifuge at 5K for 15'. Pour off supernate.

6. Resuspend pellets in a total of 125 ml (i.e. 4 x 31 ml) ice cold water & transfer cells to 4 x 50 ml Falcon tubes. Spin 3K 10' in benchtop centrifuge.

7. Resuspend pellets in a total of 20ml (i.e. 4 x 5 ml) ice cold 10% glycerol & spin at 3K for 10'.

8. Resuspend in a final volume of up to 200 μl ice cold 10% glycerol per Falcon tube depending on how many cells were lost in spins. In general, the less volume the higher the efficiencies.

9. Freeze in 40 μl aliquots in liquid nitrogen and store at –70°C. The cells are good for at least 6 months under these conditions.

Plating

1. Gently thaw cells on ice. Remove sterile cuvettes from pouches and place on ice. Place the white chamber on ice.

2. Set the Gene Pulser at 25 μF. Set the Pulse Controller to 200 Ω. Set the Gene Pulser apparatus to 2.50 kV when using the 0.2 cm cuvettes (set it to 1.50 to 1.80 kV when using the 0.1 cm cuvettes).

3. Add 1 to 2 μl of DNA to 40 μl of cells (DNA should be in a low ionic strength buffer such as TE or you may explode your cells). Mix well and let sit on ice for 30 sec. For a control use 1 μl of 10 $pg\mu l^{-1}$ stock of pBS or whatever vector you're cloning into and plate 100 μl.

4. Transfer the mixture of cells & DNA to a cold electroporation cuvette & shake the suspension to the bottom. Wipe condensation off the cuvette & place in a chilled safety chamber. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber.

5. Press both pulse buttons together until it beeps.

6. Remove the cuvette and **immediately** add 1 ml of ***SOC*** broth to the cuvette and mix by pipetting up and down a few times. It is a good idea to have the SOC in the pipette before zapping.

7. Incubate in capped cuvette for 1 hr at 37º C with shaking & then plate 200 μl per plate.

SOC (250 ml)

5 g tryptone	500 μl 5M NaCl	2.5 ml 1M $MgCl_2$
0.9 g glucose	625 μl 1M KCl	
1.25 g yeast extract	2.5 ml 1M $MgSO_4$	