Making electrocompetent *E.coli*

See allied protocol for electrotransformation

NOTE 1: Electrocompetent *E.coli* generally have a higher transformation efficiency (more colonies per μg / DNA) than chemically competent cells (**Expected ≥ 5 x 10^9 cells / μg DNA**). On the minus side the protocol is more fiddly than heat-shocking.

NOTE 2: *E.coli* strains used (detailed genotypes online)

(i)	XL1-blue	Tet^R	
(ii)	S28	Tet^R	vv. weak pellet
(iii)	DH5α	Tet^S	stronger pellet
(iv)	DH10b	Tet^S	

1. Confirm the genotype of the strain to be made competent. Determine if it has an F' (usually required for color-selection), and thus whether the seed should be grown with Tetracycline.

2. Inoculate colony into 50ml LB (+ Tet if appropriate). Grow overnight: 37°C, 300rpm.

3. Sub 1/100 into 1L LB. Note OD_{600}. Incubate 37°C, 300rpm. Measure OD_{600} every 30 – 60 mins. Harvest at $OD_{600} \approx 0.5$ (range 0.4 –> 0.6). Chill culture 15 min in an ice-bath.

NOTE 3: ALL buffers from now on are sterile and pre-chilled on ice. Keep everything ≤ 2°C.

4. Transfer culture to centrifuge flasks and collect 4000rpm, 2°C, 10m.

NOTE 4: The quality of the *E.coli* pellet will get worse after each spin (presumably the *E.coli* contacts are ion-dependent).

5. Discard supernatant (**careful**) and resuspend pellet in 250ml ddH_2O. Centrifuge 4000rpm, 2°C, 10m. Repeat this step twice.

NOTE 5: Pre-label ependorffs and store rack at –20°C. Also get some dry-ice.

6. Resuspend pellet in 40ml 10% glycerol. Transfer cells to a 50ml Falcon tube. Centrifuge 6000rpm, 2°C, 10m. Estimate packed cell volume and add an equal volume of 10% glycerol. Dispense 200μl aliquots into tubes on dry-ice and store at -80°C.

7. Estimate the competence (expressed as colonies per μg plasmid) by transforming with a known amount of plasmid. Plate numerous dilutions and count cells. Note the competence and date on the box and consider that the efficiency will drop with increasing storage, even at -80oC (best to use cells within ≤ 12 months).

NOTE 6: The number of transformants increases linearly with input DNA over a wide range (5pg -> 500ng). However it is important to keep the added volume of DNA as small as possible (particularly important for ligations) since arcing can occur at salt concentrations >1mM.