PROTOCOL / COMPETENT CELLS

(Note: Normally we work with volumes of 200ml, but for the purpose of the workshop we have reduced this quantity to 1ml, e.g. step 2 would be 200ml)

1. Pick a single colony from a prepared plate using a plastic loop
2. Add to 1ml of LB-culture, grow until fairly turbid (circa 2 hour) or in laboratory conditions to 0.4-0.5 OD_{600nm} if a spectrometer is available.
3. Place the culture on ice for 30 minutes
4. Centrifuge cells for 7 minutes at 3,500 rpm (at 4°C if possible)
5. Remove supernatant by discarding it in a supplied container - you should now have cell pellets at the bottom of the tube
6. Add 1/4 volume of 0.1M MgCl2 (e.g. 250µl for 1ml starting culture)
7. Resuspend cell pellets by pipetting
8. Centrifuge cells for 7 minutes at 3,500 rpm (at 4°C if possible)
9. Remove supernatant by discarding it in a supplied container
10. Add 1/2 volume of 0.1M CaCl2 (e.g. 500µl for 1ml starting culture)
11. Resuspend cell pellets by pipetting
12. Place on ice for 30 minutes
13. Centrifuge cells for 7 minutes at 3,500 rpm (at 4°C if possible)
14. Remove supernatant by discarding it in a supplied container
15. Resuspend cells in 1/50 volume of 0.1M CaCl2 + 15% Glycerol (14µl 0.1M CaCl2 + 6µl 50% Glycerol)
16. For starting volumes higher than 3ml prepare aliquots of 50µl (and store at -80°C or in our case on ice)

Each participant should write their name on the tube and provide an email or friend C-LAB on Facebook - we will post pictures of transformation results...

Clean up: wipe all surfaces applied with ethanol; store competent cells on ice for later test in laboratory conditions.

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