E. coli Calcium Chloride competent cell protocol

1. Inoculate a single colony into 5mL Lb in 50mL falcon tube. Grow O/N @ 37°C.
2. Use 1mL to inoculate 100mL of LB in 250mL bottle the next morning.
3. Shake @ 37°C for 1.5-3hrs.

Or

1. Inoculate a single colony into 25mL LB in a 250 mL bottle in the morning.
2. Shake @ 37°C for 4-6 hrs.

Then….

1. Put the cells on ice for 10 mins (keep cold form now on).
2. Collect the cells by centrifugation in the big centrifugue for 3 mins @6krpm
3. Decant supernatant and gently resuspend on 10 mL cold 0.1M CaCl (cells are susceptible to mechanical disruption, so treat them nicely).
4. Incubate on ice x 20 mins
5. Centrifuge as in 2
6. Discard supernatant and gently resuspend on 5mL cold 0.1MCaCl/15%Glycerol
7. Dispense in microtubes (300µL/tube). Freeze in -80°C.

Transformation of Ca++ competent cells

1. Put 1µL of circular plasmid or all of a ligation reaction of plasmid DNA in a microtube. Gently add ~100µL of competent cells. Do NO DNA control tube with cells and no DNA.
2. Incubate for 30 mins on ice.
3. Heat shock for 2 mins @ 42°C. Put back on ice.
4. Add 900 µL of LB to tubes. Incubate @ 37°C for 30 mins.
5. Plate 100-1000 µL of the cells in LBAmp or LBCarb (100µg/ml) plates. Plate 100 µL of the NO DNA control in a blood plate (to check for quality of cells). Grow O/N. U can save the rest in the cold room or freeze with 15% of Gly in case u get no colonies (v. unlikely).
6. If you need a lot of colonies or the ligation is of low efficiency, centrifuge the transformation for 1 min @ 8krpm, discard 900 µL of supernatant, resuspend on the 100 µL left and plate the whole lot.