Transformation of *E. coli* - $CaCl_2$ competents

NOTE 1: These cells are obviously not Amp^R or KanR when you start – if they are you have problems. However some strains ARE Tet^R and this selection should be maintained if you intend color selection (the F' plasmid containing some components of the LAC operon pathway is generally Tet selectable – **check**).

NOTE 2: This protocol is NOT my first choice for competent *E.coli* – it's too long and fiddley …

NOTE 3: Many of these buffers require pre-chilling on ice … *failure to do so will reduce the transformation efficiency …*

Grow an overnight culture in 2-3 ml LB.

First thing in the morning, inoculate 50ml of LB with 0.5 - 1.0 ml of the overnight.

Grow at 37°C with shaking until $OD_{600} \approx 0.4$. At this OD, the cells usually have a pearly look when swirled.

Spin down the bacteria in a 50 ml conical tube (5 minutes, 3000 rpm in a tabletop centrifuge).

Resuspend cell pellet completely in 25ml ice cold sterile 50mM $CaCl_2$. Ensure to break up clumps. Set on ice for 5-20 minutes.

Respin cells. Resuspend in 3-4 ml of ice cold 50mM $CaCl_2$.

For each transformation, use 100µl of cells. Add DNA in a volume of 10µl or less. For ligations, half the reaction is typically used. 50ng of good plasmid DNA can be used as a control and should give thousands of colonies. Mix by pipetting or tapping tube, don't vortex.

Set on ice for 20-30 minutes.

Heat shock 90 seconds at 42°C or 5 minutes at 37°C. Some strains do better with one procedure or the other.

Set on ice for 10 minutes.

Add 500µl of LB (<u>NO</u> selective agent) and incubate at 37°C for 45 minutes.

Plate on selective media (usually LB + Amp or Kan). I'd suggest three plates with significantly different amounts (5µl, 50µl and the remainder). Incubate plates overnight at 37°C.