Transformation of E. coli by $CaCl_2$

This protocol is used to generate competent cells (XL1, DH5α) (**part A**) which are then frozen in aliquots at -80°C. 50uls is generally sufficient for a single transformation (**part B**) from a ligation reaction.

A. Competent cells:

1. Grow 1 liter of cells to OD_{600} = 0.4 (2x 10ml seeds – 37deg).
2. Cool on ice for 10 mins, spin 20 mins. at 6000 rpm.
3. Resuspend in 500mls of ice cold 50mM $CaCl_2$/10mM Tris-HCl pH 7.4 (sterile filtered).
4. Incubate on ice for 1h.
5. Spin, resuspend in 5mls of 50mM $CaCl_2$, 10mM Tris-HCl pH 7.4, 20% glycerol, sterile filtered.
6. Aliquot (150uls) and freeze at -80°C.

B. Transformation:

1. Thaw cells on ice.
2. Add DNA and 100µls TCM, 100µls of cells on ice, incubate 30mins.
3. Heat shock at 42°C for 0.5 min, ice for 1.5 mins.
4. Add 2mls of LB, incubate 37°C for 1h.
5. Spin down 3k for 15 mins., plate on LB plus antibiotic.

TCM: 50mM $CaCl_2$, 30mM $MgCl_2$, 10mM Tris-HCl pH 7.4