Bacterial Transformation and Generation of Competent cells using the Calcium Chloride Method

Experimental considerations

- This method is easy and fast, and provides reasonable transformation efficiencies (10^5-10^6 colonies per ug of DNA) which are sufficient for routine subcloning experiments.
- Frozen vials can be kept at –70°C for months-years without significant loss of viability or efficiency.

Protocol

I. Preparation of Competent cells.

- Inoculate one colony into 50 ml 2xYT.
- Incubate overnight on shaker at 37°C.
- Inoculate entire overnight culture into 1 liter of pre-warmed 2xYT in a 2 liter flask.
- Incubate on shaker at 37°C for 3-4 hours.
- Spin cells for 5 minutes at 5 Krpm at 4°C (**see note).
- Resuspend cell pellet in 300 ml ice cold 100mM $MgCl_2$ (first suspend in 10 ml and then add remaining 290 ml).
- Incubate for 10 minutes on ice.
- Spin cells for 5 minutes at 5 Krpm at 4°C.
- Resuspend cell pellet in 300 ml ice cold 100mM $CaCl_2$ (first suspend in 10 ml and then add remaining 290 ml).
- Incubate for 30 minutes on ice.
- Spin down cell suspension.
- Resuspend cells in 100 ml ice cold 100mM $CaCl_2$ plus 20% Glycerol (first suspend in 10 ml and then add remaining 90 mls).
- Aliquot 0.5 ml per eppy tube on ice [keep tubes on ice until transferring to –70°C (next step)].
- Transfer aliquots to –70°C freezer.

**Note: Must use very clean centrifuge tubes since contaminating residual DNA from other experiments (e.g. Maxi-plasmid preps) causes background in cloning experiments.

II. Transformation

- Thaw a vial of competent cells on ice (or warm quickly in hand).
- Add plasmid DNA (mix by tapping with finger).
- Incubate for 30 minutes on ice.
- Heat shock: Place tubes at 37°C water bath for 2 minutes and 30 seconds.
- Immediately transfer tubes to ice for 5 minutes.
- Add 1 ml 2xYT (with no antibiotic) and mix briefly.
- Incubate samples in 37°C water bath for 45 min to 1hr.
- Plate 300ul onto an amp containing 100mm 2xYT plate.
- Incubate plate overnight at 37°C (note: 300ul of solution is generally too much to absorb completely into agar so we do not invert plate during overnight incubation – this allows the plate to dry slightly and allows the solution to soak in better).

Solutions

100 mM $MgCl_2$	500 ml
1M $MgCl_2$	50 ml
milliQ H_2O	450 ml

100 mM $CaCl_2$	500 ml
1M $CaCl_2$	50 ml
milliQ H_2O	450 ml

2xYT	1L
tryptone	16g
yeast extract	10g
NaCl	5g