Protocol: Competent Cells with $CaCl_2$

Adapted from Current Protocols in Molecular Biology

This protocol makes 4 ml of competent cells. The cells are typically stored in 110 µl aliquots. A typical transformation uses 20 µl of cells.

Material	Amount	Notes
LB with appropriate antibiotics	50 ml	
100 mM $CaCl_2$	15 ml	Ice cold
100 mM $CaCl_2$ + 15% Glycerol	4 ml	Ice cold

Note: Never vortex competent cells.
Resuspend by pipetting with large Pasteur pipettes.

The night before:

1) Inoculate a 5 ml culture and grow overnight with selection.

The day of:

2) Dilute cells ~ 1:200 into selective media.
For this example add 250 µl to 50 ml of selective media.

3) Grow the cells to an OD600 of 0.5 – 0.6.
Use a large flask, 500ml, for good aeration.
Use a baffled flask for fastest growth.
This takes about 3 hours depending on the cells.
Medium-heavy cloudiness by eye is fine.

4) Spin down the cells at 4 °C, 4000 rpm, 15 minutes.

Note: Keep the cells at 4 °C from now on.

5) Resuspend cells in 15 ml, ice-cold 100 mM $CaCl_2$.
Leave on ice 4 hours to overnight.

6) Spin down the cells at 4 °C, 4000 rpm, 15 minutes.

7) Resuspend cells in 4 ml, ice-cold 100 mM $CaCl_2$ + 15% glycerol.

8) Aliquot into pre-chilled Eppendorf tubes. Use immediately or store at -80°C.

Note: Frozen cells are only good once.
Do not refreeze cells once thawed.