Protocol For $CaCl_2$ preparation of Competent E. coli for Transformation with Plasmid DNA

1. Autoclave 1 liter of LB broth in 2 liter baffle flask and cool to room temperature.
2. Inoculate with 5 mls – 10 mls O/N Culture of E. coli started from a single colony.
3. Incubate at 37°c with shaking until the cells are in logarithmic growth 0.4 – 0.8 at 600nm optical density.
   * Should take 4 – 6 hours.
4. Wash 500 mls bottle with 70% EtOH and drip dry inverted and cool on ice.
5. Transfer cells to bottles 2/liter 500 mls each
6. Centrifuge at 4000 rpms at 4°c keeping cells cold at all times. This is extremely important.
7. Resuspend pellet of cells in 10 mls $CaCl_2$ soln ice cold. Keep on ice for 5 minutes (use 40 mls oreke tubes)
8. Centrifuge at 4000 rpms 4°c. Resuspend pellet in 10 mls $CaCl_2$ soln ice cold. Keep cells on ice for 30 minutes.
9. Centrifuge at 4000 rpms 4°c and drain pellet as dry as possible trying to keep Cold.
10. Resuspend pellet in 2.5 mls Cold $CaCl_2$ soln per tube (≈ 500 mls cells)
11. Cells should be resuspended well. Keep on ice for a few hours up to 12 – 24 hours
12. Aliquot in 50 µl aliquots. 100 aliquots per liter of cells
13. Estimate Competency using Plasmid DNA

$CaCl_2$ Soln :

pH 7.0

8.8 g $CaCl_2 \cdot 2H_2O$
3.46 g PIPES
150 mls Glycerol (100%)
850 mls ddH_2O

1000 mls

* Filter Sterilize 0.2 µm
* Cells should be aliquoted on ice and frozen immediately after aliquots.