$CaCl_2$ Competent Cells

The water source is absolutely critical for preparing competent cells. Use at least 18 Mohm water for all solutions (even the LB) in this protocol.

For maximal competent cells it is imperative that all vessels be ice cold and the cells be kept on ice throughout the entire procedure.

I. Preparing the cells

1. Dilute an overnight 1:200 in LB.

2. Aerate the culture at 30°C or 37°C until the OD_{550} is between 0.3 and 0.4. Record the OD_{550}. Warning: Do not let the OD_{550} go above 0.5 or the culture will yield poor competent cells.

3. Chill the cells on ice for 1 hour.

4. Pellet the cells at 7K for 6 minutes at 4°C.

5. Resuspend the cells in 1/4 volume of ice cold 0.1 M $CaCl_2$, and leave them on ice for at least 1 hour. To obtain optimal transforming frequency, the cells should be incubated on ice at 4°C for 12-16 hours. Failure to do so will result in a 2-5 fold reduction in transformation frequency.

6. Pellet the cells at 7K for 6 minutes at 4°C.

7. Resuspend the cells in ice cold 0.1 M $CaCl_2$; 10% w/v glycerol at a final concentration of 15 OD_{550} units/ml. Example: If the initial OD_{550} of a 50 ml culture was 0.3, then resuspend the cells in 1 ml.

8. Store the new competent cells in 0.5 to 1 ml aliquots at -70°C. 100 µl of competent cells is needed for each transformation.

II. Transforming the cells

1. Remove an aliquot of competent cells and let them thaw completely on ice.

2. Add up to 10 µl of DNA to 100 µl of competent cells. Note: DNA from ligation mixtures can be added directly to the competent cells without dialysis or precipitation to remove excess salt, ficoll, etc.

3. Incubate on ice for at least 1 hour with periodic vortexing (about every 20 minutes) so the cells remain evenly distributed and don't settle to the bottom of the tube.

4. Heat shock cells at 42°C for 3 minutes.

5. Add 100 µl of LB and plate on the appropriate selective media.