Electro 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.

Electro competent cells yield transformation frequencies that are 10 to 100 fold higher than $CaCl_2$ competent cells.

Preparing the cells (250 mL)

1. Dilute an overnight 1/200 in LB (1.25 mL into 250 mL).

2. Aerate the culture at 37°C until the OD_{550} is between 0.3 and 0.4. *Note: Some strains will yield high transforming electrocompetent cells from overgrown cells (OD_{550} from .5 to 1.0 and over). This is very strain dependent and has to be verified for each strain.*

3. Chill the cells on ice water for 10 minutes.

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

5. Discard the supernatant and resuspend the cells in ice cold 10% glycerol at ½ their original volume (125 mL).

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

7. Discard the supernatant and resuspend the cells in ice cold 10% glycerol at ¼ their original volume (62.5 mL).

8. Repeat steps 6 and 7.

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

10. Discard the supernatant. At this point the cells should be thixotropic and go easily into suspension with vortexing. If not, add a few drops of 10% glycerol until they do.

11. Store the now competent cells in 46 µl aliquots at -70°C. 40 µl of competent cells is needed for each transformation.

10% glycerol

110 mL Glycerol + 1000 mL DI H_2O + 150 mL DI H_2O rinse.

II. Transforming the cells

1. Remove an aliquot of competent cells and let them thaw completely on ice. Note: Unlike $CaCl_2$ competent cells, once thawed, electrocompetent cells can not be refrozen.

2. Add 1-2 µl of DNA, which has been suspended in a very low ionic strength buffer such a $T_{10}E_{.1}$, to 40 µl of electro-competent cells. Mix well and incubate on ice for 1 minute. *Note: DNA from ligation mixtures must be either dialyzed or precipitated if more than 1 µl is to be used to transform electro-competent cells. The presence of high salt will cause electro-competent cells to arc, thus greatly reducing their transforming frequency.*

3. Set the Gene Pulser apparatus at 25 uF and 2.5 KV. Set the Pulse Controller to 200.

4. Transfer the cell/DNA mixture to an ice cold 0.2 cm electroporation cuvette and tap the suspension to the bottom of the cuvette. Place the cuvette into the safety chamber slide (push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber).

5. Pulse once at the settings in step 3. This should produce a pulse with a time constant of 4-5 msec and a field strength of 12.5 dV/cm.

6. Remove the cuvette from the chamber and immediately add 1 ml of ice cold SOC medium. Note: The swift addition of SOC medium is crucial for maximizing the recovery of transformants.

7. Transfer the cell suspension to a culture tube and aerate at 30°C for 45 minutes.

8. Plate 10 µl, 100 µl, and 900 µl of the cell suspension on selective medium. The 900 µl sample can be pelleted and resuspended in 100 µl before plating.

SOC

2.0% bacto tryptone
0.5% bacto yeast extract
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$
20 mM glucose