PREPARATION OF COMPETENT E. coli CELLS FOR TRANSFORMATION

Shawn's Protocol

Large scale stocks of E. coli cells can be made competent for transformation with plasmid DNAs, and then stored frozen at -70°C.

1) Prepare a streak-plate of DH-1 cells on an LB plate. Incubate at 37°C overnight.

Note: This protocol is for 1-liter of competent DH-1 cells. Will yield 50 ml competent cells to be aliquotted into 200 µl or smaller aliquots.

Have prepared: 4 x 250 ml L-broth in 1-liter flasks
1100 ml ice-cold 0.1 M $CaCl_2$

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2) Inoculate two 5 ml L-broth cultures (in 15 ml snap-cap tubes) with a single DH-1 colony and shake overnight at 37°C in a New Brunswick G-25 shaker at 400 rpm.

3) Transfer the overnight cultures equally into four 1-liter flasks containing 250 ml L-broth each. Shake the flasks at 37°C until the bacteria reaches $OD_{550} = 0.5$, which will normally require approximately 2 hours.

4) Cool the bacteria on ice to roughly 5°C, then transfer to four 500 ml centrifuge bottles, and centrifuge in a Sorvall GSA rotor at 4°C for 10 minutes at 5000 rpm (10,400 x g).

5) Resuspend bacterial pellets in 200 ml ice-cold 0.1 M $CaCl_2$. Combine cells into a single bottle, and centrifuge again as above.

6) Resuspend pellet in 250 ml ice-cold 0.1 M $CaCl_2$, and keep on ice for 4-8 hours. *Note: Competent cells can be obtained with incubation times on ice as low as 20 minutes, but the longer times greatly increase the overall transformation efficiency.*

7) Recentrifuge cells as above. Resuspend pellet in 43 ml ice-cold 0.1 M $CaCl_2$ mixed with 7 ml sterile glycerol.

8) Dispense cells into 200 µl aliquots in 1.5 ml eppendorf tubes. *Aliquot in the cold room to prevent the cells from warming.*

9) Store the cells frozen at -70°C until use. Bacteria prepared in this manner can be successfully used after up to one year's storage and will still maintain a high transformation efficiency.