PREPARATION OF FROZEN STOCKS OF COMPETENT E. COLI CELLS

$CaCl_2$ Method of Transformation

This procedure can be used for a variety of different E. coli strains, including DH-1, HB101, GM119 and JM109. However, in several people's hands TG-1 cells do not appear to remain competent after freeze/thawing. The efficiency of transformation should be uniformly high, and the frozen cells should remain both competent and viable for long periods of time (I've made very large stocks and stored them frozen for up to two years).

1) Prepare a fresh plate of cells by streaking out cells from a stab or frozen stocks and growing overnight at 37°C. Pick an individual colony and set up an overnight culture in 10 ml L-broth.

2) Inoculate 5 ml of overnight culture into each of two flasks containing 500 ml L broth. Incubate at 37°C with aeration until the culture reaches OD_{550} of 0.5 (approximately 5 x 10^8 cells/ml). This should take approximately 2 hrs. **Note: The relationship between optical density and the number of viable bacteria per milliliter of culture varies from strain to strain. For example, for rec^+ strains (χ1776, MM294) 1 OD_{550}+0.2 (~5 X 10^7 cells/ml); whereas for rec- strains (DH1, HB101) 1 OD_{550}=0.5 (~5 X 10^7 cells/ml). A curve calibrating the OD_{550} and the number of bacteria per milliliter of culture should be constructed for each new strain of E. coli used. The cell density required for maximal transformation efficiency is somewhat flexible. I have used DH-1 cells at ODs ranging from 0.5 to 0.9 and have gotten highly competent cells.

3) Transfer cells to centrifuge bottles and spin in Sorvall GSA rotor at 4°C for 8 min at 8000 rpm.

4) Gently resuspend pellets in 250 ml ice cold 0.1 M $CaCl_2$ and combine into a single bottle. Spin 8 min, 8000 rpm in GSA rotor.

5) Resuspend cells in 250 ml ice cold 0.1 M $CaCl_2$ and store on ice for 4-8 hrs.*** Note: In my hands, the amount of time on ice is the most important parameter for high competency. The literature states that the competency increases over time up to 24 hrs., and then begins to decrease. I have found that anywhere between 4-8 hrs. works fine, although I have incubated more or less and it has still worked fine.

6) Centrifuge 8000 rpm for 8 min at 4°C. Resuspend pellet in 43 ml of ice cold 0.1 M $CaCl_2$ with 7 ml sterile glycerol. Distribute suspension of competent cells into convenient aliquots (0.2 ml) in cold eppendorf tubes. Freeze and store at -70°C. Save a portion of the cells to assay for viability, purity and competence. **Note: I routinely pre-chill all of the tubes, pipettes, etc. before aliquotting the cells. This makes a big difference in maintaining the competency of the cells.

TRANSFORMATION

1) Thaw cells on ice. For each transformation, only 50-100 µl of cells are necessary. Add DNA to cells (volume of DNA should not exceed 40% of cell volume). Incubate on ice for 20-30 min.
2) Place tubes in 42°C water bath for 2 min.
3) Add 1 ml L broth to tube. Incubate at 37°C for 30 min- 1hr.
4) Streak out 50-500 µl onto plates containing the appropriate antibiotics.