Preparation of Competent Cells (JM109) ---Acid Salt Method

Procedures:

1. Streak the desired bacterial strain on M-9 plates and incubate at 37°C overnight.

2. Inoculate 25 ml of LB medium (NO antibiotics!) with a single colony and incubate at 30°C overnight with vigorous shaking.

3. Inoculate 500 ml of LB medium in a 3-liter flask with 5ml of cells from the overnight culture and shake the culture at 150-200 rpm at 30°C until the A_{600} reaches 0.45-0.55.

4. Chill the cells in ice water for 2 hrs and collect by centrifugation in 150 ml Corex tube at 4000 rpm (2,500 x g) with rotor GSA (r=5.75 in) for 15-20 minutes at 4°C.

5. Resuspend the cells in 10-20 ml of ice-cold Trituration buffer and then dilute to 500 ml with the same solution and incubate the cells on ice for 45 min.

6. Centrifuge the cells at 3,300 rpm (1,800 x g) for 10 min at 4°C and gently resuspend in 50 ml of ice cold Trituration buffer.

7. Pool the cell and add 80% glycerol dropwise with gentle swirling to a final concentration of 15% (v/v). Make 0.2-1ml aliquots of the cells, freeze on dry ice and store at -70°C.

Solutions:

Trituration buffer (600 ml):

6.66 g	$CaCl_2$ (MW 110.99)	100 mM (or 8.82 g for $CaCl_2 \cdot 2H_2O$)
8.54 g	$MgCl_2 \cdot 6H_2O$ (FW 203.3)	70mM
3.27 g	$NaAc \cdot 3H_2O$ (FW 136.08)	40mM pH 5.5

This solution should be prepared fresh and filter-sterilized for each use.