Competent Cell Proceedure for Chemically Competent Cells:

Based on methodology from Hanahan (Hanahan, Jessee et al., 1991).

1. Take the frozen stock of cell type and streak out on an SOB media plate. Incubate overnight at 37 C.

2. Pick a colony off of the fresh streak plate and inoculate 5 mL of SOB media. Let grow overnight at 30 C.

3. To 100 mL of SOB in 500 mL flask add 1 mL of sterile 1M $MgCl_2$ and 1 mL of sterile 1M $MgSO_4$. Add 2 mL of the overnight culture and grow at 37 C in a shaker unitl the $O.D._{600nm}$ reaches 0.45.

4. Aliquot into sterile 50 mL tubes and chill on ice for 10 - 15 minutes.

5. Pellet cells at 3,000 rpm at 4 C for 10 -15 minutes. Drain thoroughly by inverting and tapping on paper towels to remove all traces of media.

6. Resuspend cells by pipetting in 1/3 the original culture volume in RF1 (for a 50 mL tube add 16 mL of RF1). Incubate on ice for 20 minutes and pellet the cells as in step 5.

7. Resuspend cells by pipetting in 2.5 mL of RF2 for every 50 mL tube. Incubate on ice for 15 minutes.

8. Pipet cells into 100 μL of cells into eppendorph tubes that have been on ice for 10 minutes.

9. Snap freeze cells in dry/ice ethanol bath or liquid nitrogen and transfer to -80 C.

Solutions:(Sterilize After Making):

SOB Media: (autoclave)

20 g tryptone
5 g yeast extract
0.5 g NaCl

Dissolve in 800 mL of millipure water, add 10 mL 250mM KCl, adjust the pH to 7.0, and bring to 1 Liter and autoclave. Before use add 5 mL of sterile 2 M $MgCl_2$ to 1 Liter of media.

RF1: (filter sterilize)

3 g RbCl
2.475 g $MnCl_2$ $4H_2O$
0.736 g Potassium Acetate
0.375 g $CaCl_2$ $2H_2O$
37.5 g Glycerol

Dissolve in 175 mL of millipure water, adjust pH to 5.8 with dilute acetic acid, and bring volume up to 240 mL with millipure water.

0.5 M MOPS (pH 6.8) (Filter sterilze):

Dissolve 4.18 g MOPS in 28 mL water, adjust the pH to 6.8 with 2N NaOH and add water to 40 mL. Filter sterilize.

RF2: (filter sterilize)

4 mL 0.5M MOPS (pH 6.8)
0.24 g RbCl
2.2 g $CaCl_2$ $2H_2O$
30 g Glycerol

Dissolve in 160 mL of millipure water, adjust to pH 6.8 with NaOH and add water to 200 mL.

Sore MOPS , RF1 and RF2 in -20 C freezer until use.

Hanahan D., Jessee J., et al., (1991) “Plasmid transformation of Escherichia coli and othe bacteria.” Methods in Enzymology **204:** 63-113.