Preparation of Competent Cells

(Reference: Inoue, Hiroaki, Nojima, Hiroshi, and Okayama, Hiroto. “High Efficiency Transformation of Escherichia coli with Plasmids”, Gene, 96, 23-28, 1990)

1. Streak frozen stock of SURE cells on an LB + Tet agar plate.
2. Allow the cells to grow overnight at 37 °C

3. Pick 10 large (~ 2-3 mm in diameter) colonies and place all 10 colonies into 5 ml SOB.

4. Add the SOB medium containing the colonies to 250 ml of SOB medium in a 2-l flask. (Note: 2.5 ml of the magnesium stock solution should be added to the SOB medium in the 2-l flask prior to this step)

5. Incubate the flask at room temperature with vigorous shaking.

6. Grow cells to A600 = 0.6; the specific growth rate, m, of SURE cells at R.T. ~ 0.44 h-1, and the double time at R.T. is about 1.6 h. It will take about 24 h to reach an O.D. of 0.6, but it will depend on the sizes of the colonies you picked . If you innoculate the cells say at 2 p.m., you should check the A600 in the morning when you come in, then you can calculate the exact time the cells will reach an O.D. of 0.6 using the following formula: Time (h) = 1/m . ln (0.6/A600)

7. Place flask on ice for 10 min.

8. Transfer culture into a cooled centrifuge bottle and spin at 3500 rpm (~ 2100 x g) in the JA10 rotor for 10 min at 4 °C.

9. Remove the supernatant and resuspend the pelleted cells in 80 ml of ice-cold buffer TB with a glass pipette; incubate on ice for 10 min.

10. Spin the cells down again in the same bottle at 3500 rpm for 10 min at 4°C.

11. Remove the supernatant and resuspend the pelleted cells in 20 ml of ice-cold buffer TB.

Make sure the cells are well resuspended.

12. Add DMSO to cell suspension to 7% (i.e. 1.4 ml).

13. Incubate the cells on ice for another 10 min.

14. Aliquot the cell suspension into 250 ml per eppendorff tubes and freeze the tubes quickly with liquid nitrogen.

SOB Medium:

(2% (w/v) Bacto Tryptone
0.5% (w/v) Yeast extract
10 mM NaCl

2.5 mM KCl
10 mM MgCl2
10 mM MgSO4)

To make 500 ml:

Bacto Tryptone	10 g
Yeast Extract	2.5 g
NaCl	0.29 g
KCl	0.09 g

- dissolve in 500 ml of H2O
- autoclave

Magnesium Stock Solution

1 M MgCl2	2 g
1 M MgSO4	2.5 g

- dissolve in 10 ml H2O
- filter sterlize

Before use, add 1/100 vol of Magnesium stock solution to medium (i.e. 2.5 ml to 250 ml medium).

Buffer TB:

10 mM Hepes
15 mM CaCl2
250 mM KCl
55 mM MnCl2

To make 100 ml:

Hepes (M.W. 238.3)	0.24 g
CaCl2 (M.W. 147.02)	0.22 g
KCl (M.W. 74.55)	1.86 g

- dissolve in 80 ml of H2O

- adjust pH to 6.7 with KOH
- then add 1.09 g of $MnCl_2$ (M.W. 197.9) and adjust the final volume to 100 ml with H_2O
- filter sterlize the solution