Preparation of chemo-competent E.coli KL740 cells

Materials:

E.coli KL740 cl857+（E.coli Genetic Stock Center，#14222）

LB agar plates（with and without selection marker）

LB medium

MgCl2

CaCl2，dihydrate

Glycerol

Prepare the following solutions at least one day prior to the preparation of chemo-competent cells.All solutions should be sterilized by autoclaving and cooled down to 4C before use.

·Wash Buffer1（100mM MgCI，solution）

·Wash Buffer2（50mM CaCl，solution）

·Storage Buffer（50mM CaCl2，15%v/v glycerol）

Procedure:

The following procedure is adapted from the calcium chloride method described in Sambrook et al.1989（Molecular Cloning：ALaboratory Manual).

Important：All steps should be carried out aseptically.

1. Streak E.coli KL740 cl857+ cells from a glycerol stock onto a LB agar plate and incubate for～16h at 29°C.

[Note：A constant growth temperature of 29°C is crucial to ensure a maximum level of active cla857 repressor，which allows efficient gene repression upon transformation.]

2. Inoculate 2mL LB medium in a 13mL culture tube with a single colony of KL740 cl857+ and grow cells for 16-24h at 29°C and 200rpm.

3. Next day，dilute stationary KL740 cl857+ culture 1：100 in 50mL fresh LB medium in a100mL culture flask and incubate at 29°C and 200rpm till OD550=0.5

4. Transfer entire culture into a pre-chilled，sterile 50mL Falcon. For optimum transformation efficiency，keep cells cold from here on and work as fast as possible under sterile conditions.

5. Centrifuge cells at 4400xg，4°C for 10min and discard supernatant.

6. Resuspend cells in 40mL ice-cold Wash Buffer 1. It is recommended to resuspend cells initially in 1-2mL of buffer using a pipettor. Then fill up Falcon with buffer to the recommended volume，and mix by inverting.

7. Centrifuge cells at 4400xg，4°C for 10min and discard supernatant.

8. Resuspend cells in 20mL ice-cold Wash Buffer 2.It is recommended to resuspend cells

initially in 1-2mL of buffer using a pipettor.Then fill up Falcon with buffer to the recommended volume，and mix by inverting. Incubate cell suspension for 30min on ice.Then centrifuge as before.

9. After removing the supernatant completely，resuspend cells in 2mL ice-cold Storage Buffer.

10. Aliquot the cell suspension as 150uL per 1.5mL tube on ice，and immediately store at -80℃. Sufficient competency should last at least 6 months.