Preparation of Chemical competent cell

1. Inoculate 10 µl of DH5α stock into 2 ml SOB and culture at 37˚C
2. Set up the water bath at 18˚C in cold room and pre-incubate 50 ml SOB at 18˚C
3. Grow cells to an A_{600} of 0.6 and transfer to 50 ml SOB (handle in clean bench)
4. Culture overnight (24 h) at 18˚C

1. Preparation for cell stock

1. Keep EtOH (>500 ml) at –20˚C
2. Chill two 50 ml tubes, microcentrifuge tubes, tips and vortex mixer at 4˚C
3. Prepare two ice boxes A(ice/water/NaCl) and B(dry ice/EtOH)
4. Prepare 700 µl DMSO at r.t.
5. Set up the centrifugation machine at 2,500 g at 0˚C
6. Keep 20 ml TB and 4 ml TB (2 tubes, each) on box A
7. Transfer culture to box A and chill completely for 10 min

2. Cell Stock

1. Transfer of chilled culture into two 50 ml tubes
2. Centrifuge at 2,500 g for 10 min at 0˚C
3. Remove the supernatant carefully and vortex pellets mildly
4. Resuspend pellets in 20 ml TB (in each tube) by vortex mixer and place on box A for 10 min
5. Centrifuge cells at 2,500 g for 10 min at 0˚C
6. Remove supernatant and vortex pellets mildly
7. Resuspend pellets in 4 ml TB (in each tube) by vortex mixer
8. Add 300 µl DMSO into tubes and resuspend carefully
9. Transfer 100 µl aliquot into 1.5 ml microcentrifuge tubes
10. Freeze immediately in box B for 5 min
11. Transfer tubes to deep freezer and freeze overnight at –70˚C

Transformation buffer (TB)

		Final concentration
PIPES	3.0 g	(10 mM)
$CaCl_2 \cdot 2H_2O$	2.2 g	(15 mM)
KCl	18.6 g	(250 mM)

1. Suspend in 950 ml dH_2O and adjust pH to 6.7~6.8 with 5N KOH
2. Add 10.9 g $MnCl_2 \cdot 4H_2O$ (final; 55 mM)
3. Mess up to 1L and filtrate through 0.22 µm filter

Chemical-Transformaiton

1. Thaw one tube of competent cells on ice. Keep cells cold until electroporation.

2. Dispense plasmid DNA (25 ng) to a chilled microcentrifuge tube on ice.

3. Add homogenous cell prep and mix gently.
   -Note: DNA solution should be less than 10% of competent cells solution.

4. Incubate on ice for 30 min, heat shock at 42˚C for 30 sec and return to ice for 2 min.

5. Add 500 µl of SOC and incubate at 37˚C for 1 h under vigorous agitation. Pre-incubate the LB plates with appropriate antibiotics at 37˚C.

6. Add variable volumes of your transformation mix to LB plates and spread the plates.

7. Incubate plates 12~16 h at 37˚C