Competent Cell Preparation and Transformation

[Preparing the competent cells]

Reagent

1. TSS (Transformation and Storage Solution for chemical transformation) /50 mL
   - 85% LB medium : 1.25 g x 0.85 = 1.0625 g
   - 10%(wt/vol) Polyethylene glycol (MW 8000) : 5 g
   - 5% DMSO (vol/vol) : 2.5 mL
   - 50mM $MgCl_2$ anhydrous (pH 6.5) : 0.238 g

Autoclave in a **glass bottle** or filter sterilize. Store at 4 ºC for < 2 weeks

2. **2x LB** medium /200 mL
   - LB medium : 6.2 g
   - NaCl2 anhydrous : 3.8 g

Autoclave in a 500-mL **baffled flask**.

Methods

1. Streak on a LB plate (added antibiotic if cells have antibiotic resistant). Incubate the plate at 37 ºC overnight.

2. Pick a single, well-isolated colony and inoculate it into 5 mL of LB broth (plus antibiotic). Incubate at 37 ºC overnight with shaking at 200 rpm.

3. Transfer 2 mL of the saturated overnight culture to a sterile 500-mL flask containing 200 mL of 2x LB medium (**do not add antibiotic although cells have antibiotic resistance**). Incubate the cells at 37 ºC with the shaking at 200 rpm, until OD_{600} reach **0.6 ~ 0.8**.

4. When the culture reaches an OD_{600} of 0.8, chill the flask on the ice for 20 min(completely cool down the culture) and then collect the cells by centrifugation at 1,500 rpm for 5 min at 4 ºC.

5. Resuspend the cells in 5 mL of ice-cold TSS solution. Now the competent cells are ready to be transformed.

6. Aliquot 110 uL competent cells to ice-colded 1.5 mL tube. If they are not immediately used, cells can be stored at 4 ºC for maximum of 6 hours without significant loss of competency. The same competent cells can also be stored at -70 ºC for long-term storage (pre-treat with liquid nitrogen).

[Transforming the cells]

1. Thaw -70 ºC competent cells first on ice. Add DNA (**do not exceed 10% vol. of competent cells**) to ice cold 50 uL competent cells.

2. Incubate on the ice for 20 ~ 30 min.

3. Heat shock at 42 ºC for 1 min.

4. After heat shock, put on ice for 2 min.

5. Add 200 uL of **pre-warmed** LB broth.

6. Shake and incubate at 37 ºC for 60 min.

7. Plate on the **pre-warmed** appropriate agar plates and incubate plates at 37 ºC, overnight.