E. coli Tranformation Protocol

[*E. coli* Transformation]

We used commercial competent *E. coli* cell lines DH5α, Top10, and BL21 from TIANGEN BIOTECH and Transgen. We also prepared competent cells by ourselves.

1. The protocol of preparing competent *E. coli* cells by $CaCl_2$ induction

Step1. Culture *E. coli* in 5mL LB each tube at 220 rpm under 37 °C overnight about 12-16 h.

Step2. Drop 100 μL cultured *E. coli* solution in to 10 mL new LB each tube (1:100 dilution) to culture 2-3 h more until the solution reaches about OD600≈ 0.3~0.5.

Step3.Put the *E. coli* solution on ice quietly for 10 min.Centrifuge the *E. coli* solution at 4000 rpm for 2 min at 4 °C to collect the cells and drop out supernatant.

Step4. Add 1 mL cold 0.1 M $CaCl_2$ solution to resuspend the *E. coli* cells and put tube on the ice quietly for about 30 min.

Step5. Centrifuge the preparing cell tube at 4000 rpm for 2 min at 4 °C to collect the cells and drop out supernatant.

Step6. Add 400 μL cold 0.1 M $CaCl_2$ solution and 100 μL glycerol to mix and resuspend the cells on the ice.

Step7. Distribute the competent cell solution 100 μL each tube and store at - 80°C.

2. The protocol of *E. coli* transformation

Step1. Take competent cell tube from -80 °C and put on the ice about 5min waiting for melting.

Step2. Add 1 μL plasmid solution into each tube of 100 μL competent cells and put back on the ice quietly for 15 min.

Step3. Use metal bath at 42 °C to hot shock for 90 s.

Step4. For plasmid of ampicillin resistant (Amp^+) go to Step5. For plasmid of Chloramphenicol or kanamycin resistant (Cam^+) add 200μL LB to culture at 220 rpm under 37 °C for 1 h to recover resistant.

Step5. Put the transformed *E. coli* solution on the surface of the exact resistant LB media plate and place in 37 °C for about 10-12 h (overnight).