Electroporation-Transformation E.coli BW25113 cells

Preparation of electroporational-competent E.coli BW25113 cells

Materials：

E. coli BW25113（E.coli Genetic Stock Center，#7636 ）
LB agar plates
LB medium
30% 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 4℃ before use.

Procedure：

1. Streak E.coli BW25113 cella from a glycerol stock onto a LB agar plate and incubate for 16h at 37℃.
2. Inoculate 5mL LB medium in a 13mL culture tube with a single colony of E.coli BW25113 and grow cells for 16-24 h at 37 °C and 250 rpm.
3. Next day, dilute stationary BW25113 culture 1:50 in 50 mL fresh LB medium in a 100 mL culture flask and incubate at 37 °C and 250 rpm till OD550 = 0.4~0.6.
4. Transfer entire culture into a pre-chilled, sterile 50 mL Falcon. For optimum transformation efficiency, keep cells cold from here on and work as fast as possible under sterile conditions.
5. Centrifuge cells at 4000xg~6000xg， 4 ℃ for 5 min and discard supernatant.
6. Resuspend cells in 5 or 10 mL ice-cold ddH20. It is recommended to resuspend cells initially in 1-2 mL of buffer using a pipettor. Then fill up Falcon with buffer to the recommended volume, and mix by blowing 50 times gently.
7. Centrifuge cells at 4000 xg~6000xg, 4℃ for 5 min and discard supernatant.
8. Resuspend cells in 10 mL ice-cold ddH20. It is recommended to resuspend cells initially in 1-2 mL of buffer using a pipettor. Then fill up Falcon with buffer to the recommended volume, and mix by blowing 50 times gently. Then centrifuge as before.
9. Repeat step 8 once
10. After removing the supernatant completely, resuspend cells in 1mL ice-cold ddH20.
11. Aliquot the cell suspension as 100uL per 1.5 mL tube on ice, Add 30% glycerin to each tube and mix well immediately store at -80℃.

Electroporation

Material:

Electrical competent E.coli BW25113 cells
LB medium
LB agar plates

Procedure:

1. Plasmid extraction and concentration determination.
2. Competent cells are taken from a refrigerator at the temperature of minus 80℃ and placed on ice for thaw.
3. Setting a control group and an experimental group , labeling.
4. Place 30μL unfrozen competent cells in a 1.5mL centrifuge tube.
5. In the experimental group, add 1000ng purified plasmid , mixed with mild blowing, and pre-cooled on ice together with the electric conversion cup.
6. Open the electric rotary instrument and adjust it to Manual, and adjust the voltage to 1.8kV.
7. Filling the mixture of cells and plasmids in a 1.5mL centrifugal tube into an electric conversion cup, and tapping the electrode cup gently to enable the mixture to uniformly enter the bottom of the electrode cup.
8. Push the electrode cup into the electroconverter, and press the pulse key. After hearing the beep, quickly add 950μl of LB liquid culture medium into the electric shock cup and suspend the cells again, and transfer them to a 1.5ml centrifuge tube.
9. recovery cells at 220～250 rpm, 37℃ for 1 h.
10. Centrifuge, 900ul of supernatant was discarded, the cells were re-suspended, and 50μl of the transformed product was taken, plated, and cultured overnight at 37℃.