Chemical Trandformation E.coli BW25113 cells

Preparation of chemo-competent E.coli BW25113 cells

Material:

E. coli BW25113（ CGSC, #7636)
LB agar plates
LB medium
100nM CaCl2
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:

Important:All steps should be carried out aseptically
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, 4 ℃ for 5 min and discard supernatant.
6. Resuspend cells in 5 or 10 mL ice-cold 100 nmol CaCl2. 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, 4℃ for 5 min and discard supernatant.
8. Resuspend cells in 10 mL ice-cold 100 nmol CaCl2. 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 100 nmol CaCl2.
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：

chemo-competent E.coli BW25113 cells
LB medium
LB agar plates

Procedure：

1. Thaw an aliquot of chemo-competent cells on ice.
2. Add 5μL plasmid to a 25μL aliquot of chemo-competent BW25113 cells.
3. Incubate for 30min on ice.
4. Incubate cells for 30s at 42℃ using a heat block or water bath.
5. Place the cells on ice and cool for 2min immediately.
6. Transfer cell suspension into 970μL LB medium in a 13mL culture tube and incubate for 1h at 37℃ and 250rpm.
7. Centrifuge cells for 1min at 3000x g at room temperature，remove 900μL of the supernatant，and resuspend cell sediment in the remaining medium.
8. Plate at least 50uL of that cell suspension on a pre-warmed LB agar plate containing the appropriate selection marker（and the entire remaining cell suspension on a separate plate as a back-up）and incubate the plate for 12-16h at 37℃.