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°C 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°C.
- 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 = $0.4\sim0.6$.
- 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 °C 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°C 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°C.

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 $5\mu L$ plasmid to a $25\mu L$ aliquot of chemo-competent BW25113 cells.
- 3. Incubate for 30min on ice.
- 4. Incubate cells for 30s at 42°C using a heat block or water bath.
- 5. Place the cells on ice and cool for 2min immediately.
- 6. Transfer cell suspension into $970\mu L$ LB medium in a 13mL culture tube and incubate for 1h at 37°C 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°C.