Preparation of pSIM6 Electrocompetent Cells

Materials

pSIM6-containing *E. coli*
LB medium
Ampicillin, 100 mg/mL stock solution
ice-cold sterile ddH_2O
Ice

Procedure

Culture and λ-Red Induction

1. Pick a single colony of pSIM6-containing *E. coli* from an LB agar plate containing ampicillin and inoculate it into 5 mL of LB medium supplemented with ampicillin to a final concentration of 100 μg/mL.
2. Grow the culture overnight at 30 °C with shaking at 200 rpm.
3. Inoculate 150 μL of the overnight culture into each of two 15 mL fresh LB cultures containing ampicillin, designated as the experimental and control groups.
4. Grow both cultures at 30 °C and 200 rpm until the OD_{600} reaches 0.4-0.6.
5. Incubate the experimental group in a shaking water bath at 42 °C and 220 rpm for 15 min.

Cell Collection

1. Place both the experimental group and the control group in an ice-water bath and gently swirl to cool the culture. Then incubate them on ice for 30 min.
2. Pellet the cells by centrifugation at 4 °C and 4,600 × g for 7 min, then carefully discard the supernatant.

Cell Washing

1. Gently resuspend the cell pellet in 1 mL of ice-cold sterile water, then add ice-cold sterile water to a final volume of 30 mL.
2. Pellet the cells by centrifugation at 4 °C and 4,600 × g for 7 min, then carefully discard the supernatant.
3. Add 1 mL of ice-cold sterile water and gently resuspend the cells. Transfer the cell suspension into a 1.5 mL centrifuge tube and centrifuge at 4 °C at maximum speed for 30–60 s.

Final Resuspension

1. Resuspend the final cell pellet in 200 μL of ice-cold sterile ddH_2O, mix gently, and keep on ice until use.
2. Use 50 μL of electrocompetent cells for each electroporation.