Preparation of Electrocompetent Cells for pCP20 Electroporation

Materials

Kanamycin-resistant edited *E. coli* strain
LB medium
Kanamycin, 50 mg/mL stock solution
Ice-cold sterile water

Procedure

Culture

1. Pick a single colony of the kanamycin-resistant edited strain obtained from the previous gene-editing step and inoculate it into 5 mL LB medium supplemented with kanamycin to a final concentration of 50 μg/mL.
2. Grow the culture overnight at 37 °C with shaking at 200 rpm.
3. Inoculate 150 μL of the overnight culture into 15 mL of fresh LB medium containing kanamycin.
4. Incubate at 37 °C with shaking until the OD_{600} reaches approximately 0.4–0.6.

Cell Collection

1. Incubate the 15 mL culture on ice for 15–30 min.
2. Centrifuge the cells at 4 °C and 4,600 × g for 7 min, then 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. Centrifuge the cells at 4 °C and 4,600 × g for 7 min, then discard the supernatant.
3. Gently resuspend the cell pellet in 1 mL of ice-cold sterile water, then transfer the suspension to a pre-chilled 1.5 mL microcentrifuge tube.
4. Centrifuge at 4 °C for 45–55 s at the maximum speed of the centrifuge.

Final Resuspension

1. Discard the supernatant and resuspend the cell pellet in 200 μL of ice-cold sterile water.
2. Use 50 μL of electrocompetent cells for each electroporation.