Preparation of pKD46 Electrocompetent Cells

Materials

pKD46-containing *E. coli*
LB medium
Ampicillin, 100 mg/mL stock solution
L-arabinose, 1 M stock solution
Ice-cold sterile ddH_2O
Ice

Procedure

Culture and λ-Red Induction

1. Pick a single colony of pKD46-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 0.6.
5. Add L-arabinose to the experimental group to a final concentration of 10 mM, then continue shaking both cultures at 30 °C and 200 rpm for 2 h.

Cell Collection

1. Incubate both the experimental group and the control group on ice for 30 min.
2. Pellet the cells by centrifugation at 4 °C and 4,000 × g for 10 min, then carefully discard the supernatant.

Cell Washing

1. Gently resuspend each cell pellet in 10 mL of ice-cold sterile ddH_2O by gentle pipetting.
2. Centrifuge the cells at 4 °C and 4,000 × g for 10 min, then discard the supernatant. Repeat the washing steps for a total of three washes.

Final Resuspension

1. Resuspend each 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.