Electroporation of pCP20

Materials

pCP20 plasmid DNA
Electrocompetent *E. coli* cells
Sterile, precooled electroporation cuvettes (1 mm gap)
Electroporator
LB medium
Ampicillin, 100 mg/mL stock solution
LB agar plates containing ampicillin

Procedure

Electroporation

1. Add 20–50 ng of pCP20 to 50 µL of electrocompetent cells. Gently mix the cells and DNA, then transfer the mixture into a sterile, precooled electroporation cuvette.
2. Ensure that the outside of the electroporation cuvette is dry and place the cuvette into the electroporator.
3. Electroporate the cells at 1.8 kV.
4. Immediately remove the cuvette after electroporation.

Recovery

1. Immediately add 1 mL of LB medium at room temperature to the electroporation cuvette.
2. Gently pipette the cell suspension and transfer the entire volume into a centrifuge tube.
3. Recover the cells at 30 °C and 200 rpm for 1–2 h.

Antibiotic Selection

1. Centrifuge the recovered cells at 1,900 × g for 3 min.
2. Discard the supernatant and resuspend the cell pellet in 100 µL of LB medium.
3. Spread the entire 100 µL cell suspension onto an LB agar plate containing ampicillin at a final concentration of 100 µL/mL.
4. Incubate the plates at 30 °C for 16–24 h.