# Electroporation of Linear PCR Products

## Materials

Electrocompetent *E. coli* cells
Purified linear PCR product
Sterile, precooled electroporation cuvettes (1 mm gap)
Electroporator
LB medium
Kanamycin, 50 mg/mL stock solution
LB agar plates containing kanamycin

## Procedure

### Preparation of the Electroporation Mixture

1. Transfer 50 µL of electrocompetent cells into a precooled tube.
2. Add 100–150 ng of purified linear PCR product to the cells and mix gently by pipetting.
3. Immediately transfer the mixture into a sterile, precooled electroporation cuvette.

### Electroporation

1. Ensure that the outside of the electroporation cuvette is dry and place the cuvette into the electroporator.
2. Electroporate the cells at 1.8 kV.
3. Immediately remove the cuvette after electroporation.

### Recovery

1. Immediately add 1 mL of LB medium at room temperature to the electroporation cuvette to allow the cells to recover from electroporation and begin expressing kanamycin resistance.
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 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 100 µL of the recovered cell culture onto an LB agar plate containing kanamycin at a final concentration of 30 µg/mL.
4. Incubate the plates at 30 °C for 24 h.