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Electroporation

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[1]iGEM_NTHU_Taiwan

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igemnthuth

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We use this protocol and it's working

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Abstract

Introduce the designed plasmid into DH10B to establish *E. coli* supporting orthogonal replication.

Materials

- Plasmid
- Pre-prepared DH10B competent cells
- SOB medium pre-warmed to 37 °C
- Sterile electroporation cuvette (2 mm gap)
- Antibiotic agar plates (according to the resistance gene carried by the plasmid)
- Electroporator
- Shaking incubator at 37 °C (220 rpm)
- Biosafety cabinet
- Ice bucket with ice

Preparation of the Mixture

1 Take out pre-prepared DH10B competent cells and thaw on ice (~10 minutes).

2 In a sterile workspace, add 1.0 µL of plasmid DNA (dissolved in ultrapure water, ~50–100 ng/µL) to the thawed competent cells. Do not stir, pipette harshly, or create bubbles.

3 Carefully transfer the mixture into a pre-chilled 2 mm electroporation cuvette (kept on ice ≥10 minutes). Close the lid and leave on ice for 2 minutes.

Electroporation

4 Place the cuvette into the electroporator and set the following conditions:

- **Voltage:** 2500 V (standard for 2 mm gap)
- **Time constant:** 4.5–5.5 ms (ideal range)
- **Pulse number:** 1 pulse, duration should not exceed 6 ms

5 A faint spark sound should be heard during electroporation. If the instrument displays *"Arcing"* (discharge failure), replace the cuvette immediately and check whether the sample contains salt.

Immediate Recovery

6 Immediately add 1 mL of pre-warmed (37 °C) LB medium into the cuvette and gently pipette to mix.

7 Transfer the cuvette contents into a sterile 15 mL culture tube.

8 Incubate at 37 °C in a shaking incubator at 220 rpm for 60 minutes (recovery culture).

Antibiotic Selection (see detailed steps in next protocol)

9 After 1 hour of recovery, plate 100 µL of the culture onto an LB agar plate containing the appropriate antibiotic.

10 Incubate the plate at 37 °C in an inverted position (agar side up) overnight (12–16 hours).