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Antibiotic Selection

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[1]iGEM_NTHU_Taiwan

iGEM NTHU

igemnthuth

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We use this protocol and it's working

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Abstract

Confirm that *E. coli* DH10B has successfully taken up the plasmid carrying the synthetic EcOrep operon, and select colonies with the antibiotic resistance marker via antibiotic selection.

Materials

- Recovered culture (per tube: 100 µL competent cells + 6 µL plasmid + 1 mL SOB medium; recover at 37 °C for 2 h)
- LB agar powder (containing 1% tryptone, 0.5% yeast extract, 1% NaCl, 2% agar)
- [Antibiotic] stock solution: 20 mg/mL, dissolved in 100% ethanol, stored at −20 °C
- ddH_2O
- LB agar plates: final [Antibiotic] concentration 20 µg/mL per plate (see preparation steps below)
- Sterile 1.5 mL Eppendorf tubes
- 100 mm sterile Petri dishes
- Sterile glass spreader
- Micropipettes (20 µL, 200 µL, 1000 µL)
- 37 °C incubator

Preparation of Antibiotic Agar Plates

1 Weigh 25 g LB agar powder using an electronic balance and dissolve in 1 L ddH_2O.

2 Autoclave at 121 °C, 15 psi, for 1–2 hours.

3 After autoclaving, cool the medium to ~50 °C (check with an infrared thermometer).

4 Add 1 mL of 20 mg/mL [Antibiotic] stock to 1 L of medium (final concentration: 20 µg/mL) and mix thoroughly. (*Ensure the medium has cooled sufficiently before adding antibiotic.*)

5 Pour 25 mL of the medium into each sterile Petri dish.

6 Allow to solidify at room temperature. Seal with Parafilm, label with date and antibiotic name, and store at 4 °C (up to 7 days).

Dilution and Plating of Recovered Cells

7 Mix the recovered culture thoroughly, then split into two groups:

- **Undiluted group:** Take 100 µL of undiluted culture.
- **1:10 dilution group:** Mix 100 µL culture with 900 µL SOB; from this, take 100 µL.

8 Spread each 100 µL sample evenly onto LB agar plates containing the antibiotic.

9 Use a sterile glass spreader to distribute the liquid.

10 Let the surface dry (~5 minutes), then invert the plates.

Incubation and Observation

11 Place the plates in a 37 °C incubator, inverted, and incubate for 14–16 hours.

12 On the following day, observe colony formation:

- Presence of colonies indicates successful transformation.
- If colonies are too dense on the undiluted plate, use colonies from the 1:10 dilution plate instead (adjust depending on culture density).