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Add Arabinose

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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

To control expression of the synthetic replication operon in the orthogonal replication system, this step uses an **arabinose-induced dCas9–sgRNA repression system** to reduce downstream operon component expression, thereby **improving orthogonal replicon transformation efficiency**.

Materials

- LB broth
- 10% glycerol solution (pre-chilled)
- Arabinose
- ddH_2O
- Pre-chilled electroporation cuvettes (2 mm gap)
- Pre-chilled centrifuge tubes (50 mL × 2; 1.5 mL × 1)
- Shaking incubator
- Ice-water bath and ice bucket
- Spectrophotometer (for OD600 measurement)
- Refrigerated centrifuge
- Electroporator

Pre-culture

1 Pick colonies that have successfully taken up the following plasmids:

- **EcOrep operon plasmid**
- **dCas9 + sgRNA expression plasmid** (e.g., pRT17/19/21)
- **Mutant DNAP plasmid**

2 Inoculate into **15 mL LB broth** supplemented with the **corresponding antibiotics for all three plasmids**, and incubate **overnight at 37 °C, 220 rpm** (~12 h).

Main Culture and Arabinose Induction

3 The next day, transfer **5 mL** of the overnight culture into **200 mL** fresh LB broth (**1:40 dilution**) in a **2 L shake flask**; incubate at **37 °C, 220 rpm**.

4 When **OD600 ≈ 0.2**, add **arabinose** to a **final concentration of 10 mM** (e.g., add **2 mL of 1 M stock** to **200 mL** culture) and continue incubation.

5 Grow to **OD600 ≈ 0.4**, then immediately proceed to the following steps for electroporation pre-treatment.

Preparation of Competent Cells

6 Place the culture on ice to cool for **10 minutes** (gentle swirling optional).

7 Centrifuge at **4 °C, 4500 × g for 3 minutes**; carefully discard the supernatant.

8 Wash the cell pellet with **50 mL pre-chilled 10% glycerol**; repeat the wash **twice** (total **2 washes**).

9 Finally, **resuspend** the pellet in **1 mL pre-chilled 10% glycerol** to obtain the competent cells.

10 **Aliquot** into several **100 µL** tubes (for subsequent **O-replicon electroporation**). This completes the **arabinose treatment** stage.