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Mutation

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igemnthuth[1]
[1]iGEM_NTHU_Taiwan

iGEM NTHU

igemnthuth

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Protocol status: Working
We use this protocol and it's working

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Abstract

Induce the mutator DNAP with rhamnose to drive mutations on the linear O-replicon; after the predetermined evolution time/generations, remove rhamnose to shut off mutagenesis and switch to “normal replication” to stabilize the mutations for downstream analysis.

Initial Culture (after O-replicon electroporation and initial screening)

1 Pick a single colony from the “IPTG & Kan screening” plate and inoculate into 15 mL LB + Kan (50 µg/mL).

2 Incubate overnight at 37 °C, 220 rpm for 12 h.

Start Mutagenesis

3 Transfer 5 mL of the overnight culture into 200 mL LB + Kan (50 µg/mL) in a 2 L baffled flask.

4 Grow until OD_{600} ≈ 0.2.

5 Add 400 µL of 1 M rhamnose stock (final 2 mM) to immediately induce expression of the mutator DNAP.

Directed Evolution with Passaging

6 Total duration: 48 h, with passaging every 6 h.

7 Maintain incubation at 37 °C, 220 rpm; measure OD_{600} at each 6 h interval.

8 Every 6 h: transfer 100 µL of the current culture into 10 mL fresh LB + Kan (50 µg/mL) + rhamnose (2 mM; add 20 µL of 1 M stock per 10 mL). Continue incubation at 37 °C, 220 rpm.

9 **Sample preservation at each time point (recommended):**

- 1 mL for glycerol stock (final 20%, stored at −80 °C).
- 1–2 mL saved separately for downstream PCR/sequencing.
- Continue this routine for a total of 48 h.

Stop Mutagenesis / Start Normal Replication

10 At the end of 48 h, when culture OD_{600} reaches ~1.5–2.0, collect samples into 50 mL sterile centrifuge tubes.

11 Centrifuge at 4500 × g, 4 °C, 5 min; carefully discard the supernatant.

12 Wash: resuspend pellet in 10 mL ice-cold PBS → centrifuge 4500 × g, 4 °C, 5 min → discard supernatant.

13 Resuspend in 10 mL LB + Kan (50 μg/mL) **without rhamnose**.

14 Stabilization culture: incubate at 37 °C, 220 rpm, for 12 h (normal replication phase to shut down mutagenesis and stabilize genotype).