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Stop Evolution

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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

Terminate the rhamnose-induced high-mutation-rate evolution phase by replacing the culture medium to remove the inducer, thereby shutting down expression of the mutator DNA polymerase in the OrthoRep system. This allows stabilization and selection of target mutant strains for subsequent phenotypic analysis.

Materials

- Bacterial culture after directed evolution (containing rhamnose)
- Fresh LB broth (without rhamnose)
- Corresponding antibiotic(s)
- PBS buffer
- Centrifuge
- Sterile conical tubes

Collecting the Culture

1 Take culture from the late stage of directed evolution (grown to OD_{600} = 1.5–2.0).

2 Transfer into a 50 mL sterile centrifuge tube.

Harvesting Cells by Centrifugation

3 Centrifuge at **4500 × g, 4 °C, 5 min**.

4 Carefully discard the supernatant completely.

Washing Step

5 Resuspend the cell pellet in **10 mL pre-chilled PBS**.

6 Centrifuge again at **4500 × g, 4 °C, 5 min**.

7 Completely discard the supernatant.

Resuspending Cells

8 Resuspend the pellet in **10 mL LB broth without rhamnose**.

Add Antibiotic

9 Supplement the medium with the appropriate antibiotic.

Subsequent Culture

10 Incubate the culture at **37 °C, 220 rpm for 12 h**.

11 Proceed with downstream experiments (e.g., sequencing, screening, fluorescence analysis).