

Oct 05, 2025

Stop Evolution

DOI

<https://dx.doi.org/10.17504/protocols.io.n92ld6q2og5b/v1>

igemnthuth¹

¹iGEM_NTHU_Taiwan

iGEM NTHU



igemnthuth

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.n92ld6q2og5b/v1>

Protocol Citation: igemnthuth 2025. Stop Evolution. protocols.io <https://dx.doi.org/10.17504/protocols.io.n92ld6q2og5b/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: October 04, 2025

Last Modified: October 05, 2025

Protocol Integer ID: 228983

Keywords: mutator dna polymerase, expression of the mutator dna polymerase, mutant strain, mutant strains for subsequent phenotypic analysis, mutation, evolution, rate evolution phase, strain, rhamnose, subsequent phenotypic analysis, dna, inducer

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 $OD_{600} = 1.5\text{--}2.0$).
- 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).