Engineering Cas12j3 for Enhanced Trans-Cleavage Activity Toward Sensitive Nucleic Acid Detection

Yun Chen[1, 2], Hao Qi[1, 2, *]

[1]School of Synthetic Biology and Biomanufacturing, Tianjin University, Tianjin, China

[2]State Key Laboratory of Synthetic Biology, Tianjin University, Tianjin, China

Email address:

chenyun823@tju.edu.cn (Yun Chen), haoq@tju.edu.cn (Hao Qi)

[*]Corresponding author

Abstract

The trans-cleavage activity of CRISPR nucleases is critical for the development of highly sensitive nucleic acid detection platforms. Among compact effectors, Cas12j3 shows promising programmability but relatively limited trans-cleavage efficiency, which restricts its diagnostic potential. To address this limitation, we report a directed evolution strategy to enhance Cas12j3 activity using a two-stage screening workflow that integrates GFP-based primary screening and Cell-Free Protein Synthesis (CFPS)-based functional validation.In the first stage, a mutagenesis library of Cas12j3 variants was generated and expressed in a fluorescence-based screening system. A GFP-linked cleavage reporter enabled rapid identification of mutants with enhanced cis-cleavage activity. Variants showing increased GFP signal reduction were selected as candidates for further evaluation.In the second stage, these selected mutants were quantitatively assessed for trans-cleavage activity in a CFPS system. Fluorescence reporter assays were performed to compare the catalytic performance of evolved variants with the wild-type enzyme. This two-step strategy allows rapid enrichment of functionally improved variants while minimizing false positives due to expression variability. The evolved Cas12j3 variants exhibited significantly enhanced trans-cleavage activity, demonstrating the effectiveness of this approach. Overall, this platform provides a generalizable strategy for protein engineering of compact CRISPR nucleases and advances the development of future nucleic acid detection platforms with improved sensitivity and diagnostic potential.

Keywords

Cas12j3, Trans-Cleavage Activity, GFP-Based Screening, Nucleic Acid Detection