TNPB: A POTENTIAL TOOL FOR GENOME-EDITING IN E. COLI

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At present, bacterial genome editing frequently relies on CRISPR–Cas systems, which function as RNA-guided DNA endonucleases that introduce double-strand breaks in a PAM (Protospacer Adjacent Motif)-dependent manner. Despite their high efficiency, the application of these nucleases can be restricted under certain conditions due to cytotoxicity, potential off-target activity, and PAM sequence requirements [1]. Therefore, expanding the genome-editing toolkit in *E. coli* through the introduction of novel molecular tools that exhibit DNA cleavage activity as well as a wide range of target sequences may provide a foundation for advanced genetic manipulation and broader implementation in synthetic biology [2].

The aim of this study was to determine the DNA cleavage activity of selected TnpB proteins in *E. coli*. The analysis was conducted by performing a plasmid interference assay under IPTG-driven induction, in which nuclease activity led to the inhibition of cell growth, whereas inactive proteins did not cause any growth changes.

The results demonstrate that a set of tested TnpB nucleases with varying TAM sequences is capable of cleaving DNA in *E. coli*, leading to measurable growth inhibition. Growth reduction ranged approximately from one to three orders of magnitude compared to control reactions. These findings suggest that TnpB proteins, distinguished by their target sequence diversity, may represent valuable tools for advanced bacterial genome-editing together with the expansion of the target range.

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[2] J. Luo et al., “STAGE: A compact and versatile TnpB-based genome editing toolkit for Streptomyces,” Proceedings of the National Academy of Sciences, vol. 122, no. 35, p. e2509146122, Aug. 2025, doi: 10.1073/pnas.2509146122.