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High-throughput Kinetic Profiling of Rna-guided Nucleases for Precision Genome Editing

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Genome editing tools enabling precise DNA cleavage have rapidly advanced since the discovery and engineering of programmable RNA-guided nucleases, like CRISPR-Cas9. However, it is still challenging to comprehend the mechanisms and benchmark the specificity of these nucleases. Specificity describes a nuclease's ability to distinguish a programmed target from all other DNA. The recently discovered TnpB nuclease, derived from a transposon system, represents a compact genome editing tool with high potential. However, its functionality, kinetics, and editing capabilities are not fully understood. This study aims to profile TnpB's target specificity and kinetics with NucleaSeq, a high-throughput kinetic profiling platform. Using NucleaSeq we investigated the time-resolved cleavage rates, cleavage patterns, and specificities for thousands of DNAs that mispair with TnpB's RNA guide but are nonetheless cut by TnpB. Distinct patterns of cleavage and kinetic rates were observed, driven by DNA mismatches and target modifications. Cleavage analysis of TnpB has shown more similar trends to Cas12a than to Cas9. However, the cleavage rates are significantly different. This research expands on the knowledge about the specificity of TnpB, presenting it as a possible compact alternative to more widely used CRISPR systems.