ADAPTABLE TARGET DNA LIBRARY CONSTRUCTION FOR BENCHMARKING PROGRAMMABLE NUCLEASES

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For practical application of programmable nucleases from microbial immune systems, a thorough knowledge of their processes is required. However, it might take years or decades because of the complexity of systems, current research methodologies, and resource constraints. Our approach is a plasmid-based target DNA library with a changeable target sequence with a Protospacer Adjacent Motif (PAM) flanked by barcode sequences for cleavage pattern analysis. We employ type IIS restriction endonucleases for PAM excision to change the constant PAM region while retaining variable targets and barcodes. The PAM exchange is accomplished by ligation with a short DNA sequence that matches the PAM of a different programmable nuclease. This allows for the quick adaptation of a single DNA library to test the specificity of numerous programmable nucleases. It also shortens the period between enzyme discovery and implementation.