EFFECTS OF GRNA STRUCTURE ON ASCAS12A ACTIVITY IN E. COLI

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CRISPR–Cas-based genome editing is characterized by substantial variability in editing efficiency across different genomic targets. It is hypothesized that more predictable and stable editing outcomes can be achieved by tailoring the entire guide RNA (gRNA) sequence to each specific target. To test this hypothesis, the construction of large and diverse gRNA plasmid libraries is required, together with functional assays capable of reporting gRNA-dependent cleavage efficiency at scale. In this study, gRNA activity was investigated using an Escherichia coli cleavage assay. An original gRNA plasmid library was compared with libraries subjected to Cas-mediated cleavage under induced and non-induced conditions. Cas expression was controlled by IPTG induction during bacterial growth, allowing direct comparison of cleavage-dependent library depletion. Following library propagation, plasmid populations were analyzed by high-throughput sequencing, and changes in gRNA representation were quantified. Overall, a scalable workflow was established for linking gRNA sequence variation to cleavage outcomes, thereby supporting the development of more predictable CRISPR guide design rules.