CRISPR-guided DNA polymerases enabling diversification of all nucleotides in a tunable window

The capacity to diversify genetic codes advances our understanding and engineering of biological systems. A method to continuously diversify user-defined regions of a genome without requiring the integration of nucleic acid libraries would enable forward genetic approaches in systems not amenable to high efficiency homology-directed integration, rapid evolution of biotechnologically useful activity through accelerated and parallelized rounds of mutagenesis and selection, and cell lineage tracking. Here we developed EvolvR, the first system that can continuously diversify all nucleotides within a tunable window length at user-defined loci. Our results demonstrate that EvolvR enables multiplexed and continuous diversification of user-defined genomic loci that will be useful for a broad range of basic and biotechnological applications.