Directed Evolution with Evolv-λ for Protein Engineering

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Directed evolution serves as a powerful approach for exploring protein function and engineering novel biotechnological tools. Traditional methods for targeted mutagenesis in human cells, however, are limited by reliance on base-editing deaminases, restricting the sequence space explored during evolution. We introduce Evolv-λ, a novel, unbiased mutagenesis platform that combines CRISPR-Cas9 with an error-prone variant of human DNA polymerase λ, enabling efficient and expansive genetic diversification in human cells. We evaluated Evolv-λ by rescuing the fluorescence of a mutated EGFP and performing ultra-deep sequencing to characterize mutation patterns. Evolv-λ generates mutations across 36-46 nucleotides around the target site with a frequency of 1.4e-4 substitutions per base, exhibiting no specific nucleotide bias. Moreover, it facilitates broader genetic modifications, including insertions and deletions. We further validated Evolv-λ by restoring functionality to a mutated blasticidin resistance gene and demonstrated its capacity to diversify sequences, modulating syncytia formation driven by the SARS-CoV-2 Spike protein in cultured cells. Evolv-λ thus represents a versatile and potent in vivo mutagenesis tool for human cells, with broad applications in biological research and biotechnological innovation.