Julie L. McDonald; Amanuella A. Mengiste; Robert H. Wilson; Anna V. Plank; Matthew D. Shoulders

Department of Chemistry, Massachusetts Institute of Technology; Department of Biology, Massachusetts Institute of Technology

A versatile plasmid-based system for continuous evolution in bacterial hosts

Enzyme directed evolution is a foundational strategy for advancements in medicine, agriculture, and sustainability. However, most directed evolution campaigns rely on generation of *in vitro* DNA libraries, which are limited in sequence exploration and by transformation efficiency into various bacterial species. We have developed MutaT7, a plasmid-encoded inducible chimeric protein that produces targeted mutations in a gene of interest, thereby enabling *in vivo* evolution. We have tested this system in *E. coli* for evolution of several proteins, uncovering targeted mutations that enable antibiotic resistance and enhance enzyme kinetics. Thus, when paired with a stringent selection couple, MutaT7-based evolution is a powerful tool for continuous *in vivo* evolution of target proteins. We plan to expand this system to biotechnologically-useful bacterial species with low transformation efficiencies, such as *Cupriavidus necator*.