A CELL FREE PLATFORM FOR THE DIRECTED EVOLUTION OF TOXIC ENZYMES AND PROTEINS

Will Shindel, Curie Co, USA
will.shindel@curieco.com
Erika Milczek, Curie Co, USA
Robert DiCosimo, Curie Co, USA

Key words: Directed Evolution, Toxic Enzymes, CFPS

Directed evolution has emerged as an invaluable tool for supplying industry with cost effective enzyme alternatives to precious metal catalysis and petrochemicals. However, there are several gaps in high throughput (HTP) expression limiting the types of enzymes that can be easily engineered using directed evolution. One of these gaps includes the expression and optimization of toxic proteins in timelines required for commercial development.

A directed evolution program typically combines *in vitro* and *in vivo* operations. Diversity is often generated by stochastic *in vitro* mutagenesis methods that produce a pooled library which must be transformed into an expression host to obtain monoclonal colonies. The colonies must then be induced to express the enzyme variants, typically in liquid culture, and *in vivo* or *in vitro* screening occurs. Due to the stochastic nature of the process, oversampling is required to capture most of the library's diversity. This process is time- and labor-intensive, particularly the *in vivo* steps. To circumvent these challenges, we have demonstrated a fully *in vitro* HTP platform for the directed evolution of toxic enzymes using a cell-free extract. This platform has been used to evolve an antimicrobial enzyme with ppm level efficacy starting from a nontoxic wild-type enzyme, demonstrating the platform's commercial utility.