Combinatorial mutagenesis and in vitro expression screening of enzyme variants

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We have developed a strategy for rapid, combinatorial optimization of the hot spot residues of enzymes, using lipase B from Candida antarctica (CalB) as a model enzyme. After combinatorial randomization of target locations that affect the enzymatic activity of CalB, the mutant library was transformed into E.coli cells, and the individual variant genes isolated in the colonies were expressed in a cell-free protein synthesis system to analyze the enzymatic activity of the resulting CalB variants. Through expression screening of 1,000 variant genes, we were able to identify a series of enzymes having remarkably improved enzymatic activity and thermal stability. In addition, enzymatic activity, substrate specificity and thermal stability of the variant clones selected in vitro were well reproduced when the same genes were expressed in Pichia pastoris. Therefore, we expect that the proposed strategy of in vitro expression screening can serve as a viable option for rapid screening of optimized enzymes for industrial applications.