The Eggert Laboratory is working in the field of directed evolution, an approach used for the investigation of protein structure-function relationships as well as for the optimization of diverse enzyme properties, with emphasis on their industrial applicability. To achieve this, directed or in vitro evolution mimics natural evolution and entails the production of a large number of variants by random mutagenesis and subsequently selection of the “fittest.” Depending on the improvement of molecular biology methods with respect to random mutagenesis and high-throughput screening, “evolution in the test tube” has emerged as an alternative strategy that can be applied either in fundamental research or applied sciences. The main focus of the group is the identification of novel strategies for efficient biocatalyst and strain improvement. Model targets for enzyme engineering are α/β-hydrolases and thiamin-diphosphate-dependent enzymes that are evolved with respect to solvent stability, substrate specificity, or enantioselectivity. The complex optimization of bacterial strains is accomplished by pathway engineering of the protein secretion apparatus of the Gram-positive bacterium *Bacillus subtilis*, with the objective of enhancing the amount of heterologous protein secretion. Furthermore, the research is complemented by the development of appropriate methods in variant library construction (e.g., novel mutagenesis or in vitro recombination methods and computer tools for automation) and high-throughput screening.

www.iet.uni-duesseldorf.de/Framezeiten/directed_ev&topframenuation.htm
www.insilico.uni-duesseldorf.de

The Technique

The web tool (*insilico.mutagenesis*) described here automates the laborious task of primer prediction for scanning mutagenesis experiments of a target DNA sequence (e.g., gene sequences, promoter and/or intergenic regions, operons, etc.). Therefore, the program assists the first step in constructing high-quality mutagenesis libraries, which are necessary in directed evolution experiments. Although this web tool is only one module of library creation, it can accelerate the entire process. Furthermore, the computer-based design of mutagenesis primers prevents the experimenter from introducing sequence errors that could lead to problems in the later steps of in vitro evolution such as incompatibilities in subsequent cloning steps.

*insilico.mutagenesis: a primer selection tool designed for sequence scanning applications used in directed evolution experiments, p. 679.*