Preface

Biological systems are very special substrates for engineering—uniquely the products of evolution, they are easily redesigned by similar approaches. A simple algorithm of iterative cycles of diversification and selection, evolution works at all scales, from single molecules to whole ecosystems. In the little more than a decade since the first reported applications of evolutionary design to enzyme engineering, directed evolution has matured to the point where it now represents the centerpiece of industrial biocatalyst development and is being practiced by thousands of academic and industrial scientists in companies and universities around the world. The appeal of directed evolution is easy to understand: it is conceptually straightforward, it can be practiced without any special instrumentation and, most important, it frequently yields useful solutions, many of which are totally unanticipated. Directed evolution has rendered protein engineering readily accessible to a broad audience of scientists and engineers who wish to tailor a myriad of protein properties, including thermal and solvent stability, enzyme selectivity, specific activity, protease susceptibility, allosteric control of protein function, ligand binding, transcriptional activation, and solubility. Furthermore, the range of applications has expanded to the engineering of more complex functions such as those performed by multiple proteins acting in concert (in biosynthetic pathways) or as part of macromolecular complexes and biological networks.

Not surprisingly, the growth in the ranks of practitioners of directed evolution, and also in the range of new applications, has led to a proliferation of experimental methods aimed at simplifying the process and increasing its efficiency. The purpose of this and the accompanying volume in this series is to provide a compendium of experimental protocols accessible to scientists and engineers with minimal background in molecular biology.

Directed Evolution Library Creation focuses on methods for the generation of molecular diversity. Protocols for random mutagenesis of entire genes or segments of genes, for homologous and nonhomologous recombination, and for constructing libraries in vivo in bacteria and yeast are presented. Every one of these methods has been applied for directed evolution purposes. The optimal choice depends on the many factors that characterize each evolution problem, and we have often found that any of several different methods will work. Though there may be multiple molecular solutions to any given functional problem, the library made for directed evolution must nonetheless contain at least one of those solutions. And, the higher the frequency of potential solu-

tions, the easier it is to find them. Thus, the choice of method for creating molecular diversity and its particular implementation are important. In addition to the various protocols for creating libraries, this volume also includes three chapters that describe ways to analyze libraries, particularly those made by recombination.

No directed evolution experiment is successful without a good screen or selection. *Directed Enzyme Evolution: Screening and Selection Methods* is devoted entirely to selection and screening methods that can be applied to directed evolution of enzymes. Directed evolution is not difficult, and these protocols, prepared by practitioners from many leading laboratories, should make this robust protein engineering approach accessible to anyone with a good problem.

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