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Book review

Evolutionary Methods in Biotechnology. Clever Tricks for Directed Evolution, Edited by Susanne Brakmann, Andreas Schwienhorst, Wiley-VCH, 2004 (CD-ROM included).

Evolutionary Methods in Biotechnology is an updated collection of protocols for several aspects of research in the field of the so-called Directed Evolution. Suppose an enzyme has a potential application in the industry, say, an application of detergence, but you want to improve its catalytic activity or its thermal stability. In the nature, evolution faces this kind of problems. This, possibly, can lead to a better structure when the organism containing the enzyme is subjected to a selective pressure. Directed evolutionary methods have the aim to accelerate this process and to supply the investigator with plenty of answers to mutagenesis of the template of interest. These methods are thus of interest both for the scientist working on the determination of the structure–function relationship of a protein and for the industrial researcher who wants to improve a specific functional activity or even to “create” a new one.

Chapters 2–4 deal with the methods based on a modified PCR that yields families of mutated sequences. Brackmann and Lindemann describe the generation of mutants by means of random PCR mutagenesis, a well-known protocol that uses error-prone polymerases to produce a high mutation frequency in the sequence of interest. Similar results, but with a different approach, can be obtained by DNA shuffling (Suenaga and coworkers), an interesting technique that allows mixing of sequences from similar, if not homologous, genes. StEP (staggered extension process), described by Ninkovic, creates libraries of recombined DNA sequences. Two templates virtually cross over several times through repeated switches during the PCR annealing. The result is again a library of mutants from which one can fish the structure more suitable for his purpose.

Some chapters are devoted to more updated versions of another widely known technique: phage display, i.e. the anchoring of a protein on the surface of an organism with a genetically simple and technically manageable genotype–phenotype couple. Soumillion describes a modification of the phage display called phage-displayed enzyme selection. The sequence coding for the enzyme of interest is previously mutagenized and the library of these sequences is cloned in a phage-display system. Various versions of the enzyme are expressed on the surface of phages where the more “evolved” versions of the same enzyme can be fished by using its catalytic activity. A simplified version of phage-display is depicted by Adams and co-workers. It utilizes *Escherichia coli* cells as the display system. This allows a faster selection, for example by FACS.

The intervention of RNA molecules in laboratory evolutionary methods is highlighted in Chapters 7 and 8. Fickert et al. describe a general protocol for the selection of RNA molecules (aptamers) that are able to selectively bind to a target molecule starting from a pool of degenerate oligonucleotides. The process requires an “evolutionary” adaptation of an oligonucleotide to its target. A similar strategy is depicted by Holley and Eaton for selecting catalytic nucleic acids starting from random sequences. Of industrial interest is the accurate protocol presented by Reetz, entitled “High-throughput screening of enantioselective industrial biocatalysts”.

The last three chapters are devoted to informatics techniques aimed at simulating molecular evolution or at analyzing the results of natural evolution. Tomandl and Schwienhorst present a program to design doped libraries of protein molecules while Wiederstein and co-workers deal with directed evolution in silico mutagenesis on protein structures in pdb format, and Flamm and co-workers review and apply a program on RNA folding in silico. A CD-ROM companion to the book contains the software described in these last few chapters. The reader can thus immediately apply to his research problem the software explained in detail in the text.

At the end, a chapter concerning the patenting of the invention obtained by evolutionary biotechnology (Leimkühler and Meyers) is useful as an introductory information in the jungle of protection of intellectual property.

On the whole, this book provides an overview of the tools that are used most often in directed molecular evolution research; it is a compilation of a wide range of protocols for the most important applications in this subject. The chapters are concise but well organized and complete. The book gives detailed information that can enable even a non-expert reader to develop an experimental strategy in this field.

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