
Preface

PCR is probably the single most important methodological invention in molecular biology to date. Since its conception in the mid-1980s, it has rapidly become a routine procedure in every molecular biology laboratory for identifying and manipulating genetic material, from cloning, sequencing, mutagenesis, to diagnostic research and genetic analysis. What's astounding about this invention is that new and innovative applications of PCR have been generated with stunning regularity; its potential has shown no signs of leveling off. New applications for PCR are literally transforming molecular biology. In the post-genomic era, PCR has especially become the method of choice to clone existing genes and generate a wide array of new genes by mutagenesis and/or recombination within the genes of interest. The fast and easy availability of these genes is essential for the study of functional genomics, gene expression, protein structure–function relationships, protein–protein interactions, protein engineering, and molecular evolution.

PCR Cloning Protocols was prepared in response to the need to have an up-to-date compilation of proven protocols for PCR cloning and mutagenesis. It builds upon the best-selling first edition, *PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering*, a book in the *Methods in Molecular Biology*TM series published in 1997. We divided the new edition into five parts. Part I. **Performing and Optimizing PCR**, contains basic PCR methodology, including PCR optimization and computer programs for PCR primer design and analysis, as well as novel variations for cloning genes of particular characteristics or origins, emphasizing long-distance PCR and GC-rich template amplification. Part II. **Cloning PCR Products**, presents both conventional and novel enzyme-free and restriction site-free procedures to clone PCR products into various vectors, either directionally or non-directionally. Part III. **Mutagenesis and Recombination**, addresses the use of PCR to facilitate DNA mutagenesis and recombination in various innovative approaches to generate a wide array of mutants. Part IV. **Cloning Unknown Neighboring DNA**, contains a comprehensive collection of protocols to fulfill the frequent and challenging task of cloning uncharacterized DNA flanking a known DNA fragment. Finally, Part V. **Library Construction and Screening**, addresses particular applications of PCR in library and sublibrary generation and screening. Each part also contains an overview, which summarizes the current methods available and their underlying

strategies, advantages, and disadvantages for that particular topic. These reviews are especially helpful to new researchers to orient themselves with the field and to guide them to choose a procedure that is most suitable for their experiments.

We hope that *PCR Cloning Protocols* will provide readily reproducible laboratory protocols that researchers in the field will follow closely and thereby increase their success rate in their experiments.

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