DNA Sequencing Protocols

Methods in Molecular Biology

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and Annette M. Griffin***

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Preface

The purpose of *DNA Sequencing Protocols* is to provide detailed practical procedures for the widest range of DNA sequencing methods, and we believe that all the vanguard techniques now being applied in this fast-evolving field are comprehensively covered.

Sequencing technology has advanced at a phenomenal rate since the original methods were first described in the late 1970s and there is now a huge variety of strategies and methods that can be employed to determine the sequence of any DNA of interest. More recently, a large number of new and innovative sequencing techniques have been developed, including the use of such novel polymerases as *Taq* polymerase and Sequenase, the harnessing of PCR technology for linear amplification (cycle) sequencing, and the advent of automated DNA sequencers.

DNA sequencing is surely one of the most important techniques in the molecular biology laboratory. Sequence analysis is providing an increasingly useful approach to the characterization of biological systems, and major multinational projects are already underway to map and sequence the entire genome of organisms, such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Homo sapiens*. Most scientists recognize the importance of DNA sequence data and perceive DNA sequencing as a valuable and indispensable aspect of their work. Recent technological advances, especially in the area of automated sequencing, have removed much of the drudgery that was formerly associated with the technique, whereas innovative computer software has greatly simplified the analysis and manipulation of sequence data. Large-scale sequencing efforts, such as the Human Genome Project, produce the DNA sequences of many unknown genes. Such data provide an impetus for molecular biologists to produce probes and antibodies that can be used to identify the gene product, its cellular location, and its time of appearance in the

developing cell. A function can be assigned by mutant analysis or by comparison of the deduced amino acid sequence with proteins of known function. In this way, DNA sequencing acts as a catalyst, stimulating future research into many diverse areas of science.

More than a quarter of *DNA Sequencing Protocols* is devoted to automated sequencing technology, while the other innovations, such as cycle sequencing, solid phase sequencing, chemiluminescent sequencing, and multiplex sequencing, are also fully covered. In addition, a large part of the book is devoted to basic sequencing techniques and individual chapters are dedicated to topics such as template preparation, sequencing reactions, gel pouring, and electrophoresis of reaction samples. There are also useful chapters on sequencing PCR products, lambda clones, plasmids, and cosmids.

Each contribution is written in a manner that will allow a competent scientist who is unfamiliar with the method to carry out the technique successfully at the first attempt by simply following the detailed practical procedures that have been described by the author. Any major problems or faults that can occur, the sources of the problems, and how they can be identified and overcome are clearly described. We hope that *DNA Sequencing Protocols* will provide precise instruction for the newcomer to DNA sequencing and present a wide range of new techniques to the experienced researcher.

The editors express their gratitude and appreciation to all who have helped produce this volume and in particular to the contributing authors.

Finally, we would like to dedicate this book to our own genetic recombinants, Jessica and Stephanie, with love.

Hugh and Annette Griffin

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