
Preface

DNA Sequencing Protocols provides detailed practical procedures for a range of the most commonly applied DNA sequencing methods. As well as giving detailed protocols, the present volume strives to pinpoint those areas within each of the different techniques that tend to cause the most problems, and the Notes section in each chapter provides some help in identifying and addressing these.

Technology in this field has advanced to the stage where PCR-based semiautomated fluorescent sequencing is the norm and manual Klenow fragment sequencing with incorporated radionucleotides is, for many of us, a thing of the past. Major advances, both in sequencing chemistries and the use of robotics, have enabled the complete sequencing of such complex genomes as *C. elegans*, a total of almost 100 million base pairs of DNA. The sequencing of the Human Genome (3286 million bases) is now in sight with the first draft issued in 2000, while the finished sequence should be complete by 2003. This will represent the ultimate milestone in DNA sequencing achievements and has required a massive international collaborative effort. The cornerstone sequencing techniques that have made this goal obtainable are covered in this book. Included are chapters on M13 cloning and sequencing, PCR sequencing, cycle sequencing, shotgun sequencing, primer design and primer walking techniques, solid phase sequencing, and semiautomated fluorescent sequencing. Some additional chapters, such as those discussing sequencing by capillary arrays and Web-based DNA sequence databases, deviate from the standard format, in that they provide information and a review of the relevant systems, rather than a practical bench-type guide.

The genome sequencing projects (human and of other organisms), both collaborative and private, are producing ever-increasing amounts of data. This vast amount of information will eventually result in changes to DNA sequencing with the implementation of more rapid and fully automated systems. DNA microarray technology appears to provide the answer to this, but teething difficulties have delayed the development of commercial systems for DNA sequencing. However, this technology is being used for expression analysis and for sequence-based mutation detection in defined genes. In the meantime, multichannel column-based fluorescent sequencers have filled the void and provide very rapid high-throughput systems.

DNA sequencing in one form or another is likely to be with us for a long time to come. Within the next 5–10 years, it will truly enter the era of molecular diagnostics, whether it be in defining genetic susceptibility to common diseases, such as diabetes, or classifying bacterial and viral infections for the most appropriate drug therapy and the development of novel vaccines.

Happy sequencing!

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