
Preface

The discovery of reverse transcriptase by Howard Temin and David Baltimore in 1970 launched a revolution in molecular biology that was unmatched until the advent of DNA amplification by polymerase chain reaction (PCR). Not only did the discovery overturn the “central dogma” that information coded in DNA flowed through RNA to protein, but also the activities of RNA-dependent DNA polymerase were, and are, important in medicine and other fields. The utility of these enzymes as instruments to identify coding sequences of genes and to analyze their expression was quickly realized.

The applications of cDNA technology have changed dramatically as the technology has advanced. In the early days of cDNA analysis, it revealed which genes were expressed and often the tissue specificity of gene expression. Analysis of cDNA molecules showed the chromosomal patterns of introns and exons and revealed the predicted protein sequences of countless genes. While the techniques for isolating RNA, generating cDNA, and analyzing the cloned cDNA were crude by today’s standards, they provided many insights into the workings of plants, animals, and other organisms. Not unlike the effects of PCR in biology, the cDNA revolution continues as the basic techniques are revised and new uses for the technology are developed.

Previous volumes in this series have supplied many techniques that continue to be important; in this volume, we provide current techniques that reflect the most recent advances in the construction and application of cDNA libraries. Broadly, the techniques we describe can be divided into two classes. The first class includes improved approaches to some of the most basic elements of creating cDNA libraries, while the second class is much wider and includes visionary applications of cDNA technology which were either unforeseen or technically impractical until recently.

Some of the most important advances in cDNA technology are new approaches to challenges that have been inherent in the production and analysis of cDNA libraries from the earliest days of the technology. These limits have been rolled back by dramatic technical advances in several previously limiting processes. Advances in separation of complex tissues into their several components for analysis and the ability to create suitable cDNA libraries from minuscule tissue samples, even from a single cell, have greatly expanded the range of practical experiments. A suite of technical improvements have made full-length, normalized libraries with reduced bias available. These libraries are suitable for expression analysis, and the ease of library construction has been enhanced by adopting *in vitro* recombination methods, greatly expanding the numbers of clones available for expression and increasing confidence that a library adequately represents the genes expressed in the source tissue. Analysis of the information gleaned from cDNA libraries has been continuously refined, and new bioinformatic approaches provide a more complete description of the genes transcribed in each tissue used in library construction.

The second class of additions to the array of cDNA library technology arises from visionary application of these refined techniques to new areas of research. Small RNA molecules recently and strikingly emerged as important participants in cell regulation and development; extension of cDNA libraries to this realm of small RNA has become critical in understanding these regulators. Novelty that is artificially induced, either through intentional error-prone synthesis or through shuffling of domains, is another source of new material for library construction. The ability to easily generate full-length expression

libraries has made new kinds of experiments possible, whether they rely on gain-of-function analysis in transgenic organisms or on high-throughput functional screening of libraries in test organisms. Expression libraries are also critical in the development of YSD (yeast surface display) analysis of protein interactions with ligands, a technique whose emergence may have far-reaching applications.

The ability of cDNA technology to analyze the transcriptome of an organism or tissue also points to a new era of cDNA applications. Discovery of SNP markers for genetic traits through pyrosequencing, transcriptome analysis for gene discovery or splicing analysis, and whole-genome expression analysis of organisms whose genome has not been sequenced are all techniques described in this volume.

The developments of cDNA technology described herein demonstrate that the technology continues to advance and to provide answers to fundamental questions of biology. Even more sophisticated technical improvements coupled to the scientific vision to apply the techniques to an expanded range of problems have kept the construction, analysis, and use of cDNA libraries on the cutting edge of biology, and we expect improvements will continue in the future.

We thank the publishers for inspiring the collection of this material. We heartily thank the contributors for taking time from their continuing scientific endeavors to share their acquired skills with a wider audience, and we hope that the contents of this volume will speed its readers to even more successful scientific discovery.

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cDNA Libraries

Methods and Applications

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