

Methods in Enzymology

Volume 303

*cDNA Preparation
and Characterization*

EDITED BY

Sherman M. Weissman

BOYER CENTER FOR MOLECULAR MEDICINE
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Preface

Genomic sequences, now emerging at a rapid rate, are greatly expediting certain aspects of molecular biology. However, in more complex organisms, predicting mRNA structure from genomic sequences can often be difficult. Alternative splicing, the use of alternative promoters, and orphan genes without known analogues can all offer difficulties in the predictions of the structure of mRNAs or even in gene detection. Both computational and experimental methods remain useful for recognizing genes and transcript templates, even in sequenced DNA. Methods for producing full-length cDNAs are important for determining the structure of the proteins the mRNA encodes, the position of promoters, and the considerable regulatory information for translation that may be encoded in the 5' untranslated regions of the mRNA.

Methods for studying levels of mRNA and their changes in different physiological circumstances are rapidly evolving, and the information from this area will rival the superabundance of information derived from genomic sequences. In particular, cDNAs can be prepared even from single cells, and this approach has already yielded valuable information in several areas. To the extent that reliable and reproducible information, both quantitative and qualitative, can be generated from very small numbers of cells, there are rather remarkable possibilities for complementing functional and genetic analysis of developmental patterns with descriptions of changes in mRNAs. Dense array analysis promises to be particularly valuable for the rapid expression pattern of known genes, while other methods such as gel display approaches offer the opportunity of discovering unidentified genes or for investigating species whose cDNAs or genomes have not been studied intensively.

Knowledge of mRNA structure, genomic location, and patterns of expression must be converted into information of the function of the encoded proteins. Each gene can become the subject of years of intensive study. Nevertheless, a number of methods are being developed that use cDNA to predict properties or permit the selective isolation of cDNAs encoding proteins with certain general properties such as subcellular location. This volume presents an update of a number of approaches relevant to the areas referred to above. The technology in this field is rapidly evolving and these contributions represent a "snapshot in time" of the number of currently available and useful approaches to the problems referred to above.

SHERMAN M. WEISSMAN

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