Preface

Why study RNA? This question was posed as the first edition of *RNA Methodologies* went to press in 1993. The isolation of biologically competent and chemically stable RNA continues to be a central, although somewhat unappreciated, procedure in molecular biology, which has even greater relevance today. However, much has changed since the second edition of this book was published. The completion of the human genome product has heralded the previously unknown disciplines of functional genomics and proteomics. Researchers now regularly explore the transcriptome, the proteome, the metabolome, and, lest we forget, the genome.

It is clear that the regulation of tissue-specific gene expression remains an area of intense interest fueled in no small measure by the availability of pricey microarrays printed on both nylon filters and on glass chips. These types of analyses, as well as standard quantitative PCR-based assays, are possible only when high-quality RNA is isolated from the proper biological source, is able to support reverse transcription, and is able to function in other ancillary assays. It is also worth noting here that, although microarray technology certainly has a place among contemporary research tools, the sequences that are scrutinized represent genes that have already been cloned; the technology does not directly support the detection or identification of previously uncharacterized genes, differentially spliced transcripts, or transcripts with multiple start sites.

Thus, the study of RNA as a parameter of gene expression remains a staple in many laboratories. Scrutiny of mRNA biogenesis affords a unique look at the cellular biochemistry from the perspective of the exquisite orchestration of RNA complexity. Coupled with traditional methods for assay, variations on the PCR theme continue to afford a means of unparalleled sensitivity and resolution for the characterization of transcriptional activity in the cell. mRNA biogenesis, maturity, and function are influenced transcriptionally and posttranscriptionally, and together they constitute myriad gene regulation control points. Thus, RNA stability and processing clearly play central regulatory roles in the cell.

There remains an academic imperative to unify the numerous facets of RNA characterization in a coherent start-to-finish format. The purification of high-quality RNA from biological sources is a fundamental starting point for investigations designed to give clearer definition to at least one aspect of the regulation of gene expression. Although many of the more traditional techniques, including Northern analysis, have been overshadowed by newer, more sensitive approaches, the thoughtful and expedient isolation of RNA is equally as important as the subsequent analytical and preparative manipulations of the RNA in purified form. Whether isolated from cells in culture or directly from whole tissue, only the meticulous handling of RNA will support these techniques.

This laboratory guide represents a growing collection of tried, tested, and optimized laboratory protocols for the isolation and characterization of eukaryotic RNA, with lesser emphasis on the characterization of prokaryotic transcripts. The more noteworthy additions to the third edition include various new and improved RT-PCR tricks of the trade; innovative 5′ and 3′ RACE; subtractive PCR methods; methods for improving complementary DNA (cDNA) synthesis; and informative chapters on the newer technologies of RNA interference (RNAi), microarrays, and bioinformatics. Methods such as these are especially important today in view of the unprecedented interest in quantifying the absolute abundance of specific transcripts in the cell. Clearly, the focus here is the study of RNA as a parameter of gene expression with emphasis on how these techniques can be used as stand-alone methods or in unison with other assays for the study of transcriptional and posttranscriptional regulation of eukaryotic genes. The best advice that can be offered is the following: Always think two steps ahead in an experiment, and reflect on how the method of RNA isolation affects the ensuing protocols and interpretation of data.

This text is written for the principal investigator, bench scientist, physician, veterinarian, laboratory technician, graduate student, and anyone else capable of performing basic research techniques. This resource is intended to provide a rationale to assist in the decision-making process for individuals at all levels of sophistication, from the novice to the well-seasoned scientist, and at the same

time to present realistic alternatives for achieving the same experimental goals. Many of the incorporated notations and hints are based on personal experience and pave the way for the expedient recovery of RNA from biological sources, with particular regard to those sources enriched in ribonuclease. Day-in and day-out, unsound tactics for RNA characterization result in wasted resources because of an obvious failure to understand the "what" and the "why" from the onset of the study. Although this text is by no means a comprehensive review of the fundamentals of gene expression, the recurrent themes herein are the correct way to handle and assay RNA. These pages also demonstrate clearly how a selected technique fits into the grand scheme of nucleic acid research. Although I hope that the text will be studied from cover to cover, one may pick and choose salient protocols without loss of continuity. For those readers who are new to the study of the cellular biochemistry from the perspective of RNA analysis, it may be beneficial to first read Chapter 26 (An RNA Paradigm).

Collectively, the chapters work together to embellish the RNA story, each presenting clear lessons. The liberal incorporation of flow charts, tables, and graphs facilitate learning and assist in the planning and implementation phases of a project. The investigator is limited only by his or her ingenuity.

* * *

I acknowledge, with sincere thanks and appreciation, the intellectual encouragement of the many colleagues and friends who, in some way, supported the preparation of this manuscript. The support and *patience* of my family are also publicly acknowledged and are very much appreciated.

Initium sapientiae timor Domini