

Preface

The Surprising World of RNA

Why study RNA? This question was posed as the first edition of *RNA Methodologies* went to press in 1993. The isolation of RNA continues to be a central though somewhat unappreciated aspect of molecular biology, though much has changed since the third edition of this book was published. The completion of the human genome project 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.

To state that RNA is versatile is the understatement of the century. RNA is much more than a DNA-protein go-between. RNA can exhibit catalytic activity, provide binding sites for small molecules for myriad purposes, regulate the expression of other genes, function as the backbone of ribosomes, transport amino acids, and much more. All of these roles support the notion of the widely acclaimed "RNA world hypothesis", in which RNA, and not DNA, protein, or anything else was the primary primordial information molecule. This is quite plausible because as a single-stranded molecule, RNA has an amazing ability to assume secondary structures and folding properties, imparting functionalities perhaps as diverse as its very nucleotide sequence.

Transcriptional profiling is possible only when high quality RNA is isolated from its biological source, such that it is able to support reverse transcription and any of a variety of downstream applications, including the detection of previously uncharacterized genes, differentially spliced transcripts, or transcripts with multiple start sites. Information of this nature is very important; for example, alternative splicing imparts an added level of vulnerability to mutations and the disease state, meaning that what happens at the level of RNA is often a life or death scenario.

There remains an academic imperative to unify the numerous facets of RNA characterization in a coherent start-to-finish format, though one of the major difficulties toward the realization of that goal is that a rapid succession of new techniques and variants thereof has resulted in confusing technical nomenclature. To make matters worse, not everyone uses the same terminology to describe the same techniques. Quantification these days is of ever increasing importance because of the apparent link between an abnormal abundance of a transcript (too high or too low) and a genetic disease, especially in the context

of the great interest in the use of RNA, and RNA aptamers, for *in vitro* diagnostic applications. While the very sensitive method known as real-time PCR has overshadowed Northern analysis, nuclease protection, and the nuclear runoff assay, these time-honored methods remain important, though comparatively roughhewn, tools for RNA characterization.

Thoughtful and expedient isolation of RNA is equally as important as the assays that follow. The purification of high quality RNA, what this Author affectionately refers to as eRNA (excellent RNA!), from various biological sources is the fundamental starting point for investigations designed to give clearer definition to this aspect of the regulation of gene expression. Whether isolated from cells in culture or directly from whole tissue, only the meticulous handling of RNA will support experiments that will then be used for its study.

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 fourth edition include a reorganization of chapters, placing earlier and greater emphasis on protocols. There is also information on a variety of new and improved RT-PCR tricks of the trade, including methods for improving cDNA synthesis, innovative 5' and 3' RACE, subtractive PCR methods, the isolation of RNA from plants, and updated, informative chapters on bioinformatics, high throughput analysis of gene expression, and RNA interference (RNAi), with commentary on the advantages and disadvantages of each. Emphasis is given to 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 with the greatest possible sensitivity and resolution.

This text is written for the principal investigator, bench scientist, physician, veterinarian, lab technician, graduate student, undergraduate research assistant, and anyone else capable of performing basic research techniques—there is something in it for everyone. 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 present realistic alternatives for achieving the same experimental goals. Many of the incorporated notations and hints are based upon personal experience and pave the way for the expedient recovery of RNA and the most judicious use of resources. Day-in and day-out, unsound tactics for RNA characterization result in wasted resources due to an obvious failure to understand the “what” and the “why” from the onset of the study. The best advice that I can offer: always think two steps ahead in an experiment, and reflect upon how the method of RNA isolation will impact the ensuing protocols and interpretation of data.

The recurrent themes herein are the correct way to handle and to assay RNA, and an appropriate level of background information related to the fundamentals of gene expression is likewise provided. These pages demonstrate clearly how a selected technique fits into the grand scheme of nucleic acid

research. While this Author would hope that the text 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 25 (An RNA Paradigm). Collectively, the chapters work together to embellish the RNA story, each presenting clear take-home lessons. The liberal incorporation of flow charts, tables, and representative data likewise facilitate learning and assist in the planning and implementation phases of a project. The investigator is limited only by his own ingenuity.

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