

Preface

RNA MOLECULES PARTICIPATE IN AND REGULATE a vast array of cellular processes, and the scientific community is now well into a new era in which some aspect of RNA biology—as a tool, a therapeutic, a diagnostic, or a central player in a fundamental biological process—is becoming increasingly important. Nevertheless, beginning RNA research can be daunting, and without a thorough understanding of the challenges and complexities inherent in handling this fragile nucleic acid, forays into the RNA world can be quite frustrating. For example, the introduction of many kits designed to perform everything from purification to high-throughput sequencing has been both a boon and a bane to RNA science. Although these kits have made RNA analysis accessible to nearly every laboratory, they give many researchers a false sense of security—they believe that whatever comes out of the final tube is exactly what they expect. Unfortunately, unbeknownst to the investigator, this is often not the case.

We have written this manual with all RNA scientists—novices and more advanced researchers—in mind. Chapters 1 through 3 are specifically targeted to those new to the area or those needing some “refreshing” of fundamental principles and techniques. We have emphasized “common sense” approaches and highlight the fact that it is essential to monitor the yield and quality of any RNA preparation after each step. Anyone contemplating RNA research should be comfortable with the material in these chapters before moving on to the more complex procedures in the chapters that follow.

Chapters 4 through 6 are written for those who wish to investigate RNA-related topics in more depth. Here, we describe a variety of techniques, some quite complex, designed primarily to explore specific RNA–protein interactions and elucidate the mechanisms of RNA-processing reactions. Chapter 7 describes current methodologies for using RNA interference as a tool for determining gene function, and Chapter 8 presents methods for performing increasingly popular genome-wide investigations. Finally, the Appendix includes a few methods that we have found to be useful but did not fit into any particular area, as well as recipes for commonly used reagents and some general reference information.

Almost all of the methods described in this manual are routinely used in one or more of our laboratories. In the few cases where we do not have direct experience, we have enlisted the expertise of trusted colleagues. Although we have attempted to be comprehensive, we realize that this is an impossible task. New approaches, especially in the area of genome-wide analyses, appear with high frequency. Moreover, no one method is ideally suited to any particular question. We fully realize that there are alternative approaches available for nearly every method that we describe. In addition, we acknowledge that many specialized techniques, such as circular permutation and direct approaches to RNA analysis (e.g., fingerprinting and thin-layer chromatography), have not been described at all because we believe that these methods would be of limited appeal to most RNA researchers.

It is often difficult to identify with any precision the source of particular methods because as they are adopted by different laboratories, they “evolve.” Accordingly, we have not attempted to provide extensive referencing for the protocols; only a few representative publications are cited. We apologize in advance to all of those authors who have published methods or improvements thereon who have not been cited in this manual.

Finally, we note that many "standard" molecular biology techniques, such as protein gels, western blotting, cloning, etc., are not covered in this manual. Such material is covered in great detail in the latest edition of *Molecular Cloning: A Laboratory Manual*, and readers requiring this information are strongly encouraged to consult that manual in conjunction with this one.

In *RNA: A Laboratory Manual*, we have attempted to describe a broad scope of current techniques used in RNA research. We hope that this manual will enable any researcher to approach a wide variety of problems from the most fundamental to the highly sophisticated with confidence and high expectation of success.

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