
Preface

The past decade has witnessed an explosive growth in the study of the portion of the mammalian transcriptome that does not encode protein products. This noncoding transcriptome, in fact, accounts for a large fraction of the RNA output of the cell and, until relatively recently, has been not only poorly understood and appreciated but also understudied. As we identify and begin to characterize more and more noncoding transcripts that have the potential and ability to orchestrate and control gene expression, it is important to share the molecular tools that will aid in the necessary studies. This volume of *Methods in Molecular Biology* is devoted to approaches for the study of regulatory noncoding RNAs. It offers a collection of methods that should prove of value to those interested in the discovery, localization, and functional analysis of them.

In Chapter 1, Merry, Niland, and Khalil review the current state of the field of mammalian long noncoding RNAs, pointing out many of the critical issues and discoveries that are driving the field forward and putting into context the need for appropriate methods to study the expression, location, and functions of these molecules.

There are four chapters that describe methods useful for the study of siRNAs, microRNAs, and their targets. Chapter 2 by Lee, Wagner, and Lehto describes a novel method involving polymers that enhance the delivery of small RNAs to cells. This method is applied by them for the delivery of siRNAs but could as well be applied to the delivery of microRNAs. MicroRNAs have been studied in great detail in recent years, and their importance to biology cannot be understated. However, we still have not identified many or perhaps even most of their in vivo targets. Subramanian, Li, Hara, and Lal describe biochemical methods to directly identify microRNA targets. Liu and Mourelatos describe a method to characterize microRNAs in association with their essential protein partners. Finally, Younger and Corey describe methodology to allow the identification and validation of microRNA targets that are not commonly thought to be canonical ones.

The following eight chapters are concerned with long noncoding RNAs. Many of these have been discovered using standard isolation procedures such as the isolation of polyadenylated RNAs using oligo(dT) affinity reagents. However, some important long noncoding RNAs lack poly(A) tails and require special isolation techniques. In Chapter 6, Yin, Chen, and Yang describe in detail how to isolate poly(A)-minus, ribosomal RNA-free RNAs for the detection of nonpolyadenylated long noncoding RNAs.

Long noncoding RNAs exert their effects not as naked molecules but in complexes with proteins, most of which have not been identified. Two chapters are dedicated to methods addressing long noncoding RNAs and the proteins that interact with them. Gong and Maquat and Marin-Béjar and Huarte present protocols describing how to isolate and purify long noncoding RNAs in complex with the proteins associated with them. These chapters are followed by methods detailed by Zhao, who describes how to start with an RNA-binding protein and then find its RNA targets on a genome-wide scale.

Another important way to address the function of long noncoding RNAs is to identify their subcellular location. There are three chapters that describe sensitive and accurate methods for the detection of these RNAs in cultured cells using fluorescence in situ hybridization. While the chapters by Nakagawa and Tripathi et al. employ traditional probes for

RNA detection, the chapter by Sinnamon and Czaplinski describes a quite novel type of molecular probe to enhance our ability to detect long noncoding RNAs, including those that may not be especially abundant within cells.

The function of noncoding transcripts can also be interrogated either by the introduction of these molecules or mutant versions of them into cells or by eliminating their expression or accumulation. Hirose and Mannen describe an efficient and powerful method to eliminate nuclear noncoding RNAs using modified antisense oligonucleotides.

Finally, the study of functional noncoding RNAs cannot be complete, either in vivo or in vitro, without an appreciation of some of the critical parameters of their protein–RNA interactions or of the environment in which they act. The final two chapters are devoted to such issues. Grover discusses the critical importance of divalent cations in the study of RNA function, and Goodrich and Kugel describe methods to study the affinity, kinetic stability, and specificity of RNA–protein interactions.

Taken together, the chapters presented in this volume should provide a current and state-of-the-art collection of methods and approaches that will be of value to researchers in this expanding and fascinating field.

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