
Preface

During the last few years, new discoveries in the RNA field have opened up a wealth of opportunities to specifically target mRNA for functional analysis and target validation. Contained in this volume of *Methods in Molecular Biology* are methods useful for the design and application of ribozymes, DNAzymes, and small interfering RNAs (siRNAs). In addition, a number of illustrations aiming to facilitate the understanding and application of each method is included.

Like any new field, ribozymes and siRNA research is rapidly evolving, and so too are the methods used to study, select, express, and control their structure and in vivo biological activities. *Ribozymes and siRNA Protocols, Second Edition* focuses on the latest technical advances in interfering with gene expression. It contains a short introduction followed by general and specific protocols for using hammerhead ribozymes and derivatives, DNAzymes, hairpin ribozymes, group I intron ribozymes, RNase P ribozymes, and siRNAs. Also in this volume are general methods for RNA structure analysis, delivery of oligonucleotides, and gene therapy protocols using ribozymes.

Multiple mechanisms exist by which short synthetic or expressed oligonucleotides can be used to modulate gene expression in mammalian cells. These include ribozymes, DNAzymes, and siRNAs. Although these are different strategies, all recognize their target mRNA via Watson-Crick base pairing and mediate the cleavage of cognate mRNAs. Hence, the successful use of these technologies requires good accessibility to cellular mRNA. Chapters 8, 9, 16, 19, 20, and 23 cover novel methods by which accessible sites are identified. Additionally, a PCR-based approach is used to select active siRNA in vivo (Chapter 39).

Combination of chemical and enzymatic synthesis of ribozymes and siRNAs is covered in Chapters 2 and 34, while Chapters 3–5 cover the analysis of ribozyme kinetic parameters and real-time monitoring of ribozymes and DNAzyme cleavage reactions by fluorescence detection. These chapters are followed by articles on recent technical advances in analyzing ribozyme structures and conformational transitions using nucleotide analog interference mapping and fluorescence resonance energy transfer (Chapters 6 and 7).

Using rational design and in vitro selection, a number of allosteric ribozymes that can be triggered by a variety of effectors have been engineered. Chapter 10 and 11 describe these novel molecular switches that undergo ribozyme-mediated cleavage when they bind to specific ligands. Additionally, the use of inducible promoters for switching the expression of ribozymes off or on is described in Chapter 12.

Chapters 13–18 cover the design, and in vivo and in vivo application of hammerhead ribozymes and derivatives (e.g., maxizymes). Also included is functional gene discovery using hybrid ribozyme libraries that are expected to facilitate functional genomic studies.

A group of three chapters cover the DNAzyme technology. These include design rules, target site selection, and mutation analysis. The next four chapters are on hairpin ribozymes describing, first, methods for crystallization and, second, selection of effective target sites and hairpin ribozymes for therapeutic application (Chapters 22–25).

Group I intron and RNase P ribozyme protocols are covered in Chapters 26–32. These seven chapters describe protocols for effective design, selection, and therapeutic applications. The use of ribozymes and DNAzymes in rodent models of human disease is described in Chapter 33.

A block of nine chapters (34–42) address the latest RNAi technology. This has emerged as a powerful technique for sequence-specific gene silencing in a wide variety of organisms. The chapters cover a wide range of methods and application of siRNAs. These include potential design rules, production, target site selection, plasmid/viral expression vectors, and knockout animals. These methods should benefit not only those experienced in siRNAs, but also those applying this technology for the first time.

Delivery agents from different companies and methods for the selection of cell binding peptides for specific delivery of oligonucleotides into cancer cells are described in Chapters 43 and 44. The book ends with clinical and gene therapy protocols using ribozymes (Chapters 45 and 46). Such protocols are also relevant for the future use of siRNA in humans.

It has been an honor to work with each of the authors in assembling this compilation of protocols and procedures. I congratulate them all on their achievement in making their methods available, and hope you will find *Ribozymes and siRNA Protocols, Second Edition* a useful reference easily used at the lab bench.

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