

CONTENTS

<i>Contributors</i>	<i>xi</i>
<i>Volumes in Series</i>	<i>xv</i>

Section I. Identifying MicroRNAs and Their Targets **1**

1. Identification of Viral MicroRNAs **3**

Christopher S. Sullivan and Adam Grundhoff

1. Introduction	3
2. Computational Prediction of Viral miRNA Candidates	7
3. Array Confirmation of Viral miRNA Candidates	11
4. Concluding Remarks	21
References	22

2. Robust Machine Learning Algorithms Predict MicroRNA Genes and Targets **25**

Pål Sætrom and Ola Snøve, Jr.

1. Introduction	26
2. Appropriate Use of Machine Learning	27
3. One Deterministic and One Stochastic Algorithm	33
4. miRNA Gene Prediction with Support Vector Machines	37
5. miRNA Target Prediction with Boosted Genetic Programming	43
6. Summary	46
Acknowledgments	46
References	46

3. Identification of Virally Encoded MicroRNAs **51**

Sébastien Pfeffer

1. Introduction	52
2. Purification of Rnl2 (1–249) and Adenylation of 3' Adapter Oligonucleotide	55
3. Isolation of the Small RNA Fraction	55
4. Ligation of the Purified Small RNA to Adenylated 3' Adapter	56
5. Ligation of the Small RNA-3' Adapter to the 5' Adapter	56
6. Reverse Transcription of the Final Ligation Product	57

7. First PCR Amplification of the cDNA	57
8. Pme I Digestion of the PCR Product	58
9. Second PCR Amplification	58
10. Ban I Digestion of the Second PCR Product	59
11. Concatamerization of the Ban I-Digested DNA	59
12. End Tailing of Concatamers and Cloning into T/A Vector	59
13. Sequencing and Annotation of the Library	60
14. Concluding Remarks	60
References	61
4. Computational Methods for MicroRNA Target Prediction	65
Yuka Watanabe, Masaru Tomita, and Akio Kanai	
1. Introduction	66
2. Principles of miRNA Target Recognition	69
3. Resources for Analysis of miRNA Target Genes	73
4. Software Useful for miRNA Target Prediction	74
5. Original Strategies for Prediction of miRNA Target Genes	79
6. Validation of Computational Predictions	81
7. Concluding Remarks	82
Acknowledgments	83
References	83
Section II. MicroRNA Expression, Maturation, and Functional Analysis	87
5. <i>In Vitro</i> and <i>In Vivo</i> Assays for the Activity of Drosha Complex	89
Yoontae Lee and V. Narry Kim	
1. Introduction	89
2. Assay Methods	94
Acknowledgments	105
References	105
6. Microarray Analysis of miRNA Gene Expression	107
J. Michael Thomson, Joel S. Parker, and Scott M. Hammond	
1. Introduction	107
2. Overview of miRNA Biogenesis and Effector Pathways	109
3. miRNA Expression Analysis Strategies	110
4. Considerations for miRNA Microarrays	111
5. Data Analysis and Interpretation	112

6. Validation Strategies	113
7. miRNA Microarray Protocol	113
8. Data Analysis	118
Acknowledgments	119
References	119
7. Cloning and Detecting Signature MicroRNAs from Mammalian Cells	123
Guihua Sun, Haitang Li, and John J. Rossi	
1. Introduction	124
2. miRNA Cloning	126
3. miRNA Identification	133
4. Northern Hybridization to Verify <i>In Vivo</i> Expression of miRNA	134
Acknowledgments	136
References	136
8. Approaches for Studying MicroRNA and Small Interfering RNA Methylation <i>In Vitro</i> and <i>In Vivo</i>	139
Zhiyong Yang, Giedrius Vilkaitis, Bin Yu, Saulius Klimašauskas, and Xuemei Chen	
1. Introduction	140
2. Expression and Purification of Recombinant HEN1 Proteins	141
3. Small RNA Methyltransferase Assays with Recombinant HEN1 Proteins	144
4. Reverse-Phase HPLC Analysis to Determine the Position of the Methyl Group in Products of HEN1-Catalyzed Reactions	148
5. Immunoprecipitation and HEN1 Activity Assay	149
6. Analysis of the <i>In Vivo</i> Methylation Status of miRNAs and siRNAs	151
7. Concluding Remarks	152
Acknowledgments	152
References	153
9. Analysis of Small RNA Profiles During Development	155
Toshiaki Watanabe, Yasushi Totoki, Hiroyuki Sasaki, Naojiro Minami, and Hiroshi Imai	
1. Introduction	156
2. Preparation of Low-Molecular-Weight RNA and Urea-Polyacrylamide Gel Electrophoresis (Urea-PAGE)	158
3. Cloning of Small RNAs	161

4. Classification of Small RNAs	163
5. Northern Blot Analysis	165
References	167
10. Dissecting MicroRNA-mediated Gene Regulation and Function in T-Cell Development	171
Tin Ky Mao and Chang-Zheng Chen	
1. Introduction	172
2. Characterizing miRNA Expression During T-Cell Development	172
3. Retroviral Constructs for miRNA Expression	178
4. Investigating miRNA Function in T-Cell Development	180
5. Identification and Validation of Functionally Relevant miRNA Target Genes	183
6. Materials and Reagents	187
Acknowledgments	188
References	188
Section III. MicroRNAs and Disease	191
11. Investigation of MicroRNAs Alterations in Leukemias and Lymphomas	193
George Adrian Calin and Carlo Maria Croce	
1. MicroRNA Alterations are Involved in the Initiation and Progression of Every Type of Human Cancer	194
2. Genome-Wide MicroRNA Profiling by Microarray	195
3. Identification and Validation of Targets for Differentially Expressed miRNAs	205
Acknowledgments	210
References	210
12. Discovery of Pathogen-Regulated Small RNAs in Plants	215
Surekha Katiyar-Agarwal and Hailing Jin	
1. Introduction	216
2. Sequencing-Based Approaches for the Discovery of Pathogen-Regulated Small RNAs	217
3. Hybridization-Based Approaches of Identifying and Validating Pathogen-Inducible Small RNAs	219
4. Concluding Remarks	225
References	226

13. Protocols for Expression and Functional Analysis of Viral MicroRNAs	229
Eva Gottwein and Bryan R. Cullen	
1. Introduction	230
2. miRNA Expression Cassettes	232
3. Vector Systems for Stable Delivery	233
4. Protocol: Generation of miRNA-Expressing Cell Lines Using pNL-SIN-CMV-BLR-Based miRNA Expression Vectors	235
5. Indicator Assays Establish miRNA Activity	237
6. Protocol: Preparation of Virus Mixes and Indicator Assay	237
7. Concluding Remarks	240
Acknowledgments	240
References	240
<i>Author Index</i>	245
<i>Subject Index</i>	257