

Contents

1	miRNAs Targeting and Targeting miRNAs	1
1.1	miRNA Biology	2
1.1.1	miRNAs Biogenesis	2
1.1.2	miRNAs Actions	6
1.2	miRNA Expression, Mutation and Polymorphism	8
1.2.1	miRNA Expression	8
1.2.2	miRNA Mutation	9
1.2.3	miRNA Polymorphism	10
1.3	miRNAs and Human Disease	12
1.3.1	miRNAs and Developmental Disorders	12
1.3.2	miRNAs and Apoptosis	17
1.3.3	miRNAs and Cancer	20
1.3.4	miRNAs and Cardiovascular Disease	23
1.3.5	miRNAs and Neuronal Disease	28
1.3.6	miRNAs and Viral Disease	30
1.3.7	miRNAs and Metabolic Disorders	34
1.3.8	miRNA and Epigenetics	36
1.4	miRNAs as Therapeutic Targets	38
1.4.1	Strategies for Therapeutic Modulation of miRNAs	39
1.4.2	Approaches for Therapeutic Modulation of miRNAs	41
	References	41
2	miRNA Interference Technologies: An Overview	59
2.1	New Concepts of miRNAi Technologies	59
2.1.1	“miRNAi”, A New Concept	59
2.1.2	“miRNA as a Regulator of a Cellular Function”, Second New Concept	62
2.1.3	“One-Drug, Multiple-Target”, Third New Concept	63
2.1.4	“miRNA Seed Family”, Another New Concept	64

2.2	General Introduction to miRNAi Technologies	66
2.2.1	miRNA-Targeting Technologies	66
2.2.2	Targeting-miRNA Technologies	66
2.3	miRNAi Technologies in Basic Research and Drug Design	67
	References	71
3	Synthetic Canonical miRNA Technology	75
3.1	Introduction	75
3.2	Protocols	76
3.2.1	Designing SC-miRNAs	76
3.2.2	Validating SC-miRNAs	78
3.3	Principle of Actions	86
3.4	Applications	87
3.5	Advantages and Limitations	88
	References	88
4	miRNA Mimic Technology	93
4.1	Introduction	93
4.2	Protocols	94
4.2.1	Designing miR-Mimics	95
4.2.2	Validating miR-Mimics	96
4.3	Principle of Actions	97
4.4	Applications	98
4.5	Advantages and Problems	99
	References	100
5	Multi-miRNA Hairpins and Multi-miRNA Mimics Technologies	101
5.1	Introduction	101
5.2	Protocols	102
5.2.1	Construction of Multi-miRNA Hairpins	102
5.2.2	Construction of Multi-miRNA Mimics (Chen et al. 2009) ..	105
5.3	Principle of Actions	107
5.4	Applications	107
5.5	Advantages and Limitations	108
	References	109
6	miRNA Transgene Technology	111
6.1	Introduction	111
6.2	Protocols	112
6.2.1	Conventional Transgene Methods	112
6.2.2	Artificial Intronic miRNA Methods	115
6.2.3	Cre-loxP Knock-in Methods	120
6.3	Principle of Actions	120
6.3.1	Conventional Transgene Methods	121
6.3.2	Artificial Intronic miRNA Methods	121

6.4	Applications	122
6.5	Advantages and Limitations	123
	References	125
7	Anti-miRNA Antisense Oligonucleotides Technology	127
7.1	Introduction	128
7.2	Protocols	129
7.2.1	Designing AMOs	129
7.2.2	Modifying AMOs	131
7.2.3	Monitoring Delivery Efficiency of AMOs	133
7.2.4	Evaluating Functional Effectiveness of AMOs	134
7.3	Principle of Actions	135
7.4	Applications	136
7.5	Advantages and Limitations	138
	References	140
8	Multiple-Target Anti-miRNA Antisense Oligonucleotides Technology	145
8.1	Introduction	145
8.2	Protocols	147
8.2.1	Designing MT-AMOs	147
8.2.2	Validating MT-AMOs	147
8.3	Principle of Actions	148
8.4	Applications	149
8.5	Advantages and Limitations	149
	References	150
9	miRNA Sponge Technology	153
9.1	Introduction	153
9.2	Protocols	154
9.2.1	Designing miRNA Sponges	154
9.2.2	Validating miRNA Sponges	156
9.3	Principle of Actions	157
9.4	Applications	157
9.5	Advantages and Problems	158
	References	158
10	miRNA-Masking Antisense Oligonucleotides Technology	161
10.1	Introduction	161
10.2	Protocols	162
10.3	Principle of Actions	163
10.4	Applications	164
10.5	Advantages and Limitations	166
	References	166

11	Sponge miR-Mask Technology	167
11.1	Introduction	167
11.2	Protocols	168
11.3	Principle of Actions	169
11.4	Applications	172
11.5	Advantages and Limitations	172
	References	173
12	miRNA Knockout Technology	175
12.1	Introduction	175
12.2	Protocols	176
12.2.1	Homologous Recombination Methods	176
12.2.2	Cre-loxP Methods	177
12.2.3	FLP-FRT Deletion Methods	177
12.3	Principle of Actions	179
12.4	Applications	180
12.5	Advantages and Limitations	181
	References	182
13	Dicer Inactivation Technology	183
13.1	Introduction	183
13.2	Protocols	184
13.2.1	Neomycin-Expression Cassette Methods (Homologous Recombination)	184
13.2.2	Cre-loxP Methods	185
13.3	Principle of Actions	185
13.4	Applications	185
13.5	Advantages and Limitations	187
	References	188
	Index	191