

# Contents

<b>1</b>	<b>miRNAs Targeting and Targeting miRNAs</b> .....	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
<b>2</b>	<b>miRNA Interference Technologies: An Overview</b> .....	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
<b>3</b>	<b>Synthetic Canonical miRNA Technology .....</b>	<b>75</b>
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
<b>4</b>	<b>miRNA Mimic Technology .....</b>	<b>93</b>
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
<b>5</b>	<b>Multi-miRNA Hairpins and Multi-miRNA Mimics Technologies .....</b>	<b>101</b>
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
<b>6</b>	<b>miRNA Transgene Technology .....</b>	<b>111</b>
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
<b>7</b>	<b>Anti-miRNA Antisense Oligonucleotides Technology</b>	<b>127</b>
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
<b>8</b>	<b>Multiple-Target Anti-miRNA Antisense Oligonucleotides Technology</b>	<b>145</b>
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
<b>9</b>	<b>miRNA Sponge Technology</b>	<b>153</b>
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
<b>10</b>	<b>miRNA-Masking Antisense Oligonucleotides Technology</b>	<b>161</b>
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

<b>11</b>	<b>Sponge miR-Mask Technology</b> .....	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
<b>12</b>	<b>miRNA Knockout Technology</b> .....	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
<b>13</b>	<b>Dicer Inactivation Technology</b> .....	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
	<b>Index</b> .....	191