

CONTENTS

1. The Retroelements	1
<i>Clague P. Hodgson</i>	
Introduction	1
The RNA World	2
The Evolution of Retroelements	3
Retroelements in the Expression of Eukaryotic Genes	6
The C Value Paradox, the Genomic Buffer Hypothesis, and Exon Mobility	7
Description of the Retroelements	9
LTR-Containing Elements: Retrotransposons and Retroviruses	17
VL30-Like Elements	20
2. Retrovirus-Derived Vectors	25
<i>Clague P. Hodgson</i>	
Historical Background	25
Retroviral Life Cycle	27
CIS- and TRANS-Acting Elements	33
Overcoming the Limitations of Retroviral Vectors	35
Classical Retroviral Vectors	36
Modes of Expression of Genes Introduced into Cells by Retroviral Vectors	38
Transcription from the LTR Promoter	38
Expression from Internal Transcriptional Promoters	38
Alternative Splicing in Transcript Selection	40
Internal Ribosome Entry Sites	41
Self-Inactivating Vectors	41
Alternative LTR Promoters	42
Selectable Markers	43
Reporter Genes	46
3. Retroviral Helper Cells	53
<i>Clague P. Hodgson</i>	
Introduction	53
Retroviral Host Range	55
Cell Targeting	57
Available Helper Cell Lines and Their Attributes	57
Testing of Vector Producer Cells (VPCs) for Use in Gene Therapy	60
Vectoring Techniques Using Retroviral Helper Cells	62
Safety Issues Related to Retroviral Vectoring	65
Improving Retroviral Delivery Systems	67
Optimizing Transduction Efficiency	68

4. Structure and Function of Mouse VL30 Sequences.....	73
<i>Clague P. Hodgson, Mary Ann Zink, Guoping Xu</i>	
Introduction	73
Mouse VL30 Sequences	74
The Structure of Mouse VL30 Elements	75
LTR Structure.....	77
Internal Structure of VL30 Elements	79
VL30 RNA Secondary Structure	89
Promoter Methylation and Inactivation of Expression In Vivo	91
A Plethora of LTR Transcriptional Controls	92
5. Construction, Transmission and Expression of Synthetic VL30 Vectors	103
<i>Clague P. Hodgson, Asit K. Chakraborty, Mary Ann Zink, Guoping Xu</i>	
Introduction	103
Synthetic Retrotransposon Vectors for Gene Therapy	104
Gene Insertion into the Avian Embryo Via VL30 Vectors.....	113
VL30 Vectors Appear Safe	114
Transmission of Endogenous VL30 Elements	
During Gene Therapy with Murine Vector Producer Cells	114
Expression of Synthetic Vectors in Human Cells	
That Are Targets for Gene Therapy	119
VL30 Vector RNA Expression in Transformed Cells	122
6. Future Vector Systems	129
<i>Clague P. Hodgson and Fauzia A. Solaiman</i>	
Introduction	129
Starting with a Virus	131
Starting with Synthetic Materials	136
Targeting	138
Cell Targeting Ex Vivo	138
Cell Targeting In Vivo	139
Homologous Vs. Random Integration: Positional Targeting	140
Controlling Transcription	141
Index	147