

The Development of HIV-1 Derived Gene Transfer Technology: Optimisation of Vector Safety, Processing and Production

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Submitted July 2007

Accepted December 2007

Contents

The Development of HIV-1 Derived Gene Transfer Technology: Optimisation of Vector Safety, Processing and Production	i
List of Tables	xiii
Abbreviations	xiv
Abstract	xvii
Declaration	xix
Acknowledgments	xx
1 Introduction	1
1.1 Genetics and Disease	1
1.2 Gene Therapy	1
1.3 Viruses as Gene Therapy Vectors	2
1.3.1 Adenovirus.....	2
1.3.2 Adeno-associated Virus.....	3
1.3.3 Retroviruses	4
1.3.3.1 Oncoretroviruses	5
1.3.3.2 Lentiviruses	9
1.3.3.3 Spumaviruses.....	11
1.3.4 Herpes Simplex Virus-1	12
1.3.5 Hybrid vectors.....	13
1.3.6 Synthetic Viruses	13
1.3.7 Non-viral Gene Therapy Vectors	14
1.3.8 Safety Issues Associated with Gene Therapy.....	15

1.4	Biology of HIV-1.....	16
1.4.1	Protein Complement and Structure	16
1.4.1.1	Gag.....	16
1.4.1.2	Gagpol.....	16
1.4.1.3	Env.....	19
1.4.1.4	Accessory Proteins.....	19
1.4.2	Genomic Structure	20
1.4.2.1	Trans-acting Elements.....	20
1.4.2.2	Cis-acting Elements	20
1.4.3	HIV-1 Replication.....	24
1.5	The Utilisation of HIV-1 as a Gene Therapy Vector	34
1.5.1	Overview of Vector Development.....	34
1.5.2	The Production of Clinically Relevant Amounts of Gene Therapy Vectors	38
1.5.2.1	Transient Versus Stable Production	38
1.5.2.2	Large Scale LV Purification	42
1.5.2.2.1	Ultrafiltration.....	43
1.5.2.2.2	Anion Exchange Chromatography.....	43
1.5.2.2.3	Size Exclusion Chromatography	44
1.5.2.2.4	Other Methods.....	44
1.6	WCH HIV-1 vector system	46
1.6.1	The Helper Plasmids	46
1.6.2	The Transfer Vector	48
1.6.3	Development Direction.....	51
1.7	Specific Aims of Research	51
1.7.1	Part One.....	51
1.7.2	Part Two	52
1.7.3	Part Three	53
2	Materials and Methods	54

2.1	Materials.....	54
2.1.1	Reagents, Chemicals, Kits and Plasticware	54
2.1.2	Buffers and solutions.....	59
2.1.3	Bacterial Strains and Media.....	60
2.1.4	Cell lines and cell culture products	61
2.1.4.1	Cell lines.....	61
2.1.4.2	Materials.....	61
2.1.5	Real Time PCR plastics and reagents.....	62
2.1.6	Plasmids.....	63
2.1.7	DNA oligonucleotides.....	64
2.2	Methods	69
2.2.1	Cell Culture.....	69
2.2.2	Polymerase Chain Reaction	69
2.2.2.1	PCR Mediated Mutagenesis of Splice Donor Sites.....	70
2.2.2.2	PCR mediated Oligonucleotide Assembly.....	70
2.2.2.3	PCR of Gene Sequences.....	73
2.2.2.3.1	gag.....	73
2.2.2.3.2	cPPT Modifications.....	73
2.2.2.3.3	5' U3 modifications.....	74
2.2.2.3.4	Stable Cell Line Vectors	74
2.2.3	Agarose Gel Electrophoresis.....	75
2.2.4	Phosphorylation of Oligonucleotides	75
2.2.5	Plasmid Vector Preparation	76
2.2.6	DNA Polymerase I (Klenow) Treatment of DNA.....	76
2.2.7	Phenol/chloroform Extraction and Ethanol Precipitation	76
2.2.8	Ligations.....	77
2.2.8.1	Strataclean of ligations.....	77
2.2.9	Preparation of Competent E.coli.....	77
2.2.9.1	Electrocompetent DH10 β E.coli	77
2.2.9.2	Chemically Competent MC1061 E.coli.....	78

2.2.9.3	Preparation of SURE [®] and Electro10blue [®] E.coli.....	78
2.2.10	Transformation of E.coli.....	79
2.2.10.1	Electroporation.....	79
2.2.10.2	Transfection.....	79
2.2.11	Plasmid Purification.....	79
2.2.11.1	Small scale.....	79
2.2.11.2	Large scale.....	80
2.2.12	DNA Sequencing.....	80
2.2.13	RNA Analysis.....	81
2.2.13.1	Total Cellular RNA Isolation.....	81
2.2.13.2	Cytoplasmic and Nuclear RNA Isolation.....	81
2.2.13.3	Analysis of Spliced Transcripts.....	82
2.2.14	Virus Production.....	82
2.2.14.1	Small Scale.....	82
2.2.14.2	Large Scale.....	83
2.2.15	Virus Concentration and Purification.....	84
2.2.16	Virus Assay.....	85
2.2.17	FACScan Analysis.....	86
2.2.18	Real Time PCR to Determine Virus Titre.....	86
2.2.18.1	Sample Preparation.....	86
2.2.18.2	Virus Titre Assay.....	86
2.2.19	Marker Rescue Assay.....	87
2.2.19.1	Virus Assay for Neomycin Resistant Virus.....	88
2.2.19.2	Analysis of 5' LTR status in marker rescue assay colonies.....	89
2.2.20	p24 Assay.....	89
2.2.21	Total Protein Assay.....	90
2.2.22	Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis Gels/Western Blots.....	90
2.2.22.1	Sodium Deoxycholate/Tri-Citric Acid Precipitation.....	90
2.2.22.2	SDS-PAGE Gels.....	90
2.2.22.3	Western Blot.....	91

2.2.23	Bovine Serum Albumin (BSA) Enzyme Linked Immuno-Sorbent Assay (ELISA)	92
2.2.24	Double Stranded DNA assay.....	92
2.2.25	Anion Exchange Chromatography.....	93
2.2.25.1	Toyopearl 650C	93
2.2.25.2	HiTrap Columns.....	93
2.2.25.3	MustangQ, UnoQ-1 and Sartobind Q Capsules	94
2.2.25.4	Fast Protein Liquid Chromatography	94
2.2.26	Chloride Concentration Measurement	95
2.2.27	Statistical Analysis	95
3	Establishment of Assay conditions	96
3.1	Production of Virus.....	96
3.2	Virus Assay.....	99
3.3	Titration of Vector Amount per Transfection.....	99
3.4	Real Time PCR to Determine Virus Titre	101
3.5	Marker Rescue Assay.....	106
4	Vector Development	112
4.1	Splice Site Mutation	112
4.2	The Mapping of an Inhibitory Sequence in gag	115
4.2.1	5' End of the Inhibitory Sequence.....	118
4.2.2	3' End of the Inhibitory Sequence.....	118
4.3	Modifications to the cPPT	123
4.4	Deletion of the RRE.....	128
4.5	Modifications to the 3' LTR.....	133
4.6	Modifications to the 5' LTR.....	146
4.6.1	5' U3	146

4.6.2	5' Polyadenylation Signal.....	152
4.6.3	Detailed Analysis of pRK2.....	161
4.7	Discussion	168
4.7.1	Mutation of the Splice Donor Sites	170
4.7.2	The Mapping of an Inhibitory Sequence in gag.....	171
4.7.3	Modifications to the cPPT	172
4.7.4	Deletion of the RRE	172
4.7.5	Modifications to the 3' LTR.....	173
4.7.6	Modifications to the 5' LTR.....	175
5	Large Scale Virus Production and Processing	178
5.1	Production of Virus on a Large Scale.....	178
5.2	Processing of 3-4 L of Virus Supernatant by Ultrafiltration and Ultracentrifugation....	181
5.3	Improvement in Virus Production.....	184
5.3.1	Culture of 293T Cells in DMEM/5 % FCS.....	184
5.3.2	Culture Media Supplements.....	187
5.4	Anion Exchange Chromatography	190
5.4.1	Toyopearl 650C	190
5.4.2	HiTrap™ Columns.....	198
5.4.3	Other Anion Exchange Devices	212
5.5	Discussion	219
5.5.1	Large Scale Virus Production	219
5.5.2	Large Scale Virus Processing	220
5.5.3	Culture Media Supplements.....	221
5.5.4	Anion Exchange Chromatography	222
5.5.4.1	Toyopearl 650C	222
5.5.4.2	HiTrap™ Columns.....	223

5.5.4.3	Other Anion Exchange Devices	224
6	Production of a Stable Virus Producing Cell Line	228
6.1	Helper Functions	228
6.1.1	pBCKSTREgagpollstml	228
6.1.2	pBCKSTREvsv-g	229
6.1.3	Expression from pBCKSTREvsv-g and pBCKSTREgagpollstml	229
6.1.4	pBCKStatzeo	232
6.1.5	pBCKSpgkrevbGH	234
6.1.6	pSMARTheelper	236
6.1.7	Expression of pSMARTheelper	236
6.2	Transfer Vector	242
6.3	Discussion	245
6.3.1	Helper Functions	245
6.3.2	The Transfer Vector	247
6.3.3	Other Considerations for the Production of a Stable Virus Producing Cell Line	248
7	Concluding Remarks	252
8	References	255

List of Figures

<i>Figure 1.1- Overview of Retroviral Replication</i>	6
<i>Figure 1.2 - Structure of a mature HIV-1 virion</i>	17
<i>Figure 1.3 - HIV-1 polyprotein processing</i>	18
<i>Figure 1.4 - Genomic Structure and Transcription products of HIV-1</i>	21
<i>Figure 1.5 - HIV-1 cis-acting Sequences</i>	23
<i>Figure 1.6 - Conversion of the single stranded RNA genome to a double stranded DNA intermediate</i>	26
<i>Figure 1.7 - Upregulation of RNA Polymerase II</i>	30
<i>Figure 1.8 - The Export of RRE containing mRNA from the nucleus</i>	31
<i>Figure 1.9 - The helper plasmids used for the development of in vivo gene therapy protocols</i>	47
<i>Figure 1.10 - Structure of the HIV-1 transfer vector</i>	49
<i>Figure 2.1- PCR mediated mutagenesis</i>	71
<i>Figure 2.2 - PCR mediated oligonucleotide assembly</i>	72
<i>Figure 3.1 – Virus production in 6 and 12 well plate formats</i>	97
<i>Figure 3.2 – Comparison of 6 and 12 well virus production</i>	98
<i>Figure 3.3 – Comparison of 12 and 24 well virus assay</i>	100
<i>Figure 3.4 – Titration of vector amount per transfection</i>	102
<i>Figure 3.5 – Relationship between IU added to NIH3T3 cells as determined by FACScan and Real Time PCR</i>	107
<i>Figure 3.6 – Relationship between IU added to A549 cells as determined by FACScan and Real Time PCR</i> ...	108
<i>Figure 4.1 – Cloning of pHIV1SDm2.1</i>	113
<i>Figure 4.2 – Effect of Splice Site Mutation on Virus Titre</i>	114
<i>Figure 4.3 – Analysis of splicing patterns in splice site mutation vectors</i>	116
<i>Figure 4.4 - Titre analysis to examine the requirement for gag within pHIV1SDm2+3bp</i>	117
<i>Figure 4.5 – Cloning of 5' mapping of gag inhibitory sequence vectors</i>	119
<i>Figure 4.6 – Effect of gag length on virus titre</i>	120
<i>Figure 4.7 – Schematic of the gag sequences to be analysed to determine the 3' end of the inhibitory sequence</i>	121
<i>Figure 4.8 – Three part ligation strategy used to isolate pHIV1SDm2+3bpg325-450 and pHIV1SDm2+3bpg325-525</i>	122

<i>Figure 4.9 – Second cloning strategy used to create vectors for the mapping of the 3'end of the inhibitory element in gag.....</i>	<i>124</i>
<i>Figure 4.10 – Mapping of 3'end of gag inhibitory sequence.....</i>	<i>125</i>
<i>Figure 4.11 – Construction of pHIV1SDm2cppt.....</i>	<i>126</i>
<i>Figure 4.12 – Effect of duplicate cPPT on virus titre.....</i>	<i>127</i>
<i>Figure 4.13 – Construction of pRK1extcppt.....</i>	<i>129</i>
<i>Figure 4.14 – Effect of extended cPPT sequence on viral titre.....</i>	<i>130</i>
<i>Figure 4.15 – Cloning of RRE deleted vectors</i>	<i>131</i>
<i>Figure 4.16 – Comparison of RRE containing and RRE deleted vectors for virus titre.....</i>	<i>132</i>
<i>Figure 4.17 – Modifications to the 3'LTR.....</i>	<i>135</i>
<i>Figure 4.18 – Cloning of pHIV1SDm2+3bpINT19.....</i>	<i>136</i>
<i>Figure 4.19 – Effect of 3'U3 deletions on virus titre.....</i>	<i>137</i>
<i>Figure 4.20 – Comparison of 12 and 19 bases of 3'U3 in pHIV1SDm2bGH and pHIV1SDm2SV40.....</i>	<i>138</i>
<i>Figure 4.21 – The construction of pHIV1SDm2g325-400SV40INT19</i>	<i>141</i>
<i>Figure 4.22 – Effect of combination gag and 3'U3 modifications on virus titre.....</i>	<i>142</i>
<i>Figure 4.23 – Examination of colonies isolated from the marker rescue assay of pRK1neo, pHIV1SDmneo and pHIV1SDm2neoLTR.....</i>	<i>145</i>
<i>Figure 4.24 – Schematic showing the deletions to the modulatory region of 5'U3.....</i>	<i>147</i>
<i>Figure 4.25 – Three part ligation strategy used to incorporate deletions into 5'U3</i>	<i>148</i>
<i>Figure 4.26 – Second strategy used to create 5'U3 deletion vectors</i>	<i>150</i>
<i>Figure 4.27 – Effect of 5'U3 deletions on virus titre.....</i>	<i>151</i>
<i>Figure 4.28 – Insertion of the SV40 pA signal into the 5' LTR.....</i>	<i>153</i>
<i>Figure 4.29 – Effect of combination 5'U3 and 5'SV40 modifications.....</i>	<i>154</i>
<i>Figure 4.30 – Cloning of pRK1bGH</i>	<i>156</i>
<i>Figure 4.31 – Cloning of pRK1bGHDSE</i>	<i>157</i>
<i>Figure 4.32 – Effect of Insertion of 5' bGH pA Signal Insertion on Virus Titre.....</i>	<i>158</i>
<i>Figure 4.33 - Transfer of 5' bGH into 5'U3 deletion vectors.....</i>	<i>159</i>
<i>Figure 4.34 - Effect of combination 5' U3 and 5' bGH modifications on virus titres.....</i>	<i>160</i>
<i>Figure 4.35 – Comparison of pHIV1SDm2 and pRK2.....</i>	<i>162</i>
<i>Figure 4.36 – Titration of pHIV1SDm and pRK2</i>	<i>164</i>

<i>Figure 4.37 – Schematic of pA signal readthrough assay</i>	166
<i>Figure 4.38 – Cloning of pRK2prt</i>	167
<i>Figure 5.1 – Production of LV in 2 layer cell factories</i>	180
<i>Figure 5.2 – Large scale processing by ultrafiltration and ultracentrifugation</i>	183
<i>Figure 5.3 – SDS-PAGE of virus processing samples</i>	185
<i>Figure 5.4 – Western Blot of virus processing samples</i>	186
<i>Figure 5.5 – Effect of supplement addition on virus titre</i>	188
<i>Figure 5.6 – Effect of Glutamine Concentration of Virus Titres (n=3). The glutamine concentration in both the plating DMEM/5 % FCS and collecting OptiPro SFM were varied to determine its effect on virus titre</i>	189
<i>Figure 5.7 – Effect of NaCl concentration on virus titre</i>	191
<i>Figure 5.8 – Anion exchange chromatography using viral supernatants collected in DMEM/10 % FCS or OptiPro SFM</i>	194
<i>Figure 5.9 – Anion Exchange Chromatography using Toyopearl 650C</i>	195
<i>Figure 5.10 – Analysis of protein concentration during virus purification using Toyopearl 650C</i>	196
<i>Figure 5.11 – Capacity of Toyopearl 650C</i>	197
<i>Figure 5.12 – FPLC using a Toyopearl 650C column</i>	199
<i>Figure 5.13 – Virus recovery from different anion exchange resins</i>	201
<i>Figure 5.14 – Recovery of virus applied to QFF and QXL columns</i>	202
<i>Figure 5.15 – Capacity of QFF column</i>	203
<i>Figure 5.16 – 20 mL LV applied to QFF column</i>	205
<i>Figure 5.17 – 15 mL LV applied to a 1 mL QFF column</i>	206
<i>Figure 5.18 – Analysis of protein composition of virus peaks</i>	208
<i>Figure 5.19 - 10 mL LV applied to a 1 mL QFF column</i>	209
<i>Figure 5.20 – Percentage recovery of virus when applied using a syringe to a 1 mL QFF column</i>	211
<i>Figure 5.21 – Elution profile of 10 mL LV applied to a QFF column and eluted using a step gradient</i>	213
<i>Figure 5.22 – Virus recovery with different anion exchange devices</i>	215
<i>Figure 5.23 – Elution profile of 40 mL LV applied to a MustangQ membrane and eluted with a gradient of 0-2 M NaCl in PBS</i>	216
<i>Figure 5.24 – Detailed analysis of run 1 of 40 mL LV applied to a MustangQ membrane and eluted with a gradient of 0-2 M NaCl in PBS over 20 mL</i>	217

<i>Figure 6.1 – Cloning strategy used to make pBCKSTREgagpollstml</i>	230
<i>Figure 6.2 - Cloning strategy used to make pBCKSTREsv-g</i>	231
<i>Figure 6.3 – Testing of pBCKSTREsv-g and pBCKSTREgagpollstml</i>	233
<i>Figure 6.4 – Cloning strategy used to construct pBCKStatzeo</i>	235
<i>Figure 6.5 – Cloning strategy used to construct pBCKSpkrevbGH</i>	237
<i>Figure 6.6 – Structure of pSMARThelper</i>	238
<i>Figure 6.7 – Testing of pSMARThelper</i>	240
<i>Figure 6.8 – Testing of pBCKStatzeo and pBCKSpkrevbGH</i>	241
<i>Figure 6.9 – Cloning of pFPRK2</i>	243
<i>Figure 6.10 – Transient expression of pFPRK2</i>	244

List of Tables

<i>Table 2.1 - Primer combinations used to construct various splice site mutations</i>	<i>70</i>
<i>Table 2.2 - Primer combinations used to produce various 5' ΔU3 fragments.....</i>	<i>74</i>
<i>Table 2.3 - Template and primer combinations used to amplify sequences required for the construction of the stable cell line vectors.....</i>	<i>75</i>
<i>Table 2.4 - Amount of reagents used for virus production in 6 and 12 well plates</i>	<i>83</i>
<i>Table 2.5 – Amount of reagents used for virus production in 150 mm plate, 245 mm square plate and 2 layer cell factory.....</i>	<i>84</i>
<i>Table 3.1 – Sequence of the probe and primers of the Real Time PCR Assays designed by Applied Biosystems</i>	<i>104</i>
<i>Table 3.2– Trial of marker rescue assay.....</i>	<i>110</i>
<i>Table 4.1– Marker rescue assay of vectors containing various 3' LTR modifications.....</i>	<i>140</i>
<i>Table 4.2 – Marker rescue assay of pRK1neo, pHIV1SDmneo and pHIV1SDmneoLTR</i>	<i>144</i>
<i>Table 4.3 – The sequence of the primers and probe used for the detection of pA signal readthrough by real time PCR.....</i>	<i>165</i>
<i>Table 4.4 – Rate of pA signal readthrough in pHIV1SDm, pRK2 and pRK2prt</i>	<i>169</i>
<i>Table 5.1 – Summary of the different ultrafiltration concentrated virus preparations used to examine the anion exchange devices.....</i>	<i>192</i>
<i>Table 5.2 – Summary of the similarities and differences between QFF and MustangQ.....</i>	<i>226</i>

Abbreviations

ψ	Encapsidation Signal
AAV	Adeno-associated Virus
Ad	Adenovirus
ADA	Adenosine Deaminase Deficiency
ANOVA	Analysis of Variance
AP-1	Activator Protein-1
APOBEC3	Alipoprotein B Editing Complex-3
ARP-1	Alipoprotein Regulatory Protein-1
ATCC	American Type Culture Collection
AXN	AXN Sepharose™ Fast Flow
bGH	bovine Growth Hormone
BSA	Bovine Serum Albumin
CA	Capsid Protein
cppt	Central Polypurine Tract
CRS	<i>cis</i> -Repressive Sequence
DEAE	DEAE Sepharose™ 4 Fast Flow
DMEM	Dulbecco's Modified Eagle Medium
dsDNA	Double Stranded DNA
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
Ets	Ets Family of Transcription Factors
EYFP	Enhanced Yellow Fluorescent Protein
FCS	Foetal Calf Serum
FPLC	Fast Protein Liquid Chromatography
g325	325bp of <i>gag</i>
G418 sulphate	Geneticin
gDNA	Genomic DNA
GFP	Enhanced Green Fluorescent Protein
hAd	Helper Dependent Adenovirus
hCMV	Human Cytomegalovirus Promoter
HIV-1	Human Immunodeficiency Virus type-1

hPGK	Human Phosphoglycerate Kinase Promoter
HSV-1	Herpes Simplex Virus-1
IN	Integrase
INT	Integrase Binding Site
IU	Infectious Units
LEF	Lymphoid Enhancing Factor
LPC	Lysophosphatidylcholine
LTR	Long Terminal Repeat
LV	Lentivirus
MA	Matrix Protein
MHC-1	Major Histocompatibility Complex Class I
MoMLV	Moloney Murine Leukaemia Virus
NAPDH	Nicotinamide Dinucleotide Phosphate
NC	Nucleocapsid Protein
NFAT-1	Nuclear Factor of Activated T-cells-1
NF- κ B	Nuclear Factor κ B
NP40	Nonident® P40
pA	Polyadenylation
PB	Primer Binding Site
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PIC	Pre-integration Complex
PIPES	Piperazine-N,N'-bis[2-ethane sulfonic acid]
Polybrene	Hexadimethrin Bromide
PPT	3' Polypurine Tract
PR	Protease
QFF	Q Sepharose™ Fast Flow
QXL	Q Sepharose™ XL
RD114	Modified Cat Endogenous Retrovirus
RNAPII	RNA polymerase II
RRE	Rev Response Element
RT	Reverse Transcriptase
SA	Splice Acceptor

SD	Splice Donor
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis
SIN	Self Inactivating
SIV	Simian Immunodeficiency Virus
SP-1	Specificity Protein-1
SV40	Simian Virus 40
TAR	<i>Trans</i> -Acting Responsive RNA element
TCF-1	Transcription Factor-1
TTBS	0.05% Tween 20 in Tris Buffered Saline
USE	Upstream Element
USF-1	Upstream Stimulating Factor-1
VSV-G	Vesicular Stomatitis Virus glycoprotein G
whvpre	Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element
X-GCD	X-linked Chronic Granulomatous Disease
X-SCID	X-linked Severe Combined Immunodeficiency

Abstract

Vectors derived from Human Immunodeficiency Virus type 1 (HIV-1) are being widely developed for gene therapy applications, principally because they are able to transduce both dividing and non-dividing cells and result in stable, long term gene expression. However, these vectors are difficult to produce in high titres and sufficient volumes for large scale experiments and clinical application. Therefore, an investigation into methods to improve the production of HIV-1 derived gene transfer vectors was undertaken.

One factor that limits the production of recombinant virus is the amount of viral genomic RNA available for packaging into virions. Therefore, a transfer vector was modified with the aim of increasing the amount of genomic RNA produced. Substitution of the polyadenylation (pA) signal, mutation splice donor sites and removal of unnecessary sequences were all examined. pA signal readthrough was quantified to determine the effect of these modifications on the rate of pA signal readthrough.

Insertional mutagenesis and vector mobilisation are recognised risk factors with all integrating vectors. Self inactivating (SIN) vectors, which contain a deletion of U3 sequences in the 3' LTR, demonstrate a reduced rate of mobilisation. Transduction with these vectors results in a provirus containing no viral promoter elements, with transcription of the transgene being controlled from an internal promoter. However, LTR repair of SIN vectors occurs at an appreciable frequency. Therefore, the extent of this deletion was maximised and the effect on the frequency of the repair examined.

The production of lentiviral gene therapy vectors by large-scale transient transfection is both time consuming and technically difficult. Therefore, methods to increase the scale of production without compromising virus titre were developed. This resulted in fewer transfections and less handling of the cells when making virus on a large scale (3-4 L). In order to process the virus on this scale in a single day (i.e. 8 hours), new concentration and purification methods were established. The protocol consisted of low speed centrifugation, 0.45 μm filtration, 750 kDa ultrafiltration, 0.8 μm filtration and ultracentrifugation. However, the use of ultracentrifugation means that this protocol is not amenable to further scale up. Therefore, the replacement of the ultracentrifugation step with anion exchange was investigated. A number of different resins and anion exchange devices were investigated, two of which show promise for large scale purification of HIV-1 derived gene transfer vectors.

In an ideal world, HIV-1 derived gene transfer vectors would be produced using stable packaging cell lines engineered to produce the desired virus. However, previous attempts to produce such a cell line with the desired properties have had limited success and have generally used outdated helper systems. Therefore, in an attempt to combine the efficiency advantages of having a single helper plasmid with the safety advantages of expressing each protein separately, a single packaging construct that contained separate transcription units for each of the required proteins was produced. Transcription of cytotoxic proteins was controlled by inducible promoters. Initial results suggest that such a system is technically feasible but that further work is required to optimise the expression of helper functions.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to thesis copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

Rachel Marie Koldej

Acknowledgments

I would like to thank my supervisors Associate Professor Donald S Anson and Professor Keryn Williams for their guidance, support and patience throughout my PhD. You have always ensured that my work is of high quality.

To all the private donors who have supported my work through donations to the Adelaide Cystic Fibrosis Gene Therapy Group, thankyou for your continued belief and support of the work that the group is doing.

Thankyou to the University of Adelaide for providing me with the opportunity to undertake my PhD and supporting me with an Australian Postgraduate Award. I would also like to thank the members of the Department of Genetic Medicine, Women's and Children's Hospital, for providing me with a stimulating and supportive place to conduct my research.

To all the other members of the Adelaide Cystic Fibrosis Gene Therapy Group; Dr David Parsons, Alice Stocker, Karlea Kremer, Dr Julie Bielicki, Stanley Tan, Chantelle McIntyre, Edward Wong, Nocolle Woods, Patricia Cmielewski and Dr Chuanhe Lui. Thankyou for your support and critical analysis of my research.

First and foremost, thankyou to my family for their love and support. You have always encouraged me to follow my dreams and it is only with your support that I have managed to get this far.