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

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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	*cis*-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 *gag*
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-κB	Nuclear Factor κB
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	*Trans*-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 cyotoxic 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

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