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(54) **HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY**

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C12N 5/078	(2010.01)
A61P 31/18	(2006.01)
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(57) **ABSTRACT**

The present invention relates generally to immunization and immunotherapy for the treatment or prevention of HIV. In particular, the methods include in vivo and/or ex vivo enrichment of HIV-specific CD4+ T cells.

14 Claims, 40 Drawing Sheets

Specification includes a Sequence Listing.

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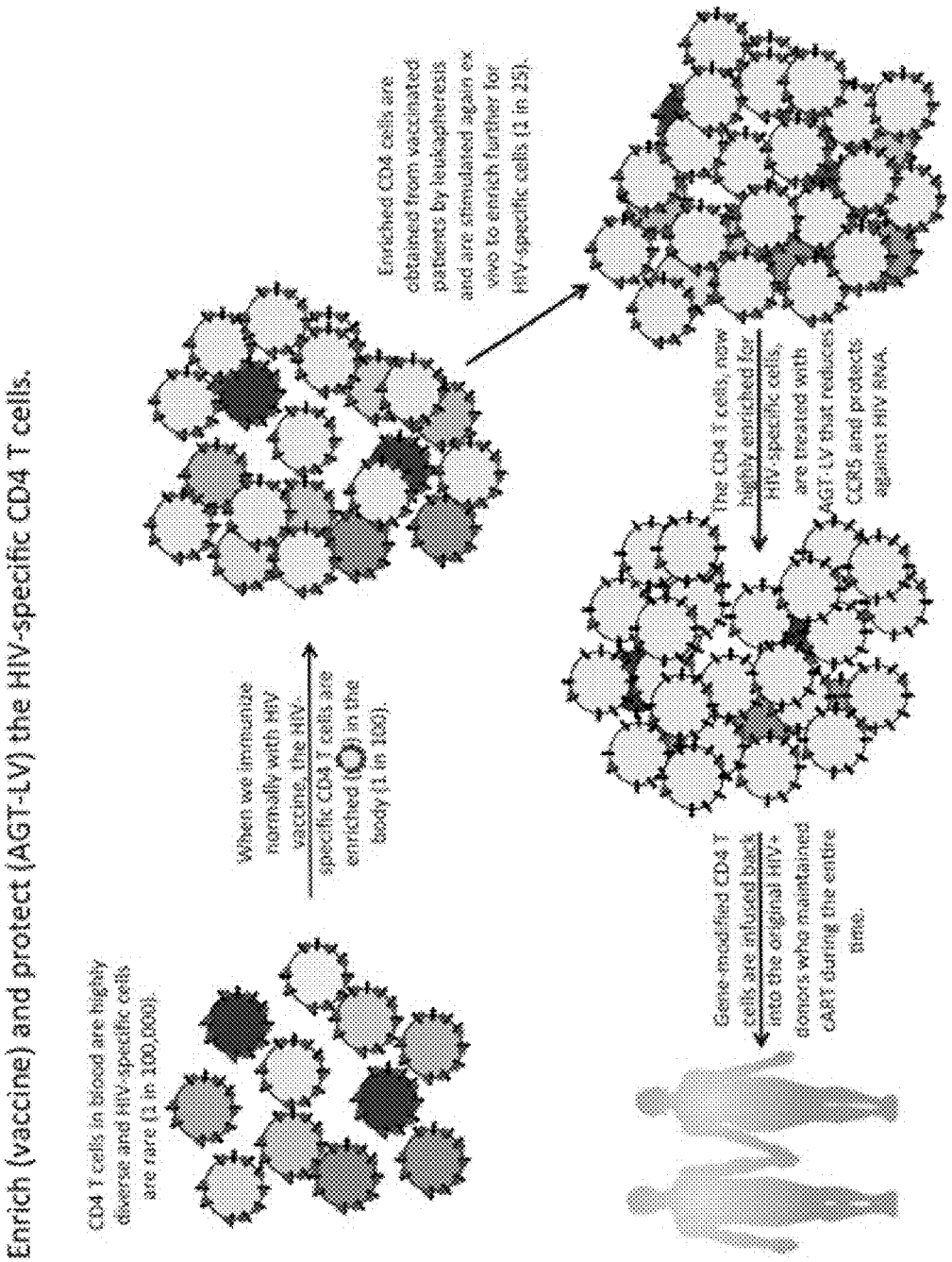
Enrich (vaccine) and protect (AGT-LV) the HIV-specific CD4 T cells.
CD4 T cells in blood are highly diverse and HIV-specific cells are rare (1 in 100,000).
When we immunize normally with HIV vaccine, the HIV-specific CD4 T cells are enriched (○) in the body (1 in 100).
Enriched CD4 cells are obtained from vaccinated patients by leukapheresis and are stimulated again ex vivo to enrich further for HIV-specific cells (1 in 25).
The CD4 T cells, now highly enriched for HIV-specific cells, are treated with AGT-LV that replaces CCR5 and protects against HIV RNA.
Gene-modified CD4 T cells are infused back into the original HIV+ donors who maintained CART during the entire time.

FIG. 1

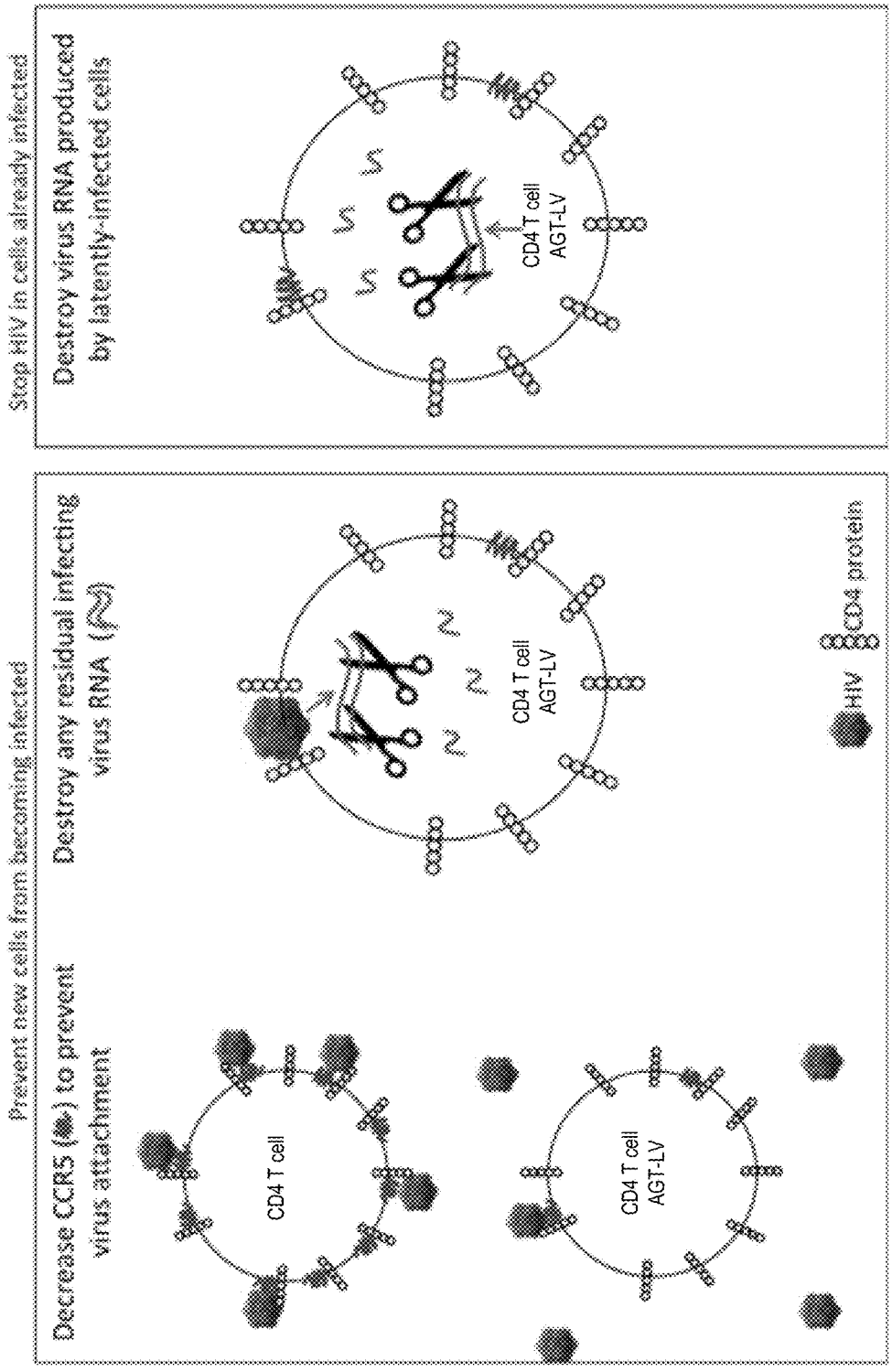
Prevent new cells from becoming infected
Decrease CCR5 (●) to prevent virus attachment
CD4 T cell
CD4 T cell AGT-LV
Destroy any residual infecting virus RNA (≈)
CD4 T cell AGT-LV
HIV
CD4 protein
Stop HIV in cells already infected
Destroy virus RNA produced by latently-infected cells
CD4 T cell AGT-LV

FIG. 2

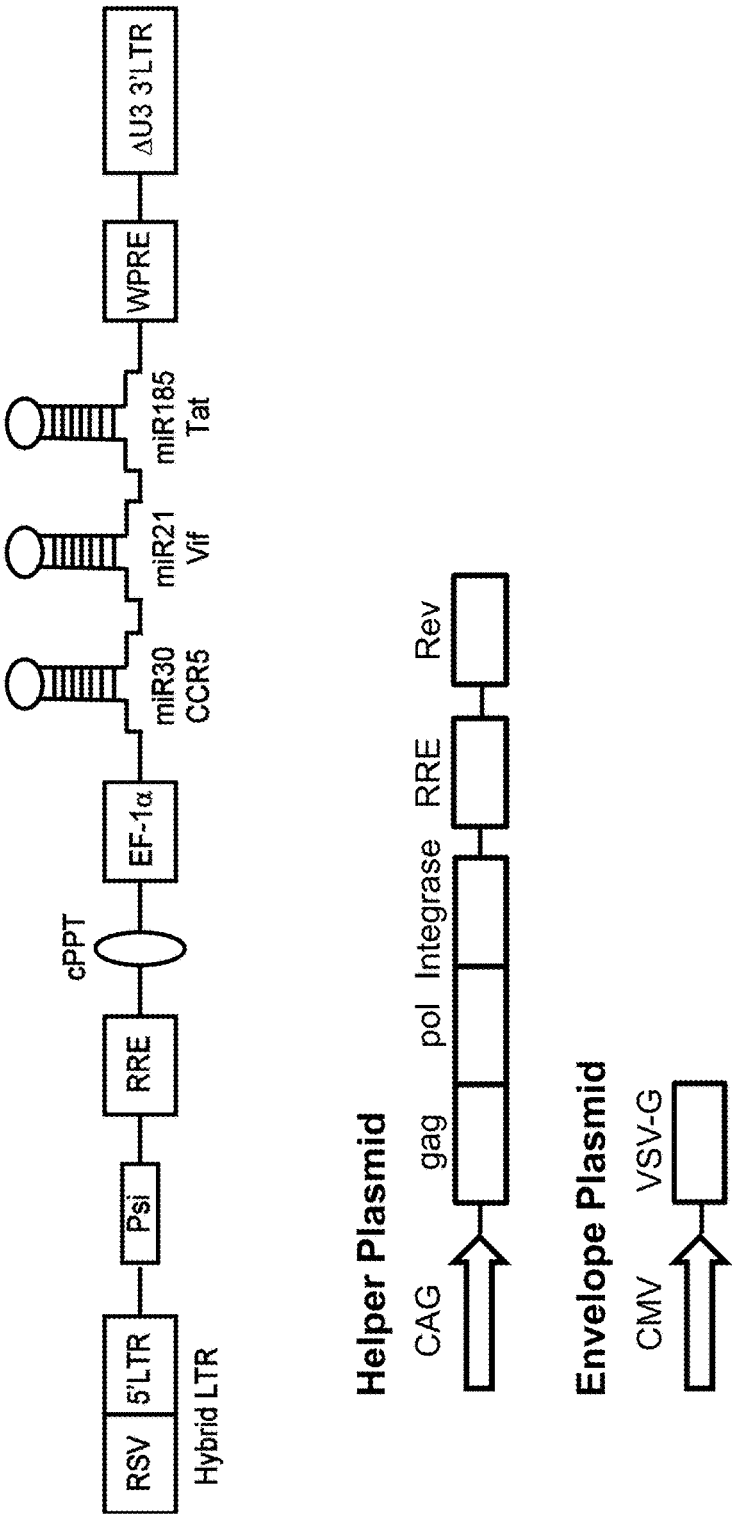
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
EF-1α
miR30
CCR5
miR21
Vif
miR185
Tat
WPRE
ΔU3 3'LTR
Helper Plasmid
CAG
gag
pol
Integrase
RRE
Rev
Envelope Plasmid
CMV
VSV-G

FIG. 3

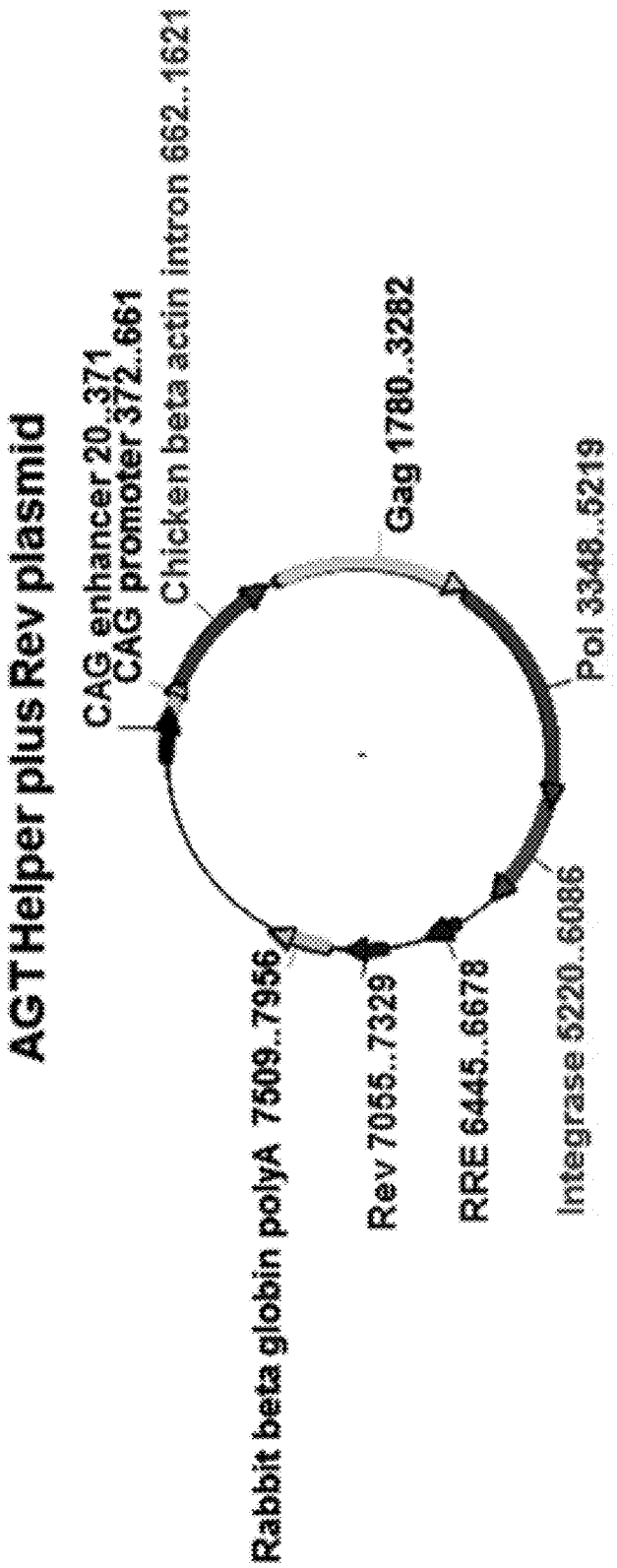
AGT Helper plus Rev plasmid
CAG enhancer 20..371
CAG promoter 372..661
Chicken beta actin intron 662..1621
Gag 1780..3282
Pol 3348..6219
Integrase 6220..6086
RRE 6445..6678
Rev 7055..7329
Rabbit beta globin polyA 7509..7956

FIG. 4A

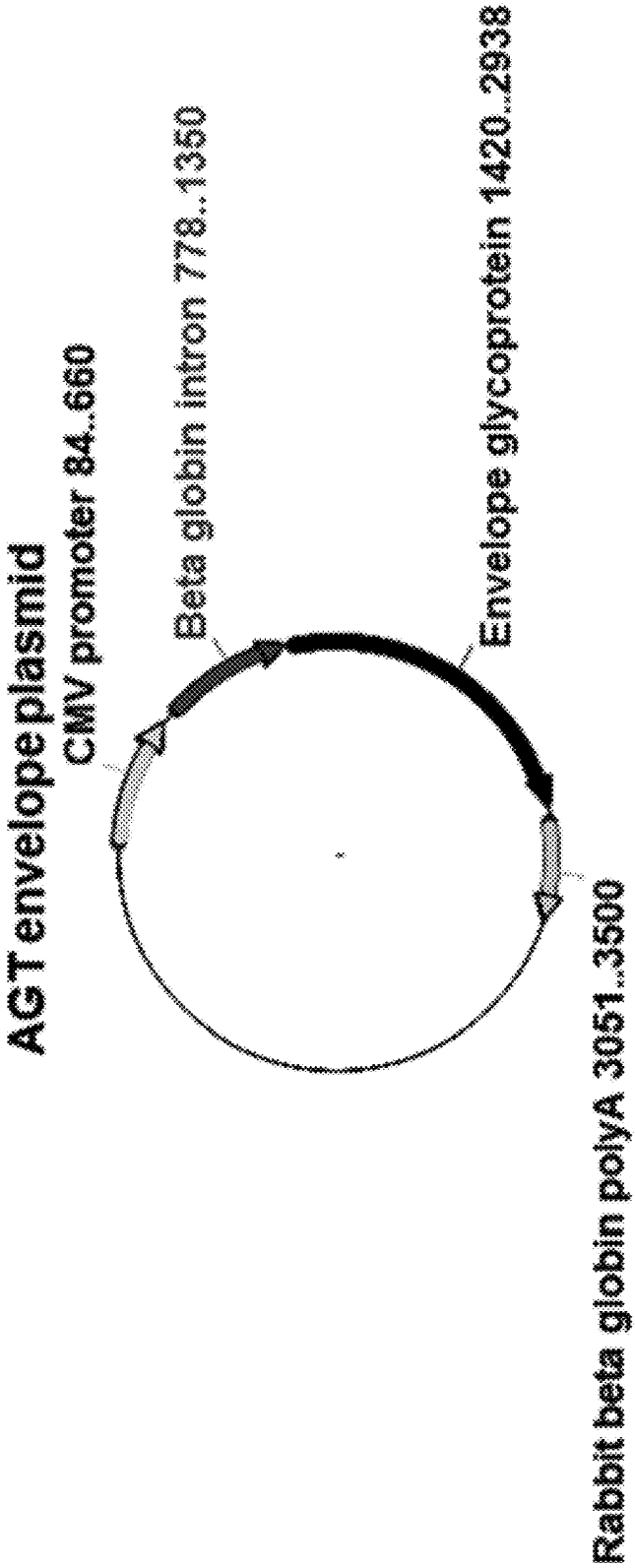
AGT envelope plasmid
CMV promoter 84..660
Beta globin intron 778..1350
Envelope glycoprotein 1420..2938
Rabbit beta globin polyA 3051..3500

FIG. 4B

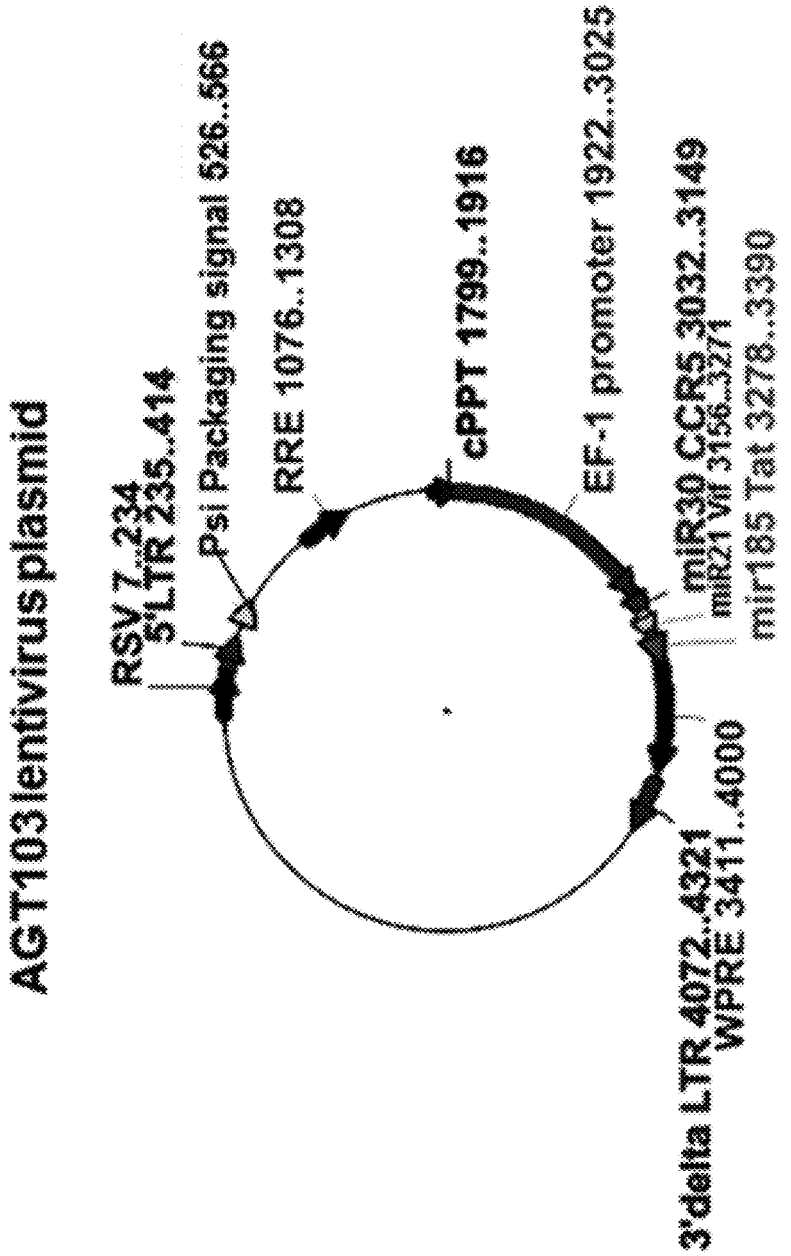
AGT103lentivirusplasmid
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
RRE 1076..1308
cPPT 1799..1916
EF-1 promoter 1922..3025
miR30 CCR5 3032..3149
miR21 Vif 3156..3271
mir185 Tat 3278..3390
3'delta LTR 4072..4321
WPRE 3411..4000

FIG. 4C

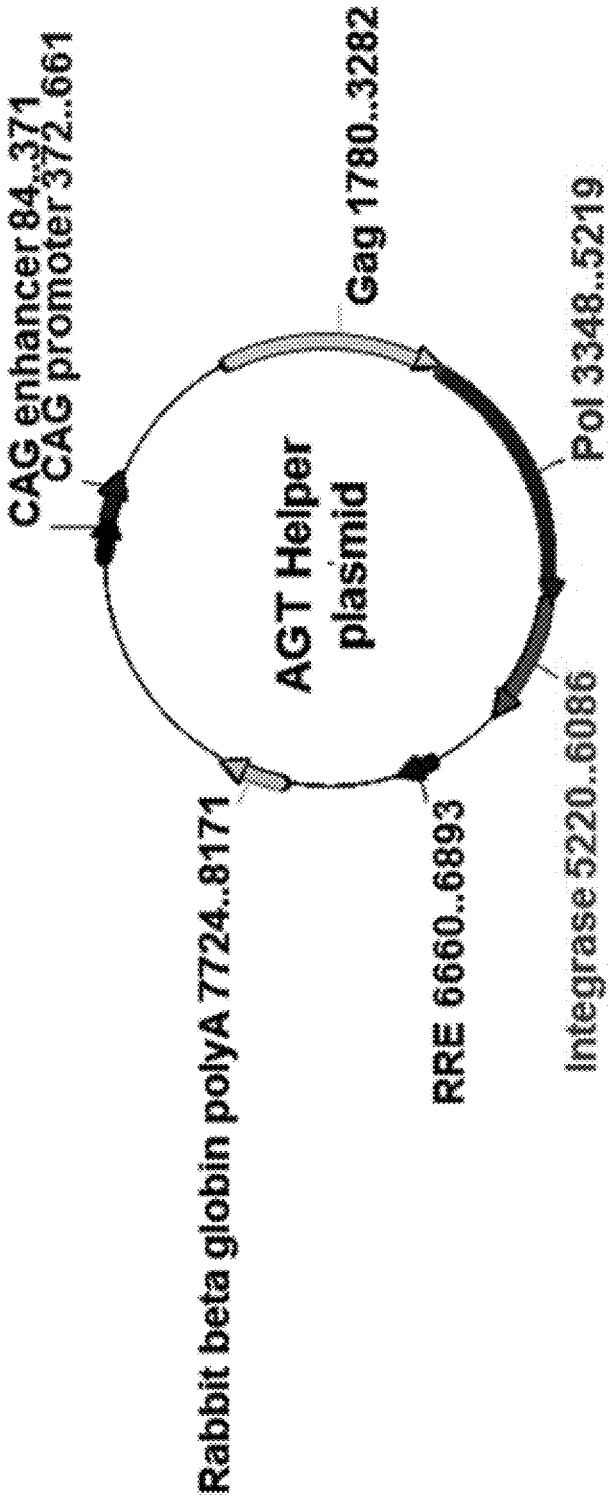
CAG enhancer 84..371
CAG promoter 372..661
Gag 1780..3282
Pol 3348..5219
Integrase 5220..6086
RRE 6660..6893
Rabbit beta globin polyA 7724..8171
AGT Helper plasmid

FIG. 5A

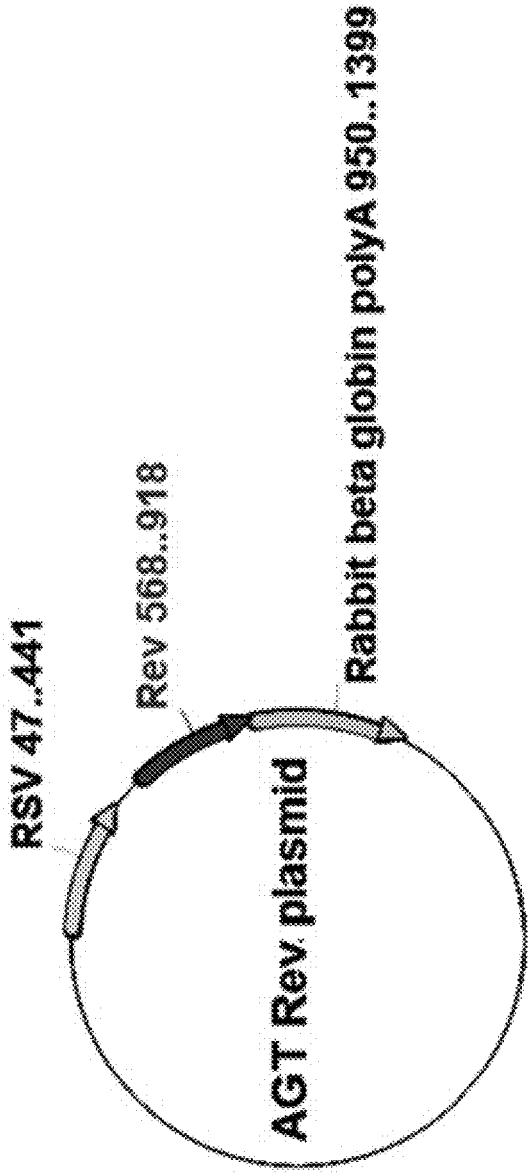
RSV 47..441
Rev 568..918
Rabbit beta globin polyA 950..1399
AGT Rev plasmid

FIG. 5B

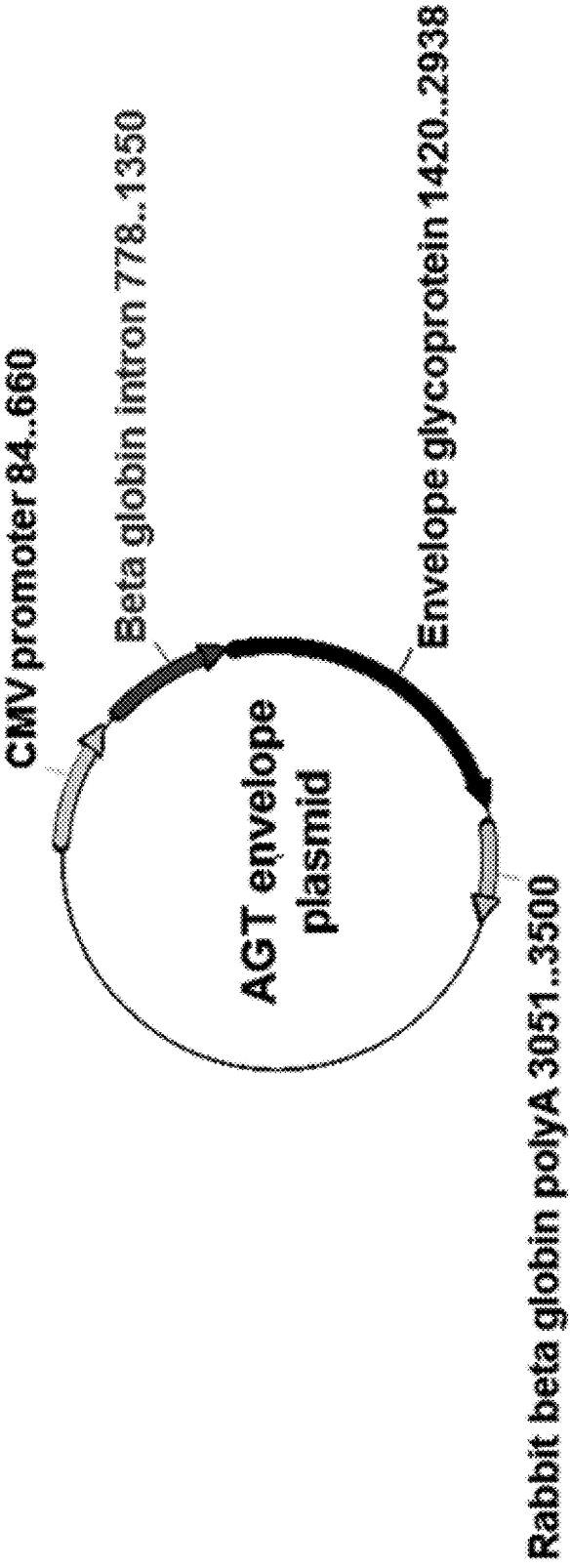
CMV promoter 84..660
Beta globin intron 778..1350
Envelope glycoprotein 1420..2938
AGT envelope plasmid
Rabbit beta globin polyA 3051..3500

FIG. 5C

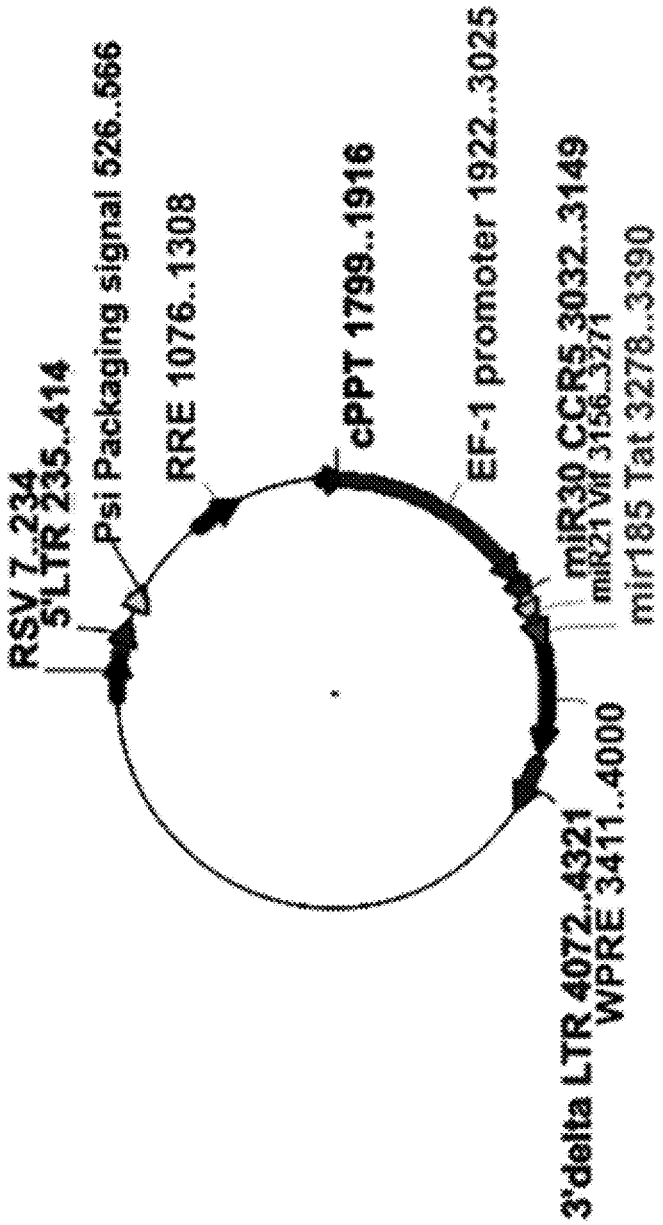
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
RRE 1076..1308
cPPT 1799..1916
EF-1 promoter 1922..3025
miR30 CCR5 3032..3149
mil21 VII 3156..3271
mir185 Tat 3278..3390
3'delta LTR 4072..4321
WPRE 3411..4000

FIG. 5D

Elongation Factor-1 alpha (EF1-alpha) promoter

CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT
TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA
CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGG
GTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCC
CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTC
GTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGC
GCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCT
TTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTG
GGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGA
GCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCT
CGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAG
CGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA
GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATG
TGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGT
CGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACT
GAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATC
TTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAT
GTACA

miR30 CCR5

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATG
GGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTT

miR21 Vif

CCCGGG*CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATC*
TCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA

miR185 Tat

GCTAGC**GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCC**
TCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC

FIG. 6

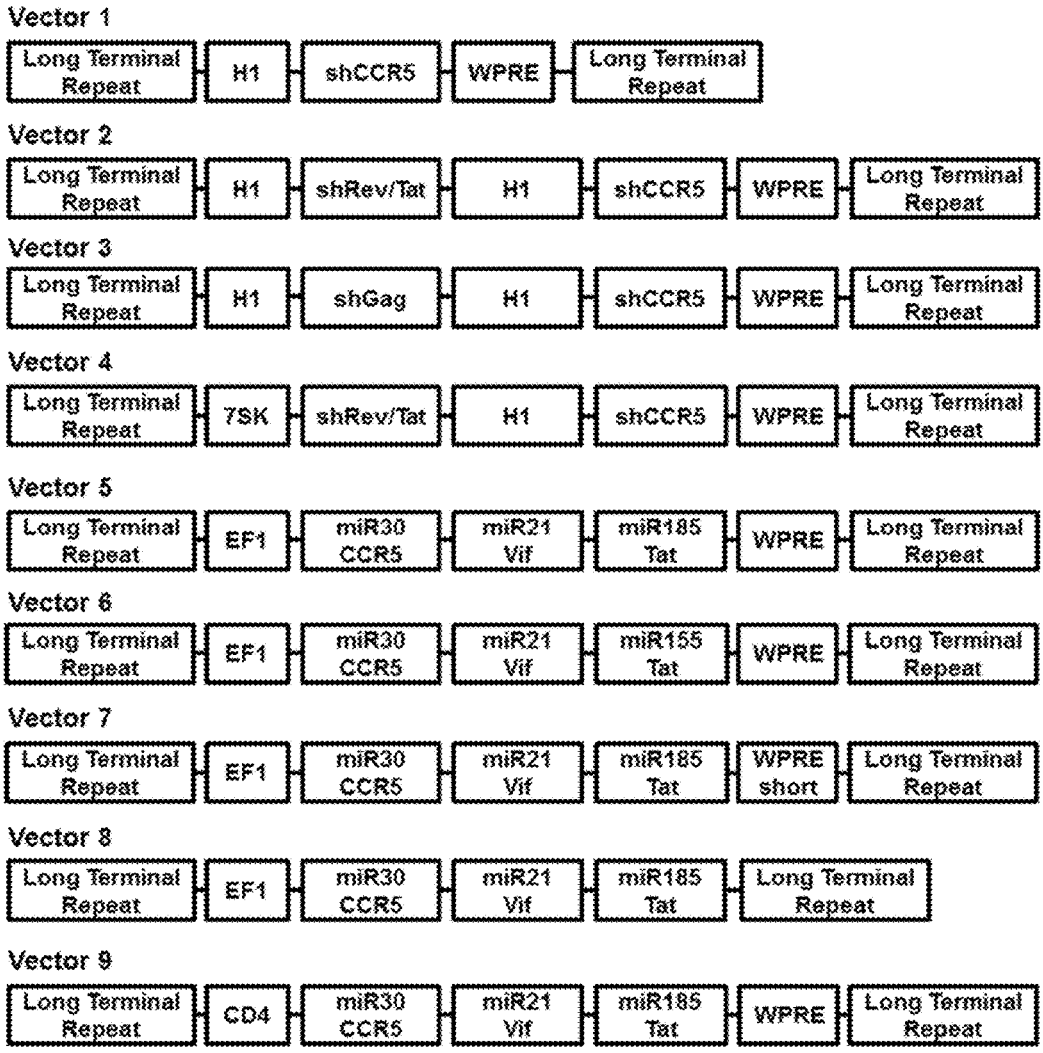
Vector 1
Long Terminal Repeat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 2
Long Terminal Repeat
H1
shRev/Tat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 3
Long Terminal Repeat
H1
shGag
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 4
Long Terminal Repeat
7SK
shRev/Tat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 5
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE
Long Terminal Repeat
Vector 6
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR155 Tat
WPRE
Long Terminal Repeat
Vector 7
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE short
Long Terminal Repeat
Vector 8
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
Long Terminal Repeat
Vector 9
Long Terminal Repeat
CD4
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE
Long Terminal Repeat

FIG. 7

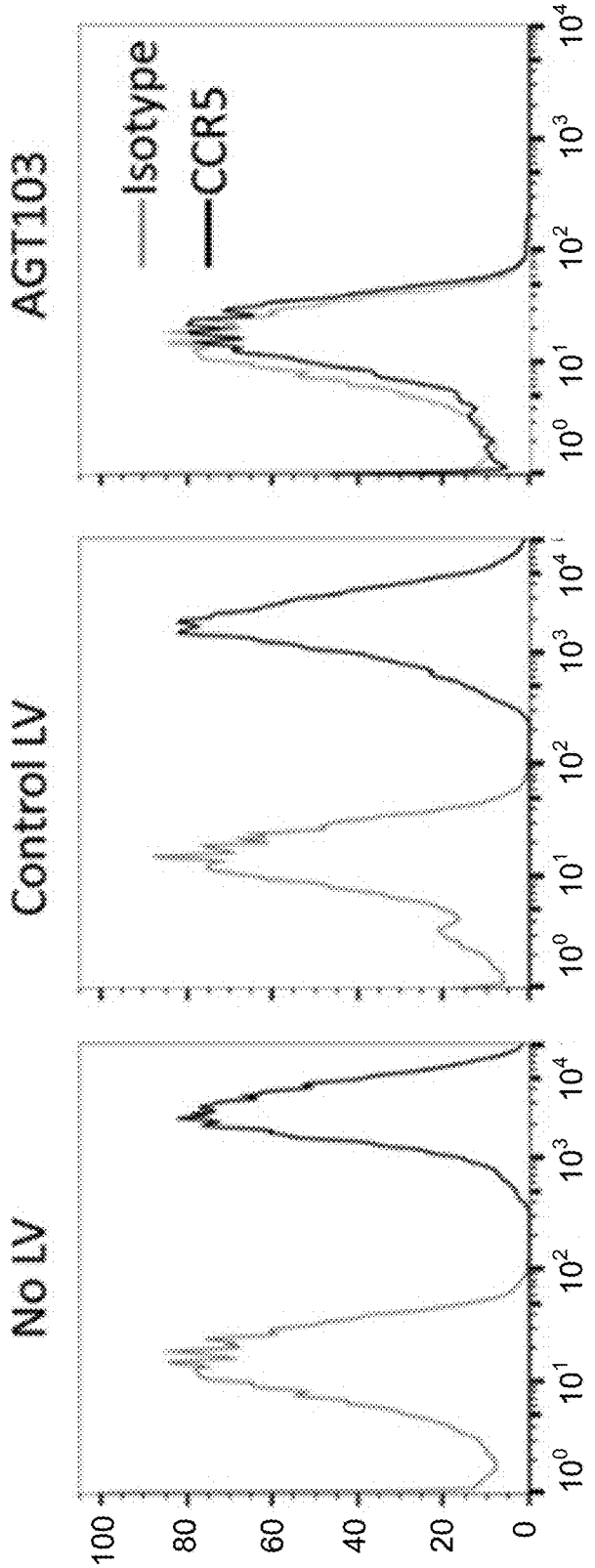
No LV
Control LV
AGT103
Isotype
CCR5
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4

FIG. 8A

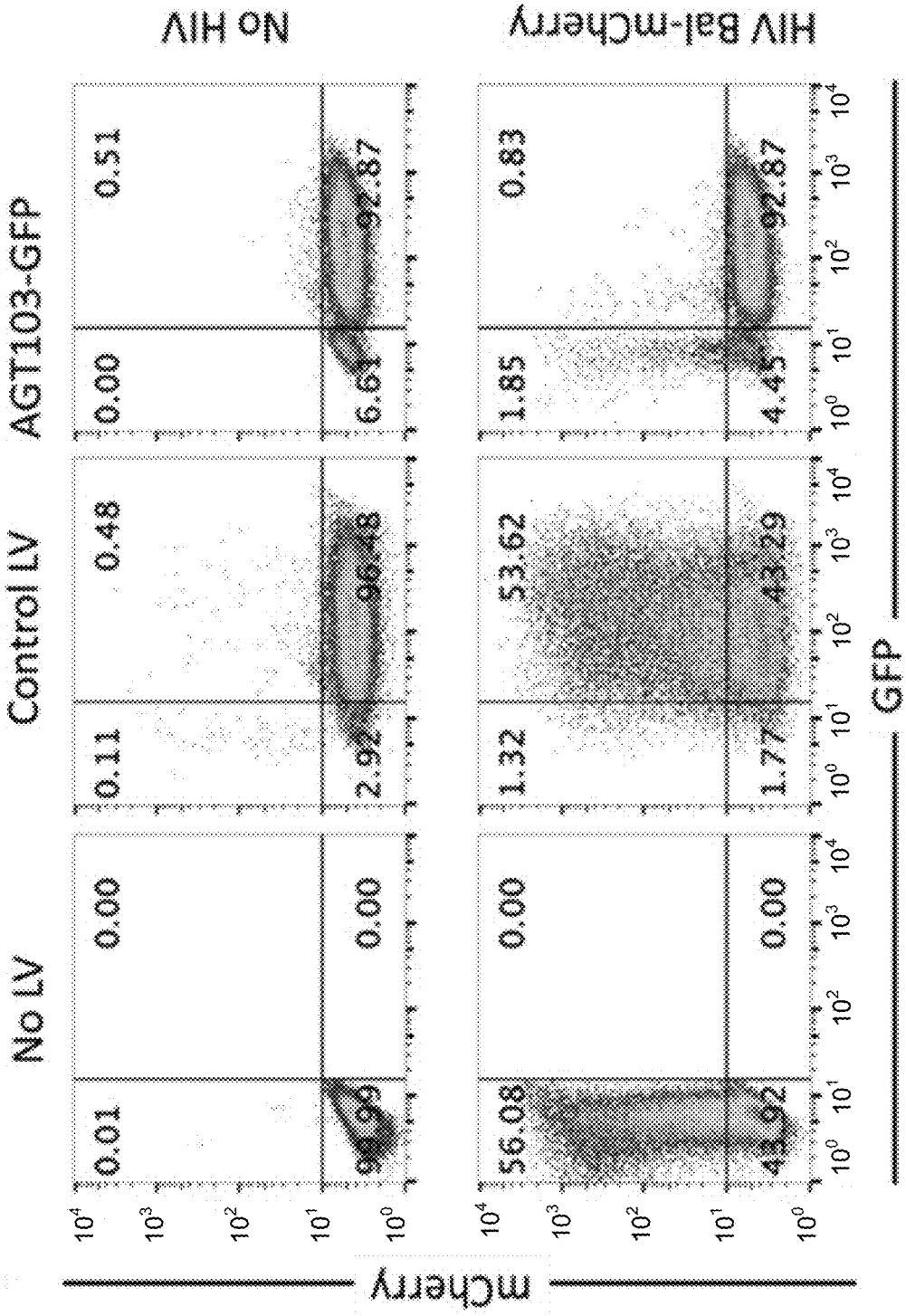
No HIV
HIV Bal-mCherry
No LV
Control LV
AGT103-GFP
mCherry
GFP
0.01
0.00
0.11
0.48
0.00
0.51
99.99
0.00
2.92
96.48
6.61
92.87
56.08
0.00
1.32
53.62
1.85
0.83
43.92
0.00
1.77
43.29
4.45
92.87

FIG. 8B

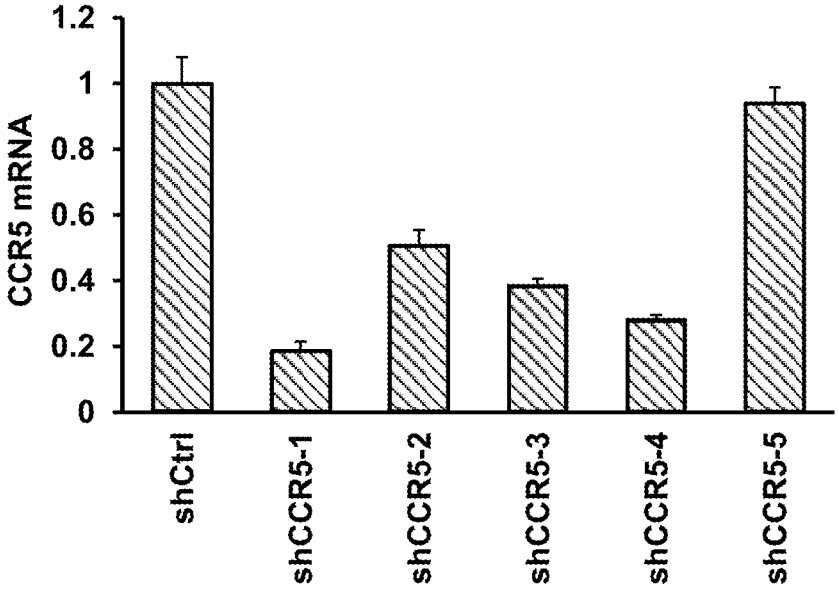
1.2
1
0.8
0.6
0.4
0.2
0
CCR5 mRNA
shCtrl
shCCR5-1
shCCR5-2
shCCR5-3
shCCR5-4
shCCR5-5

FIG. 9A

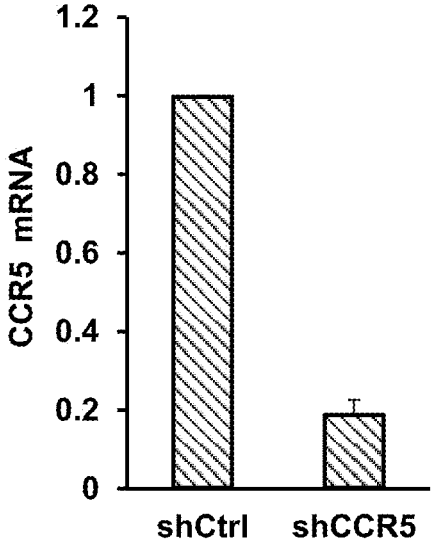
1.2
1
0.8
0.6
0.4
0.2
0
CCR5 mRNA
shCtrl
shCCR5

FIG. 9B

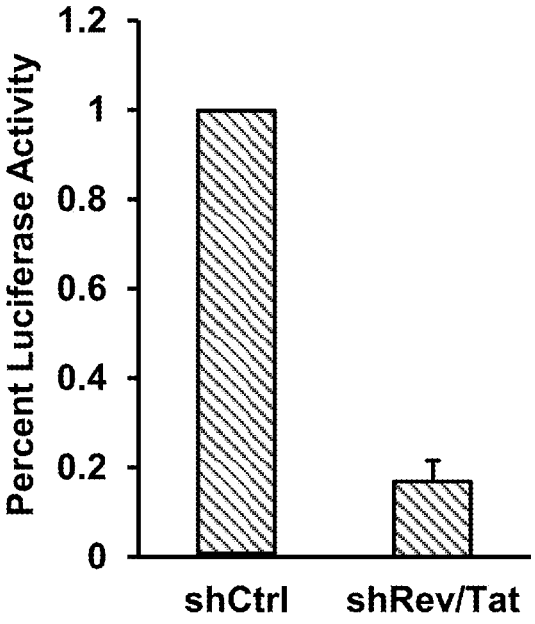

FIG. 10A

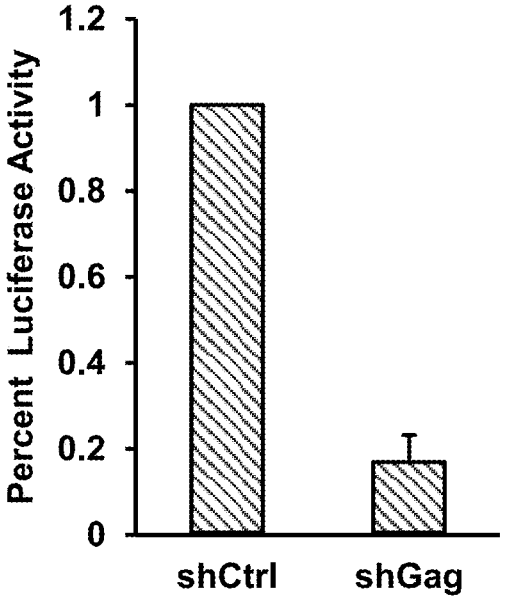

FIG. 10B

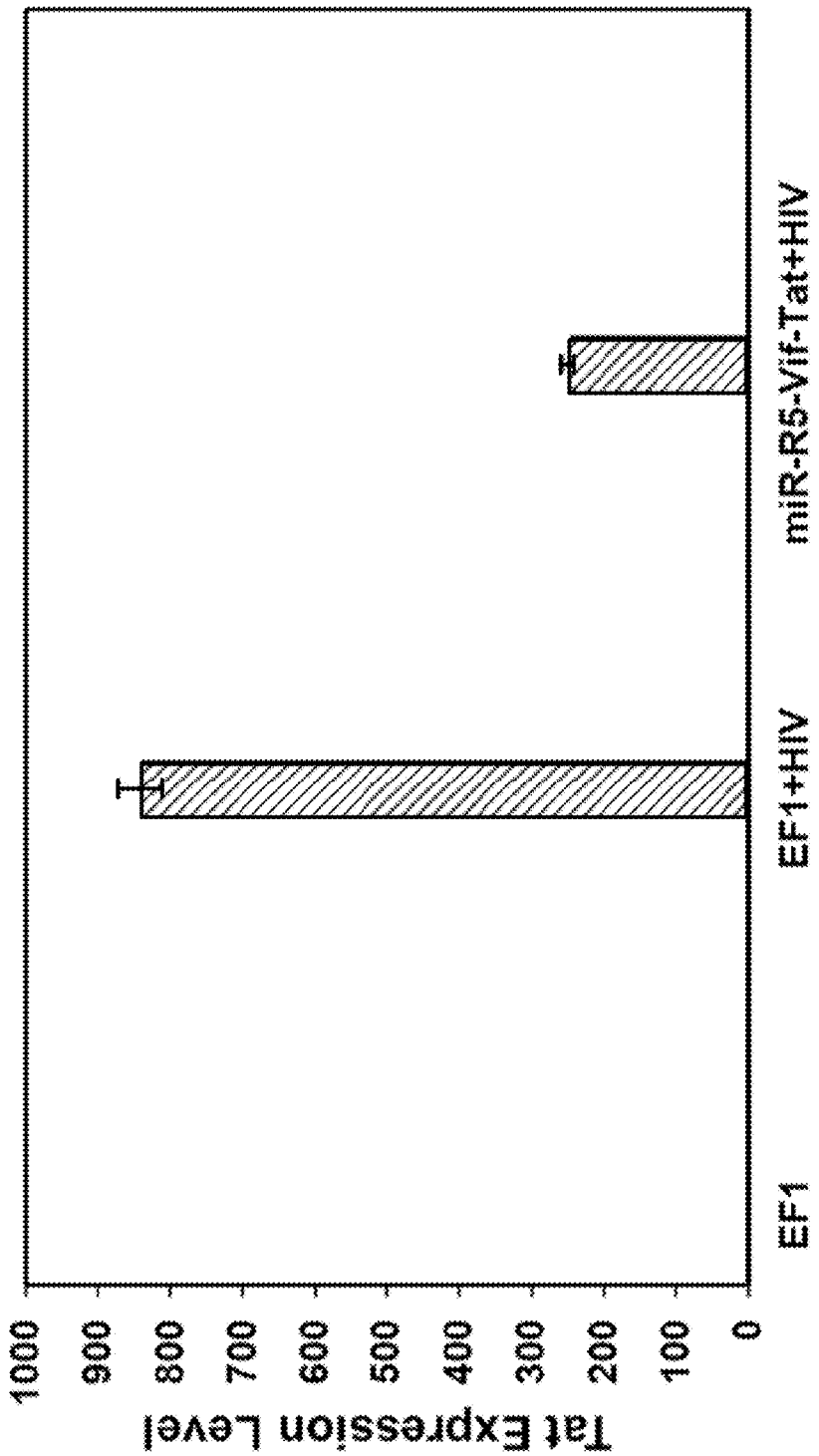
Tat Expression Level
1000
900
800
700
600
500
400
300
200
100
0
EF1
EF1+HIV
miR-R5-Vif-Tat+HIV

FIG. 11

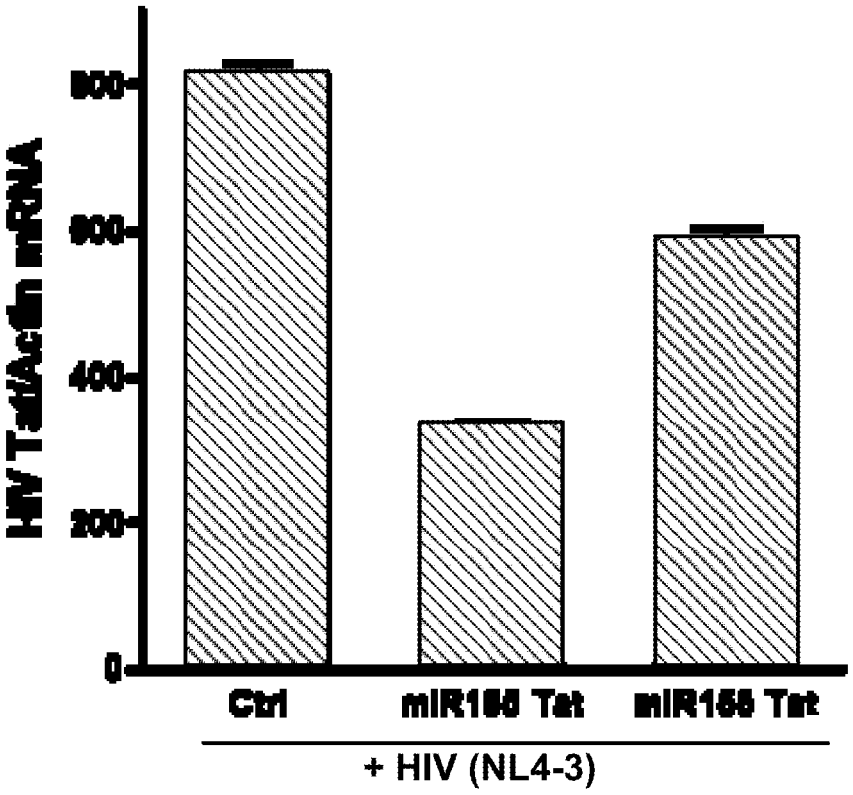

FIG. 12A

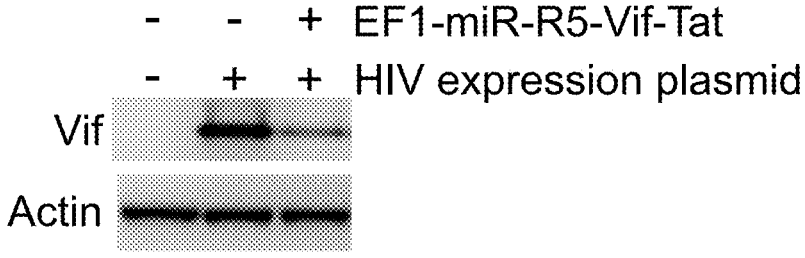

FIG. 12B

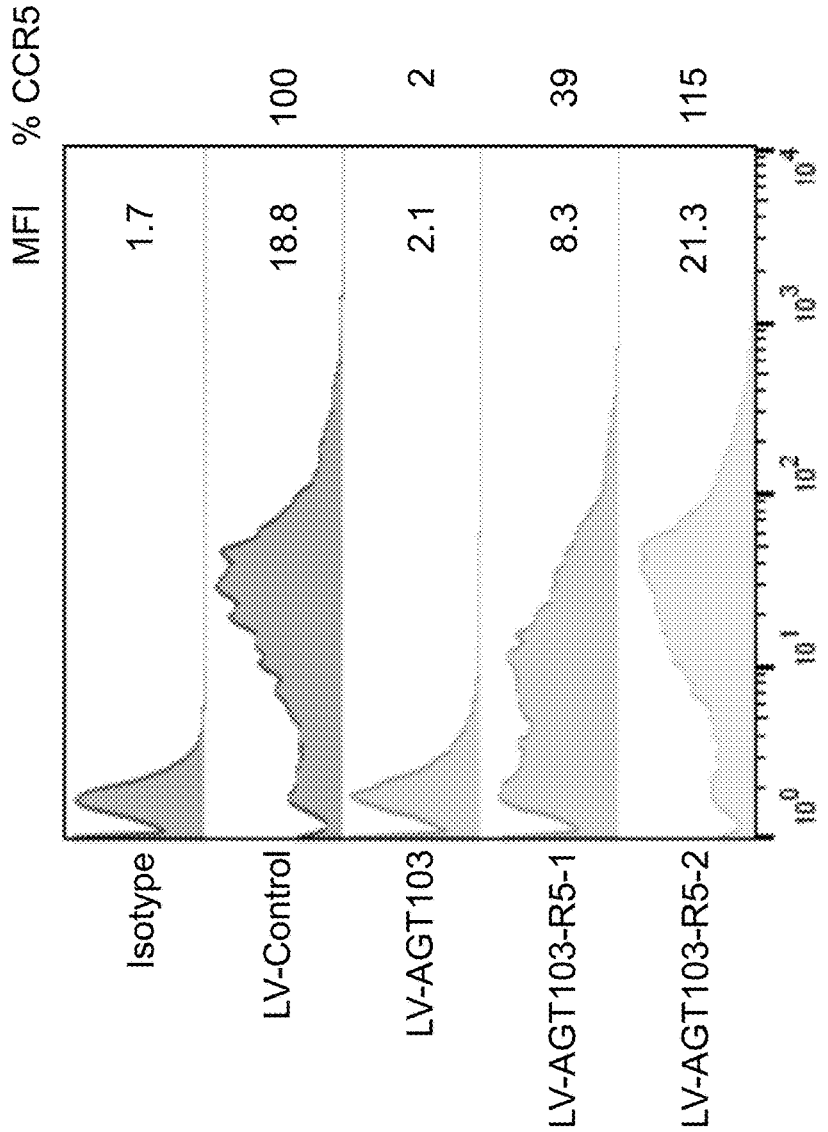
MFI
% CCR5
1.7
18.8
100
2.1
2
8.3
39
21.3
115
Isotype
LV-Control
LV-AGT103
LV-AGT103-R5-1
LV-AGT103-R5-2
FL4-H

FIG. 13

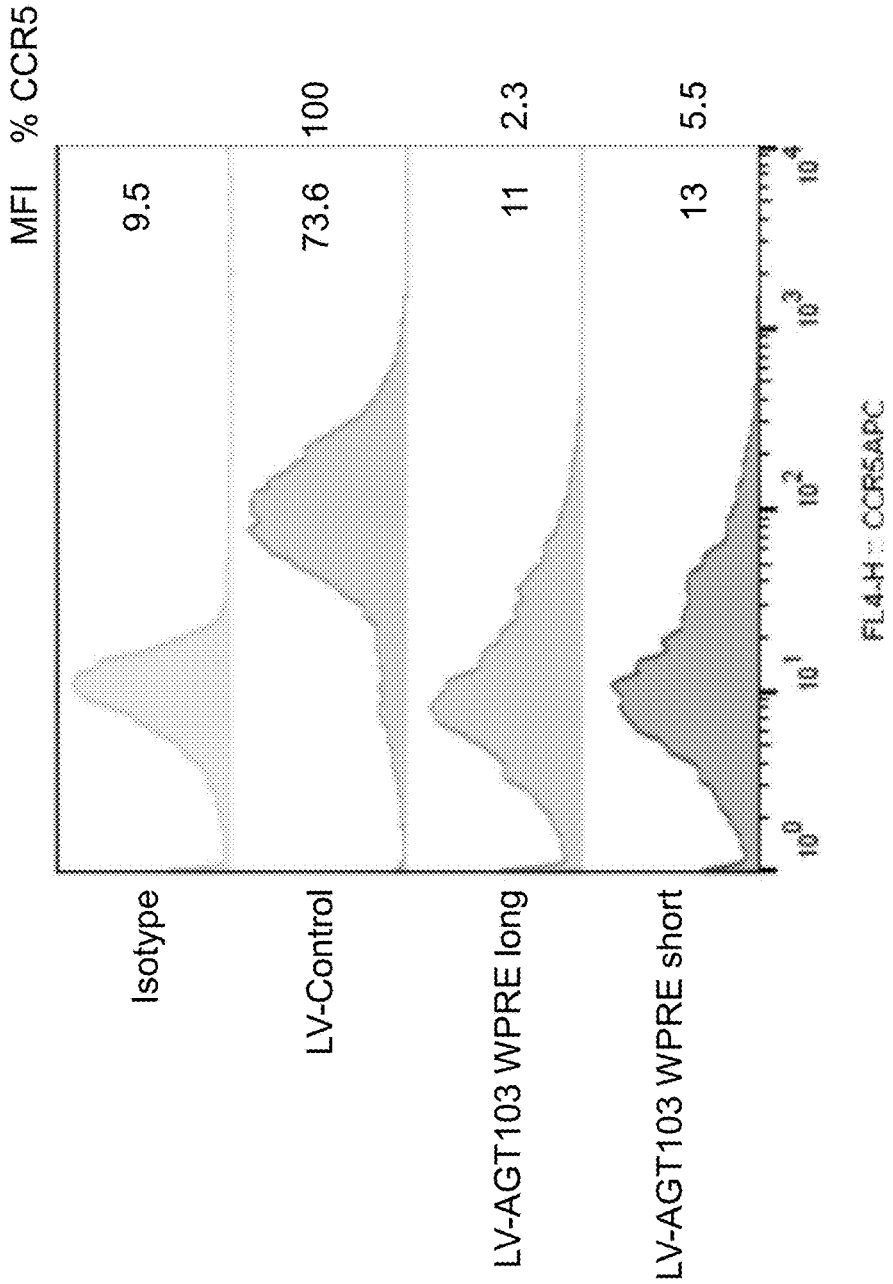
MFI
% CCR5
9.5
73.6
11
13
100
2.3
5.5
Isotype
LV-Control
LV-AGT103 WPRE long
LV-AGT103 WPRE short
FL4-H :: CCR5APC

FIG. 14

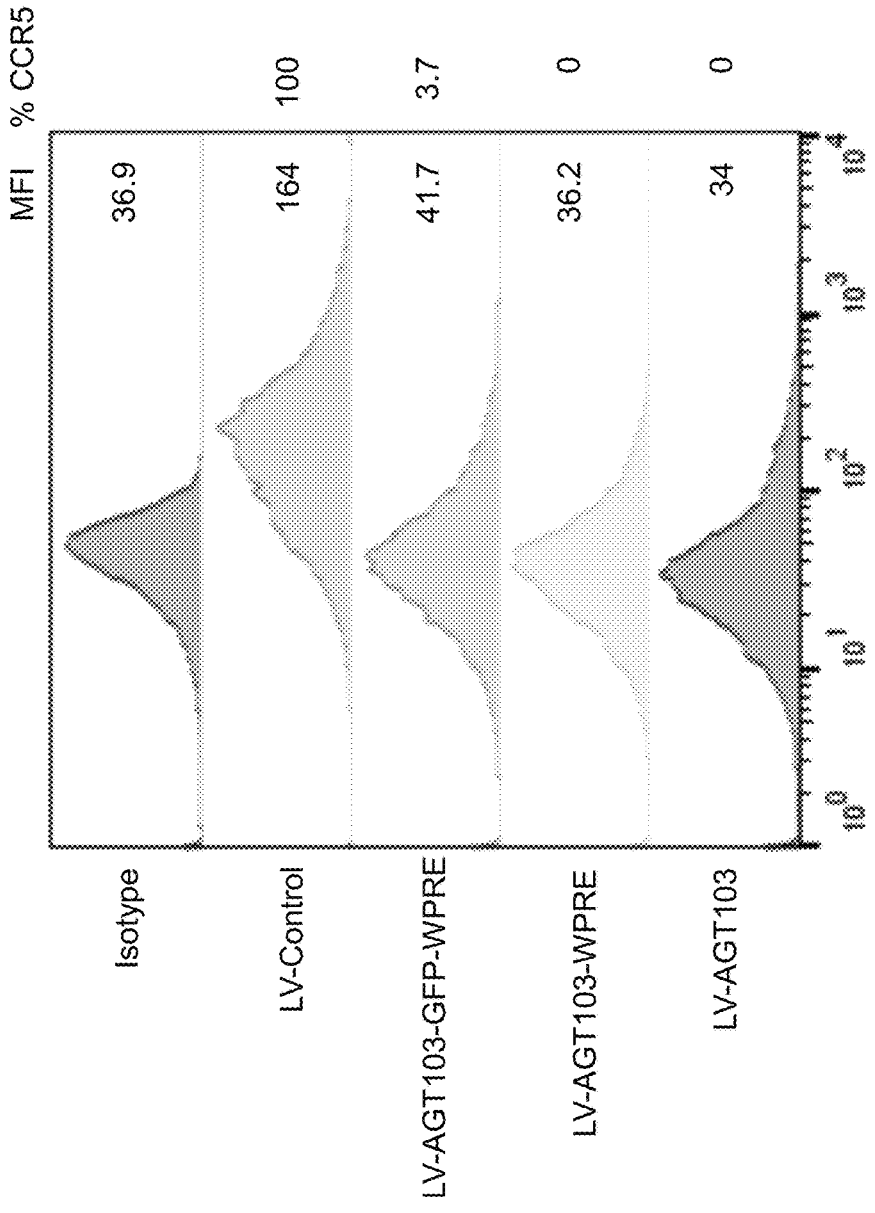
MFI
% CCR5
Isotype
36.9
LV-Control
164
100
LV-AGT103-GFP-WPRE
41.7
3.7
LV-AGT103-WPRE
36.2
0
LV-AGT103
34
0
10^0
10^1
10^2
10^3
10^4
FL4-H :: CCR5 APC

FIG. 15

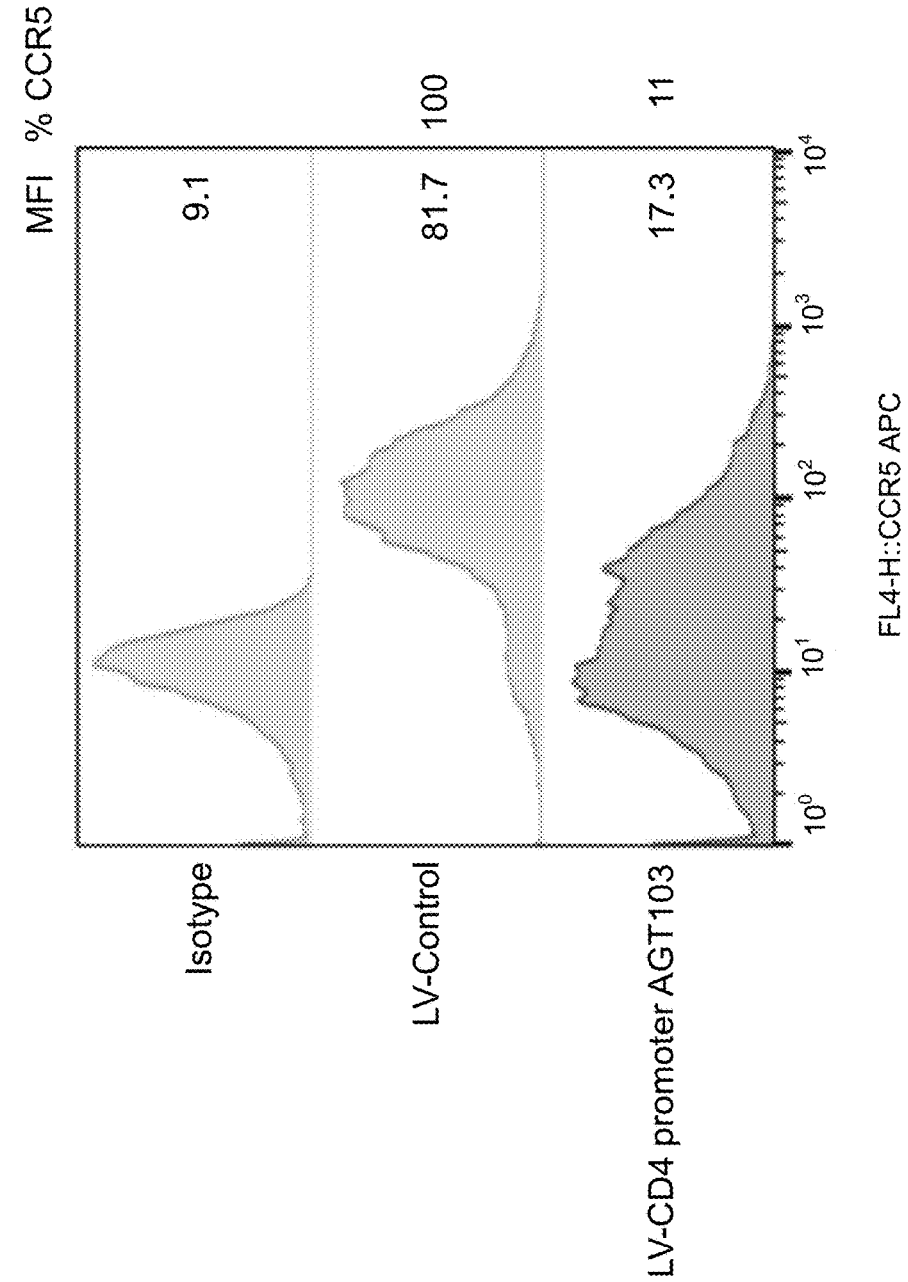
MFI
% CCR5
9.1
81.7
17.3
100
11
Isotype
LV-Control
LV-CD4 promoter AGT103
10^0
10^1
10^2
10^3
10^4
FL4-H::CCR5 APC

FIG. 16

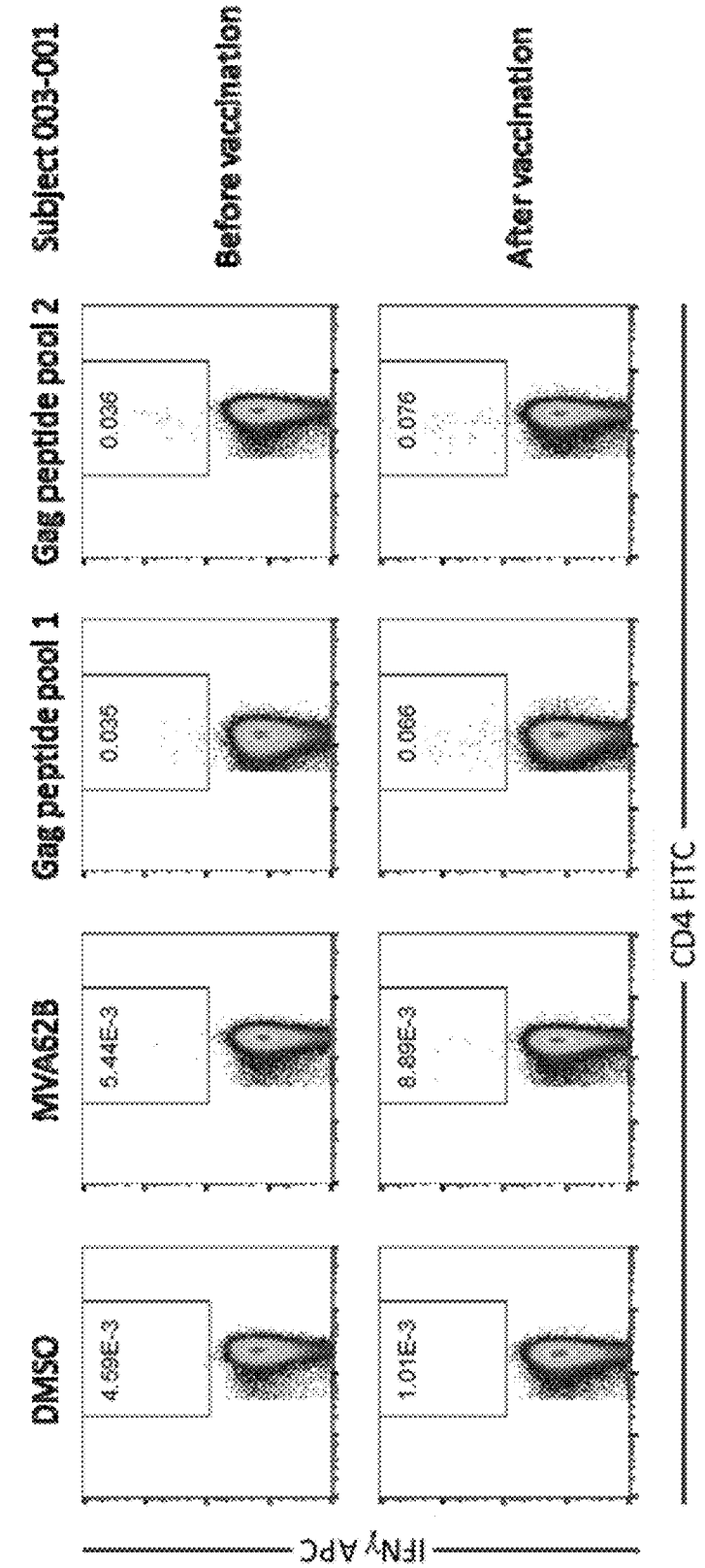
Subject 003-001
Before vaccination
After vaccination
DMSO
MVA62B
Gag peptide pool 1
Gag peptide pool 2
4.99E-3
5.44E-3
0.035
0.036
1.01E-3
8.89E-3
0.066
0.076
IFNγ APC
CD4 FITC

FIG. 17

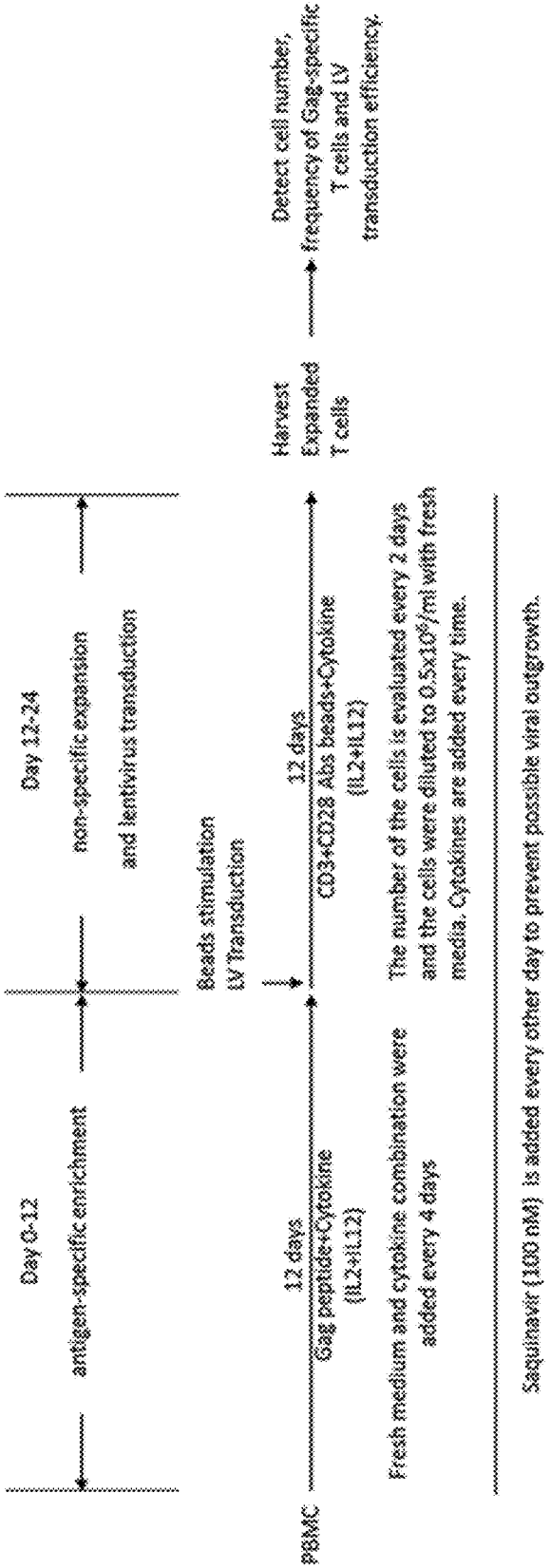
Day 0-12
antigen-specific enrichment
Day 12-24
non-specific expansion
and lentivirus transduction
Beads stimulation
LV Transduction
12 days
CD3+CD28 Abs beads+Cytokine
(IL2+IL12)
Harvest
Expanded
T cells
Detect cell number,
frequency of Gag-specific
T cells and LV
transduction efficiency.
PBMC
12 days
Gag peptide+Cytokine
(IL2+IL12)
Fresh medium and cytokine combination were
added every 4 days
The number of the cells is evaluated every 2 days
and the cells were diluted to 0.5x10^6/ml with fresh
media. Cytokines are added every time.
Saquinavir (100 nM) is added every other day to prevent possible viral outgrowth.

FIG. 18A

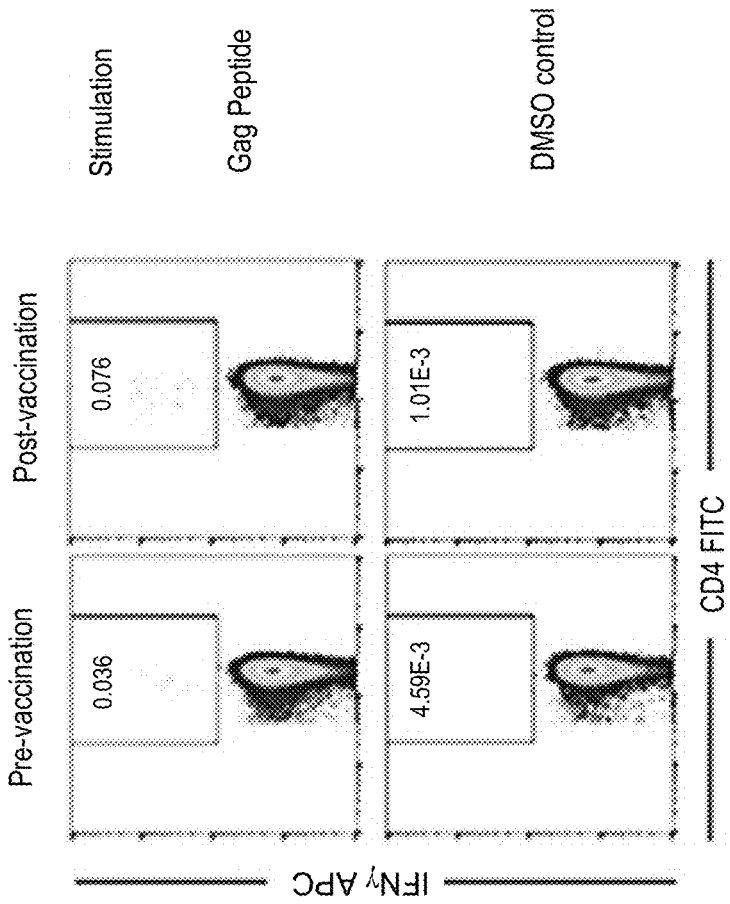
Stimulation
Gag Peptide
DMSO control
Post-vaccination
Pre-vaccination
0.076
1.01E-3
0.036
4.59E-3
IFNγ APC
CD4 FITC

FIG. 18B

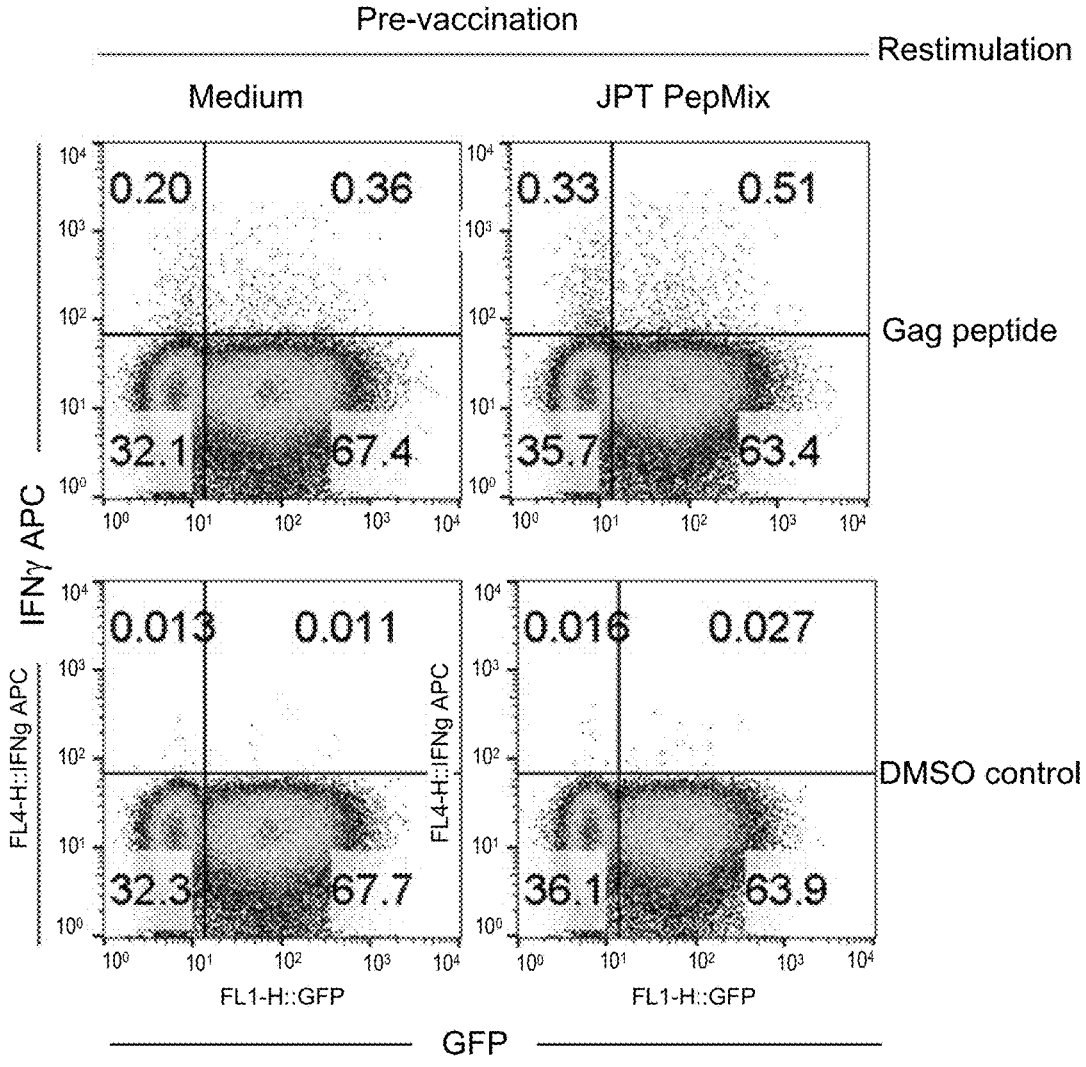
Pre-vaccination
Restimulation
Medium
JPT PepMix
0.20
0.36
32.1
67.4
0.33
0.51
35.7
63.4
Gag peptide
0.013
0.011
32.3
67.7
0.016
0.027
36.1
63.9
DMSO control
IFNγ APC
FL4-H::IFNg APC
FL1-H::GFP
GFP

FIG. 18C

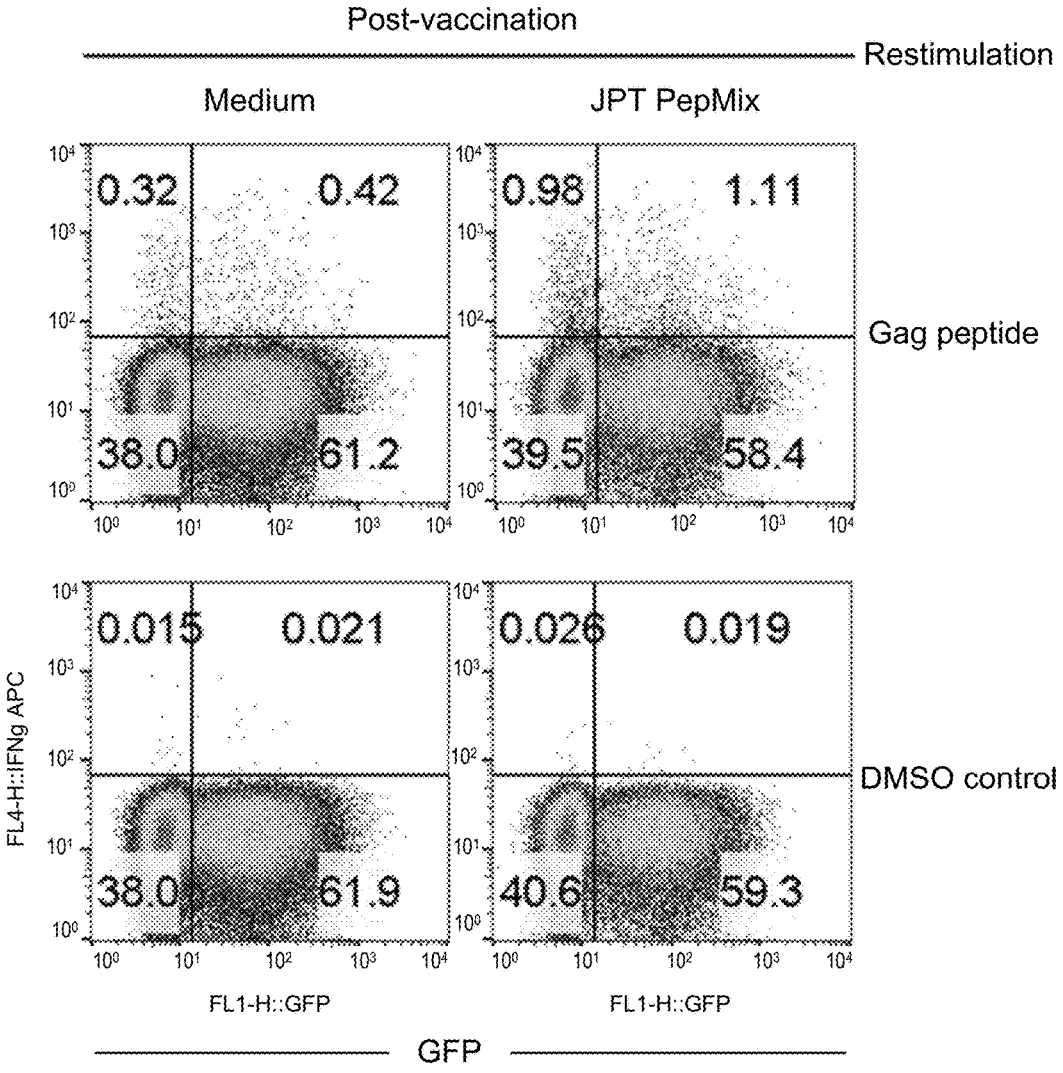

FIG. 18C CONTINUED

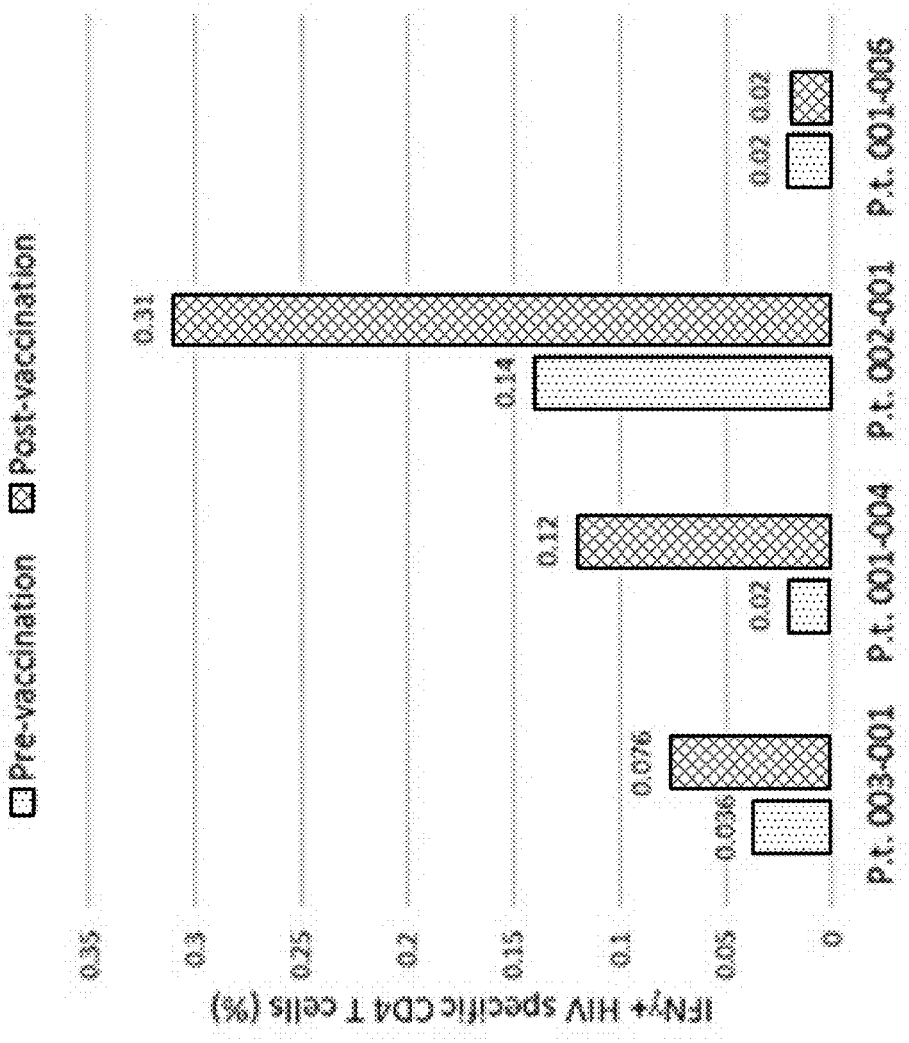
Pre-vaccination
Post-vaccination
IFNγ+ HIV specific CD4 T cells (%)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.31
0.14
0.12
0.02
0.076
0.036
0.02
0.02
P.t. 003-001
P.t. 001-004
P.t. 002-001
P.t. 001-006

FIG. 18D

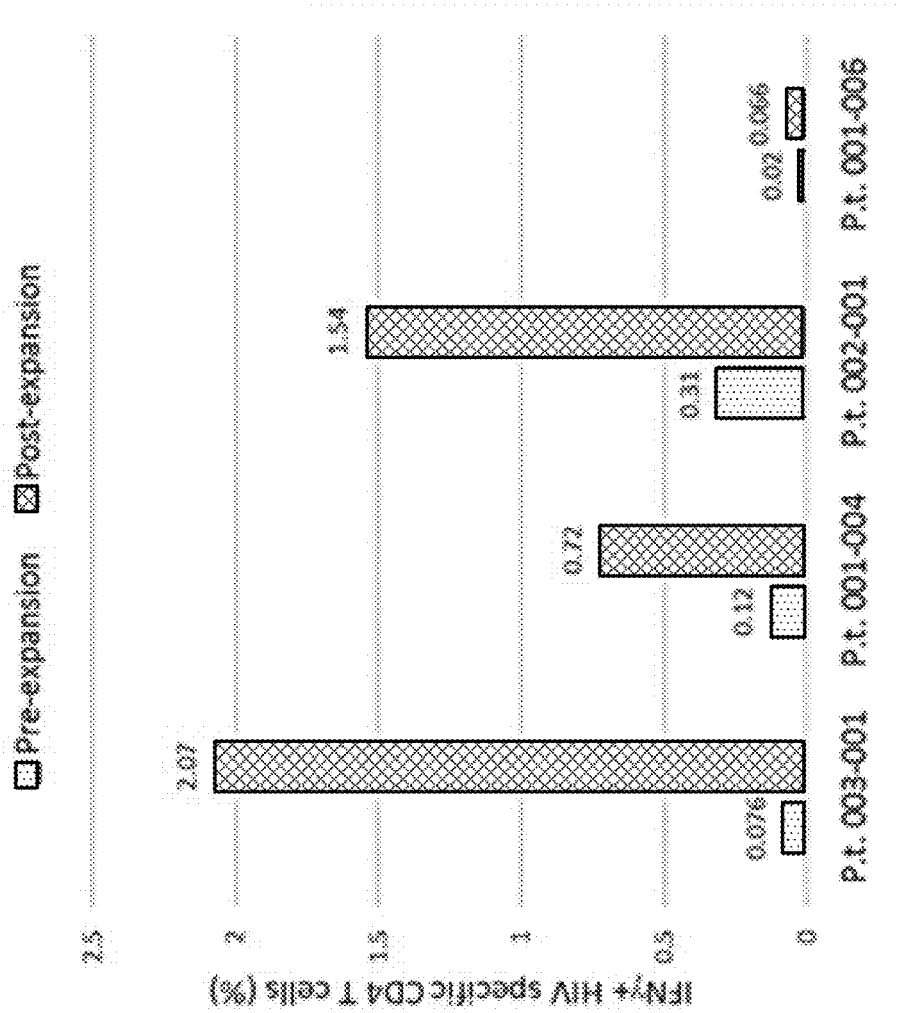
Pre-expansion
Post-expansion
IFNγ+ HIV specific CD4 T cells (%)
2.5
2
1.5
1
0.5
0
2.07
0.076
0.72
0.12
1.54
0.31
0.02
0.066
P.t. 003-001
P.t. 001-004
P.t. 002-001
P.t. 001-006

FIG. 18E

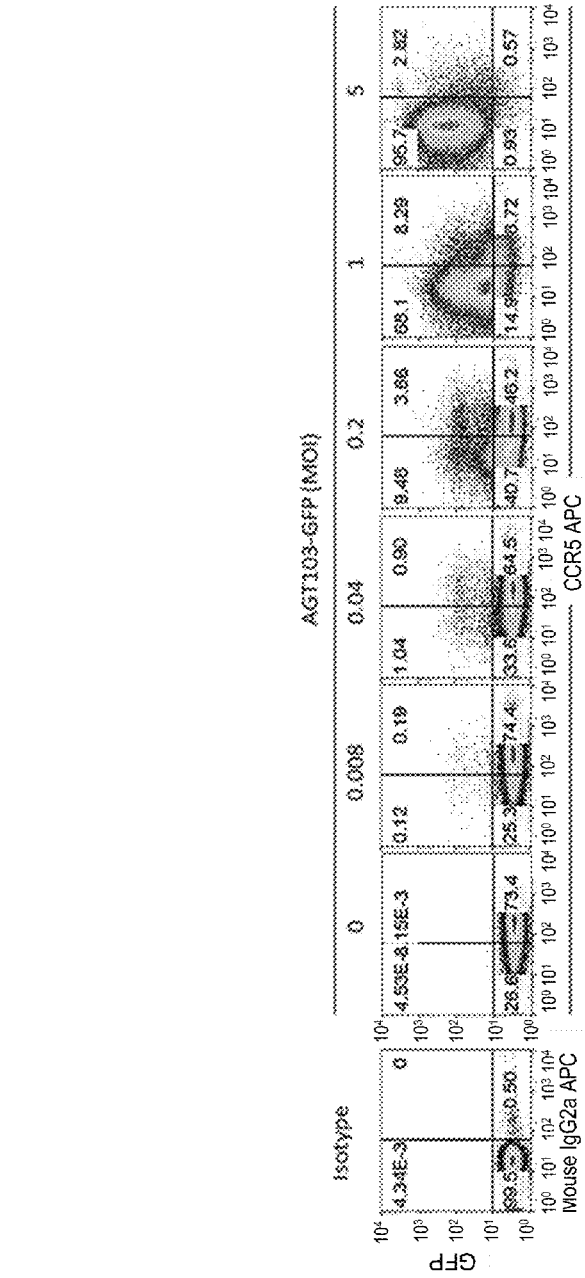
AGT103-GFP (MOI)
Isotype
0
0.008
0.04
0.2
1
5
GFP
Mouse IgG2a APC
CCR5 APC

FIG. 19A

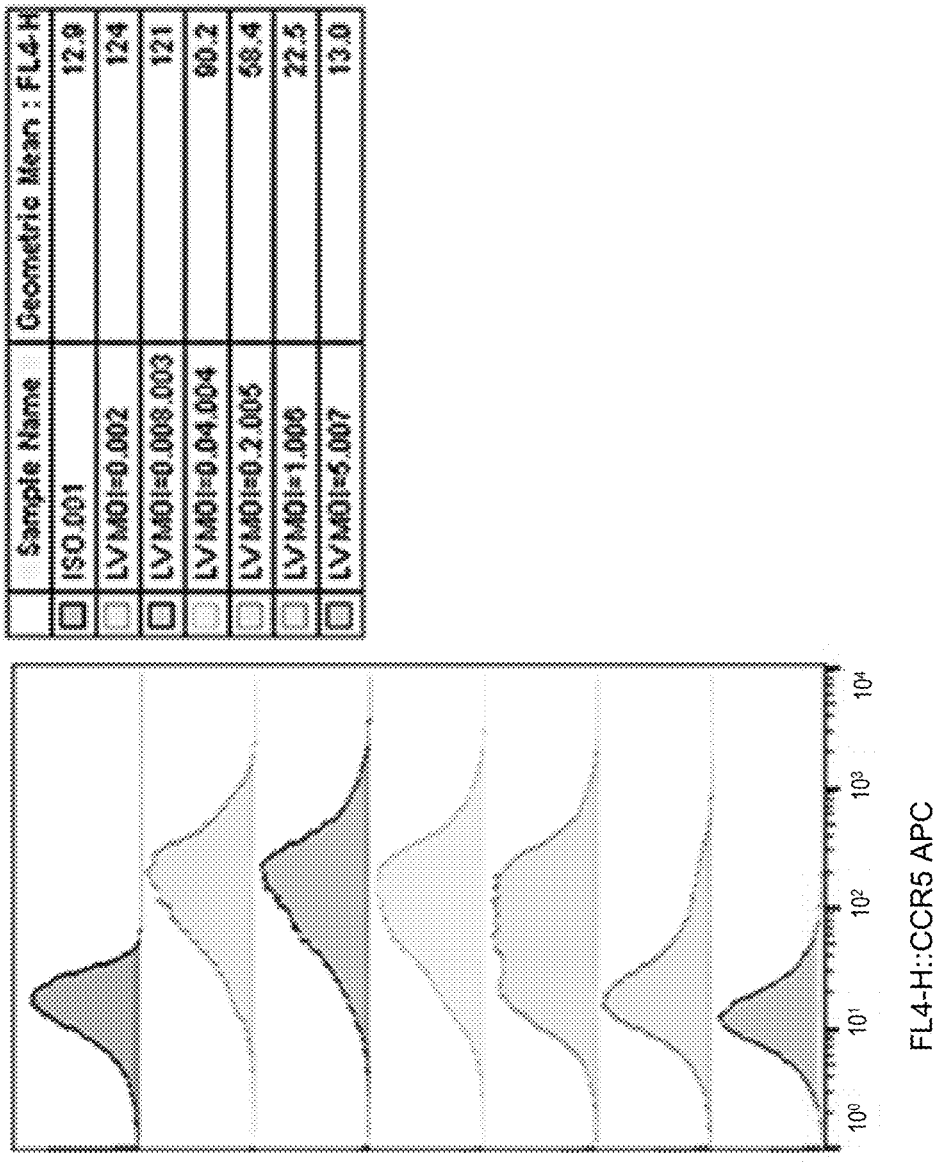
Sample Name
Geometric Mean : FL4-H
ISO.001 12.9
LVM0i=0.002 124
LVM0i=0.008.003 121
LVM0i=0.04.004 60.2
LVM0i=0.2.005 58.4
LVM0i=1.006 22.5
LVM0i=5.007 13.0
10^0
10^1
10^2
10^3
10^4
FL4-H::CCR5 APC

FIG. 19B

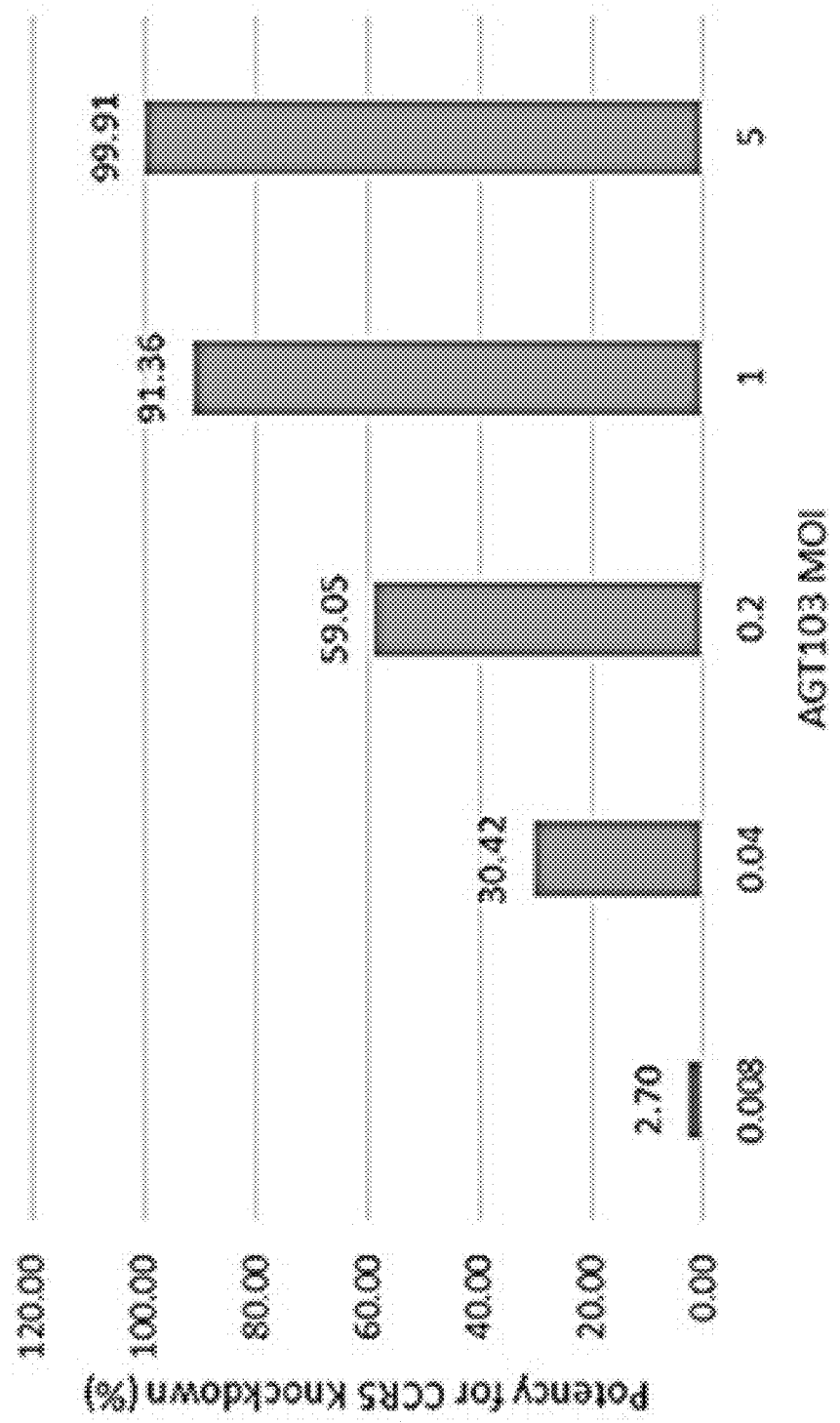
Potency for CCR5 knockdown (%)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
99.91
91.36
59.05
30.42
2.70
5
1
0.2
0.04
0.008
AGT103 MOI

FIG. 19C

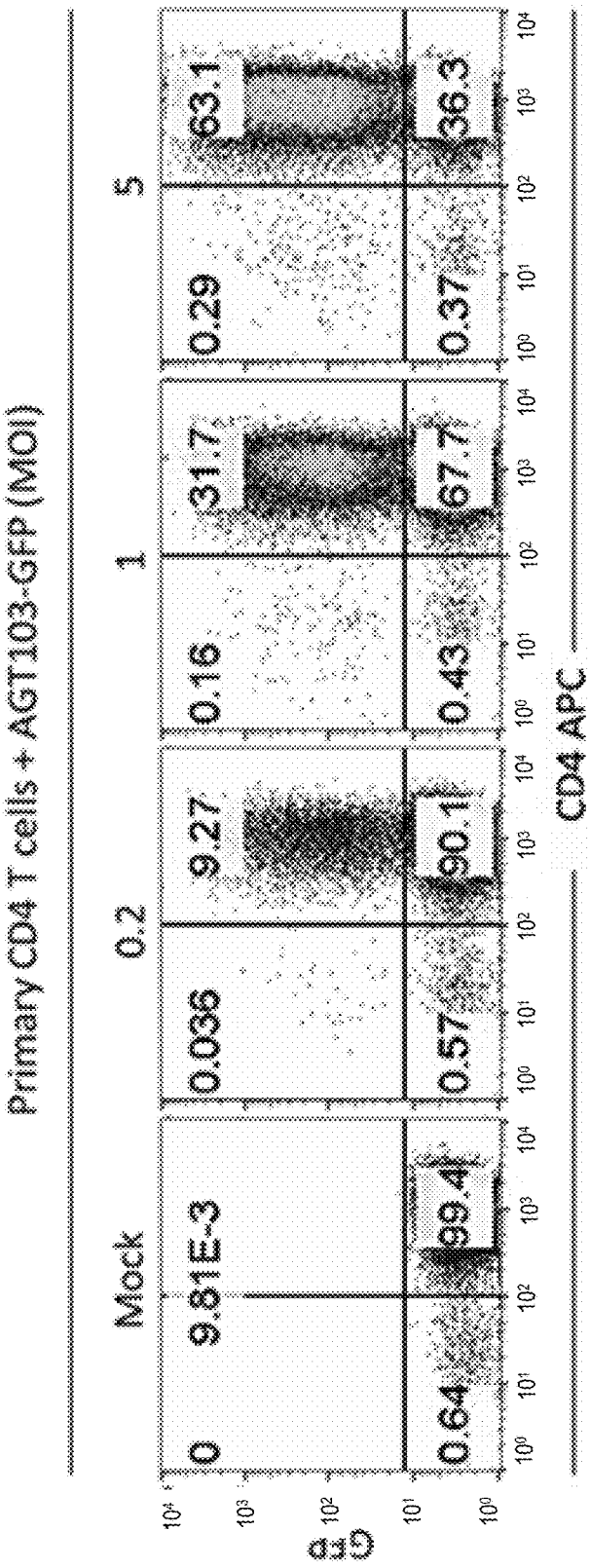
Primary CD4 T cells + AGT103-GFP (MOI)
Mock
0.2
1
5
0
9.81E-3
0.035
9.27
0.16
31.7
0.29
63.1
0.64
99.4
0.57
90.1
0.43
67.7
0.37
36.3
GFP
CD4 APC

FIG. 20A

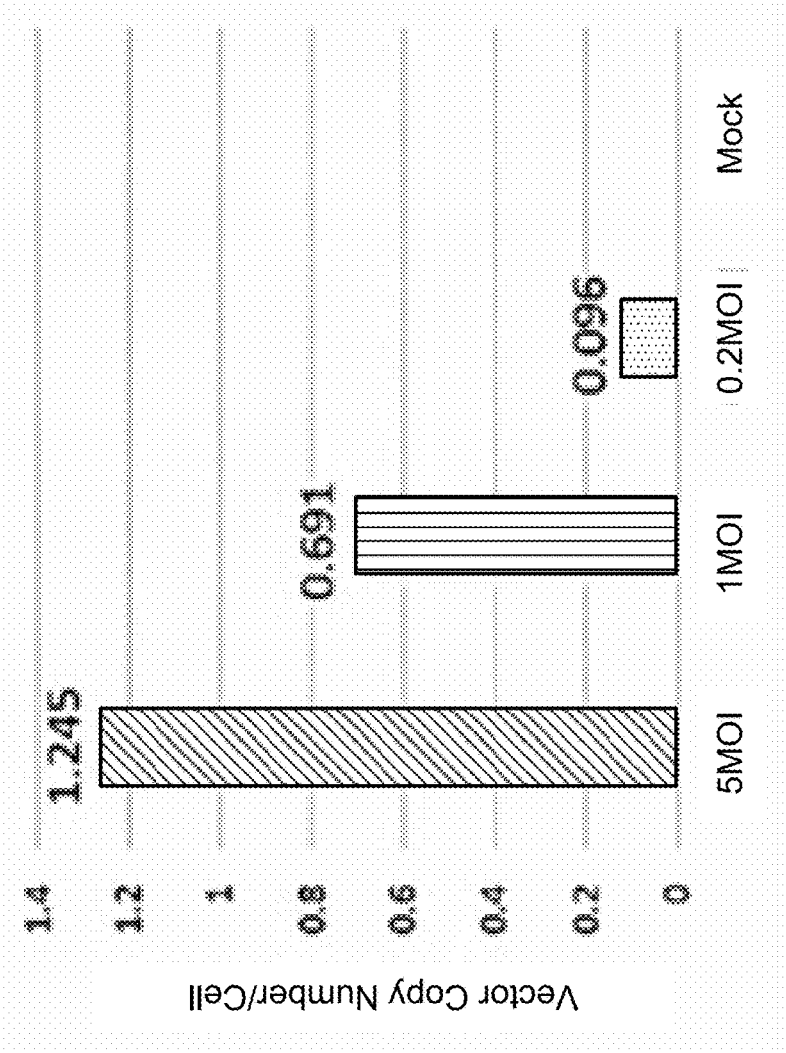
Vector Copy Number/Cell
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.245
0.691
0.096
5MOI
1MOI
0.2MOI
Mock

FIG. 20B

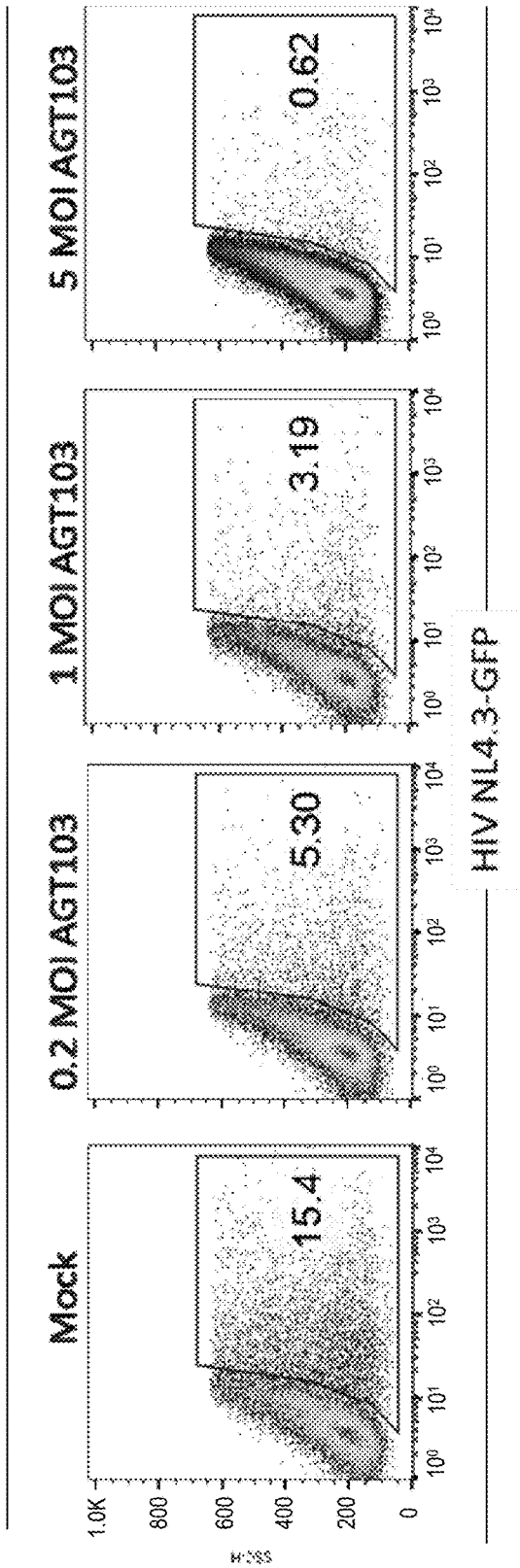
C8166 cells infected by supernatant from HIV-infected Primary CD4 T cells
Mock
0.2 MOI AGT103
1 MOI AGT103
5 MOI AGT103
15.4
5.30
3.19
0.62
HIV NL4.3-GFP

FIG. 21

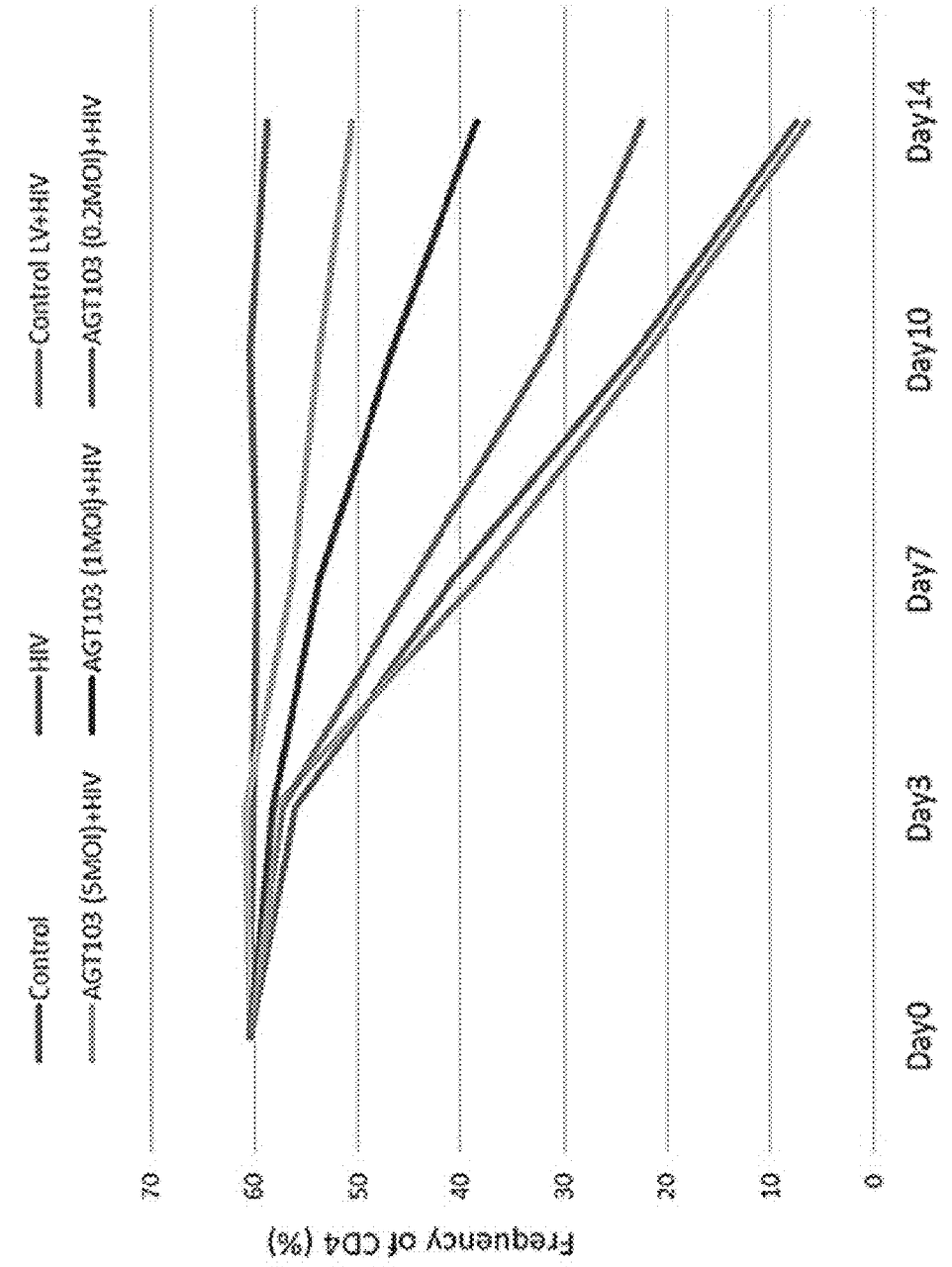
Control
HIV
Control LV+HIV
AGT103 (5MOI)+HIV
AGT103 (1MOI)+HIV
AGT103 (0.2MOI)+HIV
Frequency of CD4 (%)
70
60
50
40
30
20
10
0
Day0
Day3
Day7
Day10
Day14

FIG. 22

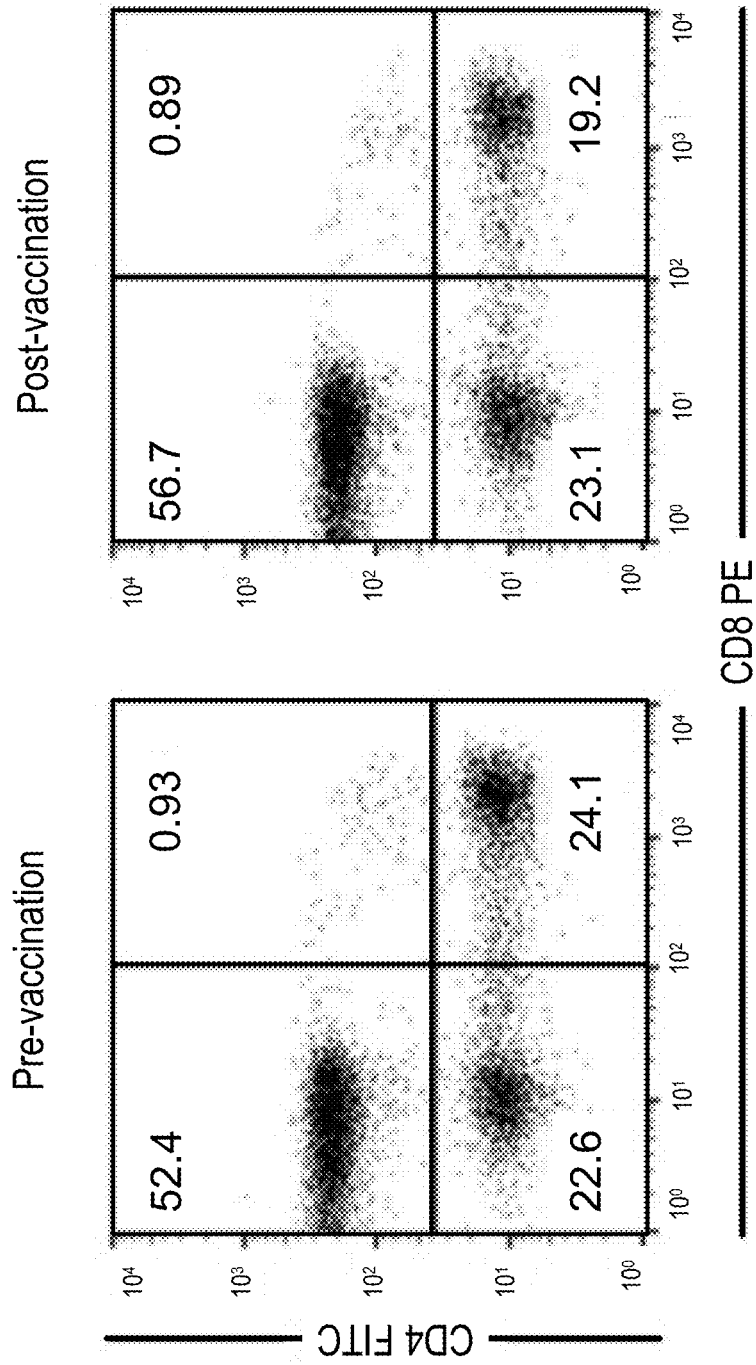
Pre-vaccination
Post-vaccination
52.4
0.93
22.6
24.1
56.7
0.89
23.1
19.2
CD4 FITC
CD8 PE
10^0
10^1
10^2
10^3
10^4

FIG. 23A

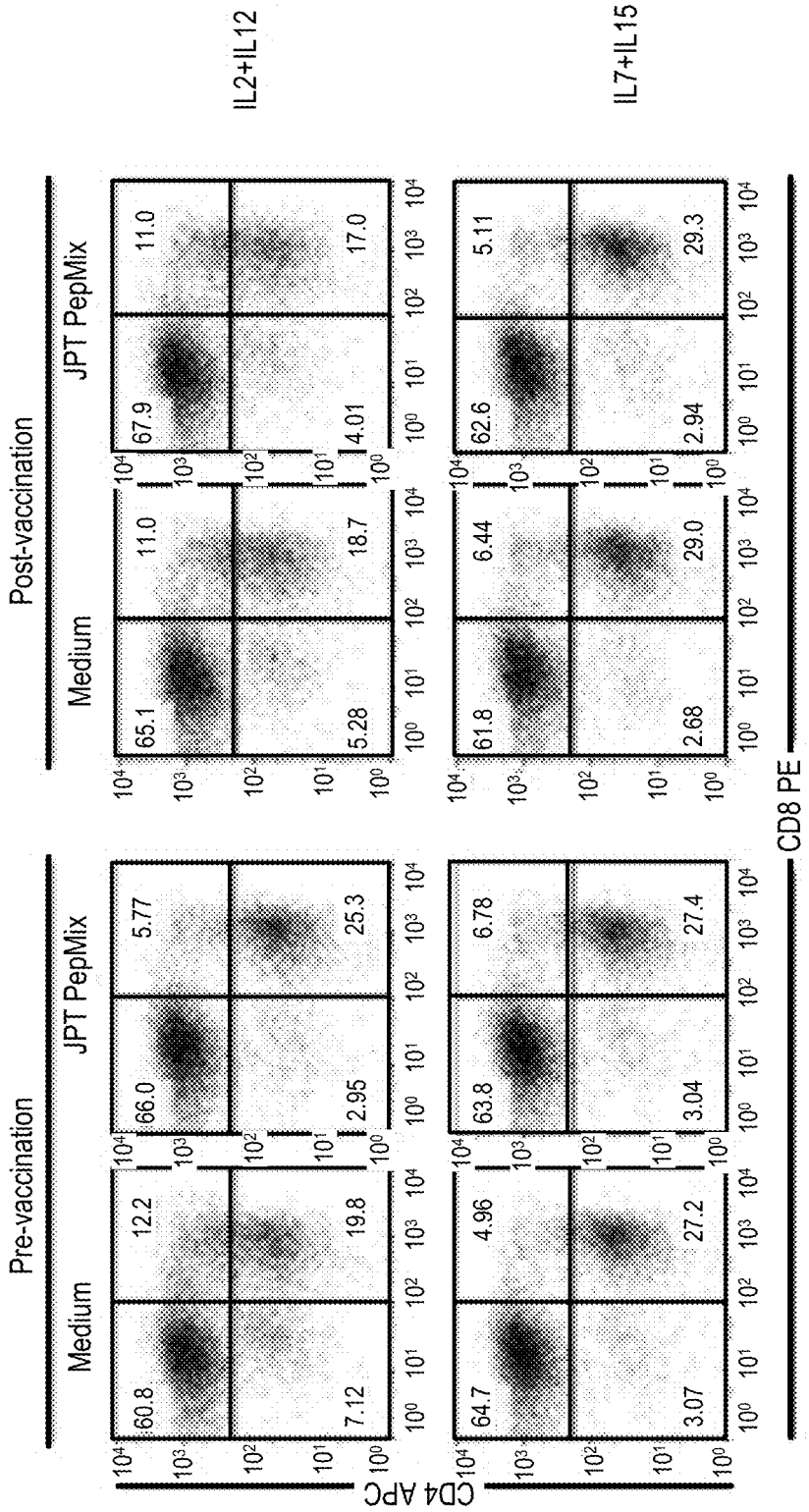
Pre-vaccination
Post-vaccination
Medium
JPT PepMix
IL2+IL12
IL7+IL15
CD4 APC
CD8 PE
60.8
12.2
66.0
5.77
7.12
19.8
2.95
25.3
64.7
4.96
63.8
6.78
3.07
27.2
3.04
27.4
65.1
11.0
67.9
11.0
5.28
18.7
4.01
17.0
61.8
6.44
62.6
5.11
2.68
29.0
2.94
29.3

FIG. 23B

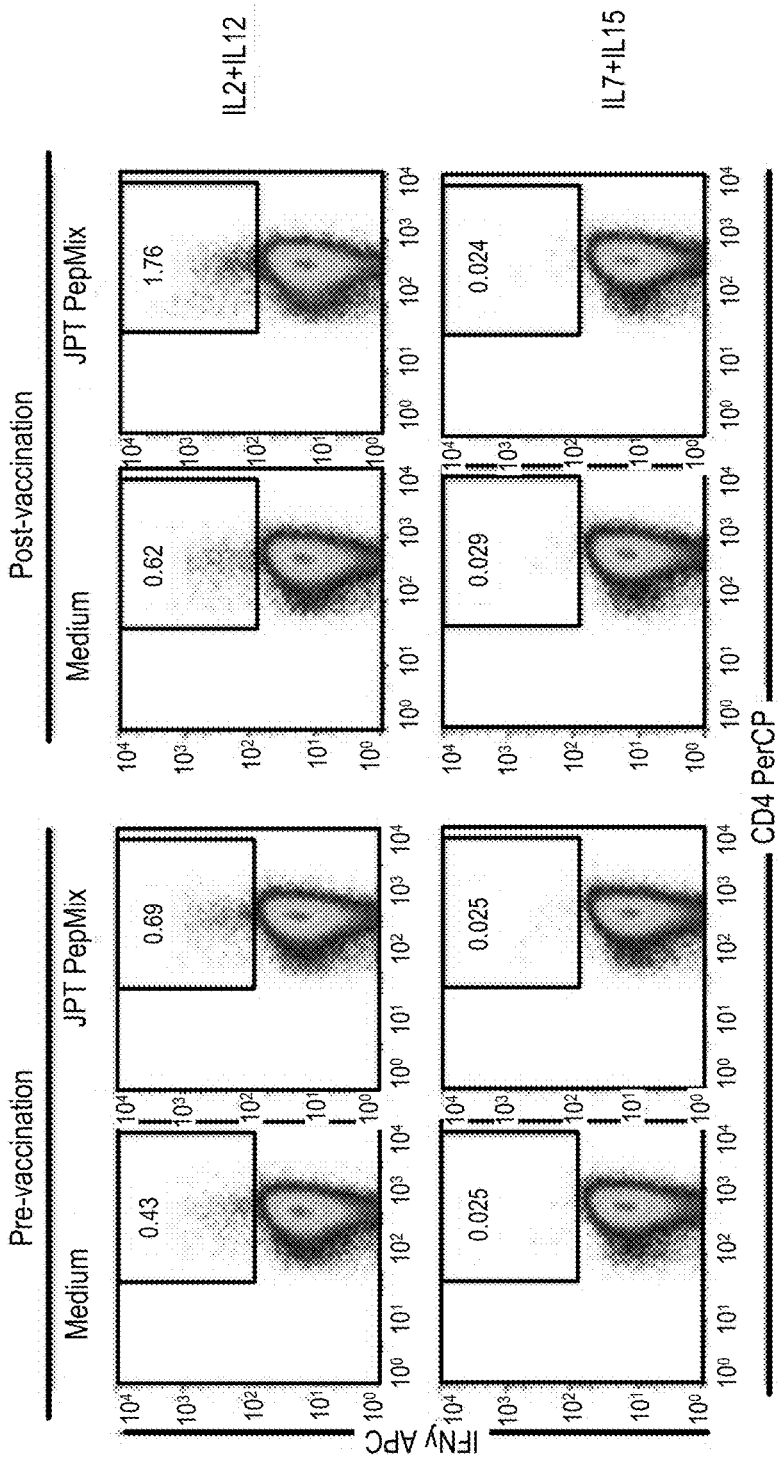
Pre-vaccination
Post-vaccination
Medium
JPT PepMix
IL2+IL12
IL7+IL15
0.43
0.69
0.025
0.025
0.62
1.76
0.029
0.024
IFNγ APC
CD4 PerCP

FIG. 23C

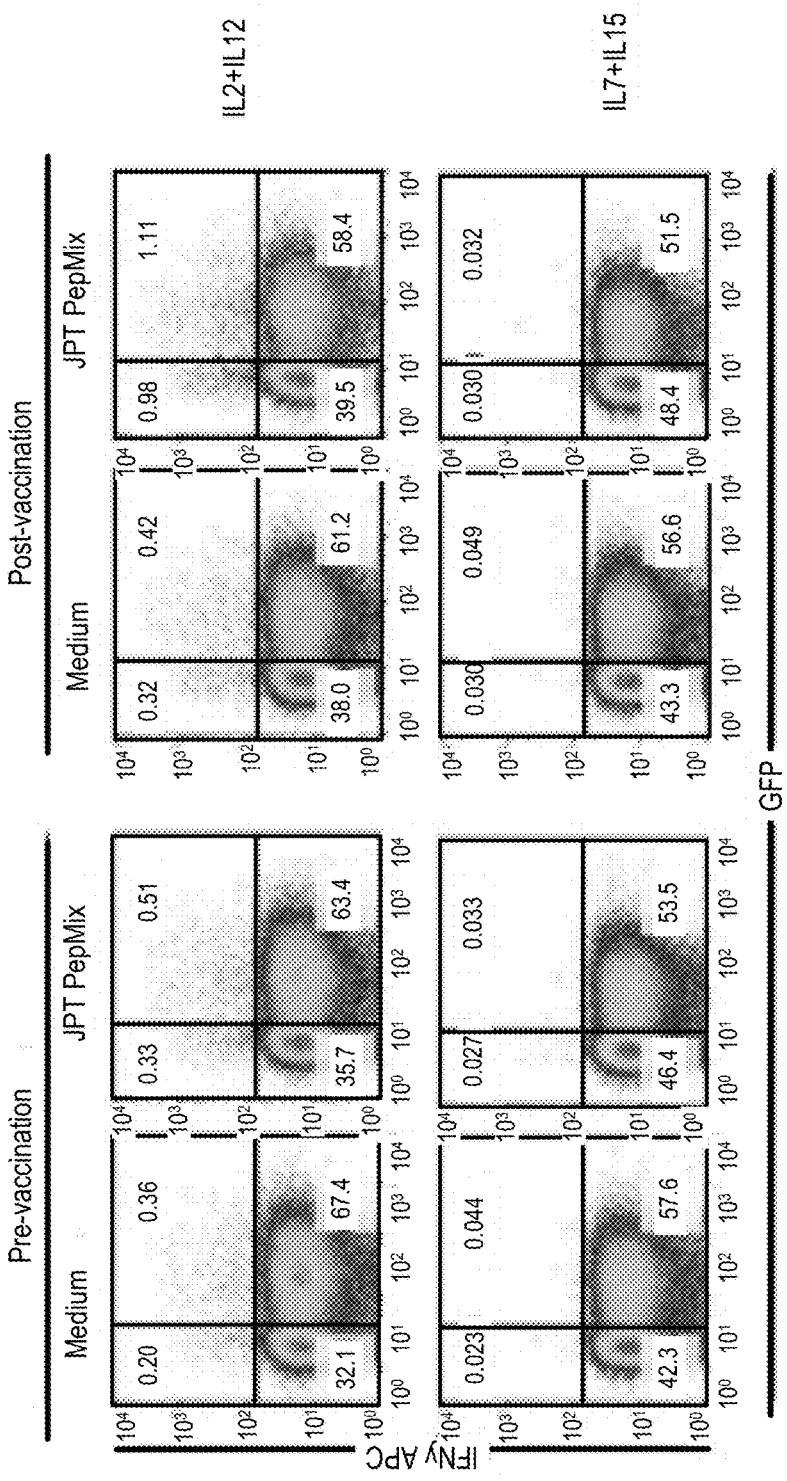
Pre-vaccination
Post-vaccination
Medium
JPT PepMix
IL2+IL12
IL7+IL15
IFNγ APC
GFP
0.20
0.36
0.33
0.51
32.1
67.4
35.7
63.4
0.023
0.044
0.027
0.033
42.3
57.6
46.4
53.5
0.32
0.42
0.98
1.11
38.0
61.2
39.5
58.4
0.030
0.049
0.030
0.032
43.3
56.6
48.4
51.5

FIG. 23D

HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/668,223 filed on Aug. 3, 2017, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY" which is a continuation of International Application No. PCT/US17/13019 filed on Jan. 11, 2017, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY" which claims priority to: U.S. Provisional Patent Application No. 62/360,185 filed on Jul. 8, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", U.S. Provisional Patent Application No. 62/385,864 filed on Sep. 9, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", and U.S. Provisional Patent Application No. 62/409,270 filed on Oct. 17, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY," the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to the field of immunization and immunotherapy for the treatment and prevention of HIV. In particular, the disclosed methods of treatment and prevention relate to the administration of viral vectors and systems for the delivery of genes and other therapeutic, diagnostic, or research uses.

BACKGROUND OF THE INVENTION

Combination antiretroviral therapy (cART) (also known as Highly Active Antiretroviral Therapy or HAART) limits HIV-1 replication and retards disease progression, but drug toxicities and the emergence of drug-resistant viruses are challenges for long-term control in HIV-infected persons. Additionally, traditional antiretroviral therapy, while successful at delaying the onset of AIDS or death, has yet to provide a functional cure. Alternative treatment strategies are needed.

Intense interest in immunotherapy for HIV infection has been precipitated by emerging data indicating that the immune system has a major, albeit usually insufficient, role in limiting HIV replication. Virus-specific T-helper cells, which are critical to maintenance of cytolytic T cell (CTL) function, likely play a role. Viremia is also influenced by neutralizing antibodies, but they are generally low in magnitude in HIV infection and do not keep up with evolving viral variants in vivo.

Together this data indicates that increasing the strength and breadth of HIV-specific cellular immune responses might have a clinical benefit through so-called HIV immunotherapy. Some studies have tested vaccines against HIV, but success has been limited to date. Additionally, there has been interest in augmenting HIV immunotherapy by utilizing gene therapy techniques, but as with other immunotherapy approaches, success has been limited.

Viral vectors can be used to transduce genes into target cells owing to specific virus envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for the transfer of genes into many different cell types including whole T cells or other immune cells as well as embryos, fertilized eggs, isolated tissue samples, tissue targets in situ and cultured cells. The ability to introduce and express foreign or altered genes in a cell is useful for therapeutic interventions such as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy.

Gene therapy is one of the ripest areas of biomedical research with the potential to create new therapeutics that may involve the use of viral vectors. In view of the wide variety of potential genes available for therapy, an efficient means of delivering these genes is needed to fulfill the promise of gene therapy as a means of treating infectious and non-infectious diseases. Several viral systems including murine retrovirus, adenovirus, parvovirus (adeno-associated virus), vaccinia virus, and herpes virus have been proposed as therapeutic gene transfer vectors.

There are many factors that must be considered when developing viral vectors, including tissue tropism, stability of virus preparations, stability and control of expression, genome packaging capacity, and construct-dependent vector stability. In addition, in vivo application of viral vectors is often limited by host immune responses against viral structural proteins and/or transduced gene products.

Thus, toxicity and safety are key hurdles that must be overcome for viral vectors to be used in vivo for the treatment of subjects. There are numerous historical examples of gene therapy applications in humans that have met with problems associated with the host immune responses against the gene delivery vehicles or the therapeutic gene products. Viral vectors (e.g., adenovirus) which co-transduce several viral genes together with one or more therapeutic gene(s) are particularly problematic.

Although lentiviral vectors do not generally induce cytotoxicity and do not elicit strong host immune responses, some lentiviral vectors such as HIV-1, which carry several immunostimulatory gene products, have the potential to cause cytotoxicity and induce strong immune responses in vivo. However, this may not be a concern for lentiviral derived transducing vectors that do not encode multiple viral genes after transduction. Of course, this may not always be the case, as sometimes the purpose of the vector is to encode a protein that will provoke a clinically useful immune response.

Another important issue related to the use of lentiviral vectors is that of possible cytopathogenicity upon exposure to some cytotoxic viral proteins. Exposure to certain HIV-1 proteins may induce cell death or functional unresponsiveness in T cells. Likewise, the possibility of generating replication-competent, virulent virus by recombination is often a concern. Accordingly, there remains a need for improved treatments of HIV.

SUMMARY OF THE INVENTION

In one aspect, a method of treating cells infected with HIV is provided. The method variously includes contacting peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting is carried out ex vivo; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. The method may further include infusing the transduced PBMC into a subject. The subject may be a human. The stimulatory agent may include any agent suitable for stimulating a T cell response in a subject. In embodiments, the stimulatory agent is a peptide or mixture of peptides, and

in embodiments includes a gag peptide. The stimulatory agent may also include a vaccine. The vaccine may be a HIV vaccine, and in embodiments, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In further embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In further embodiments, the at least one genetic element may include a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence includes any HIV sequence suitable for targeting by a viral delivery system. In embodiments, the HIV RNA sequence includes one or more of a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element includes any genetic element capable of being expressed by a viral delivery system. In embodiments, the at least one genetic element includes a microRNA or a shRNA. In further embodiments, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a subject is disclosed. The method variously includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and obtaining peripheral blood mononuclear cells (PBMC). The method further includes contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. In embodiments, the method further involves infusing the transduced PBMC into a subject. The subject may be a human. The first and second stimulatory agents may be the same or different. The first and second stimulatory agents may include one or more of a peptide or mixture of peptides. In embodiments, at least one of the first and second stimulatory agents includes a gag peptide. The at least one of the first and second stimulatory agents may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element may include a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least

90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector is disclosed. The lentiviral vector includes at least one encoded genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5. The at least one encoded genetic element may also comprise at least one small RNA capable of targeting an HIV RNA sequence. In another aspect, the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one encoded genetic element may include a microRNA or a shRNA. The at least one encoded genetic element may include a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein preferably optimized for infecting a cell; and at least one helper plasmid for expressing genes of interest. In embodiments, the genes of interest include one or more of gag, pol, and rev genes. In embodiments, the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line. In further embodiments, a lentiviral particle is produced by the packaging cell line. In embodiments, the lentiviral particle is capable of modulating production of a target of interest. In embodiments, the target of interest is any of

chemokine receptor CCR5 or an HIV RNA sequence. The system may further include a first helper plasmid and a second helper plasmid. In embodiments, a first helper plasmid expresses the gag and pol genes, and a second helper plasmid expresses the rev gene.

In another aspect, a lentiviral particle capable of infecting a cell is provided. The lentiviral particle includes an envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. In embodiments, the envelope protein may be optimized for infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is provided. The modified cell includes any cell capable of being infected with a lentiviral vector system for use in accordance with present aspects and embodiments. In embodiments, the cell is a CD4+ T cell that is infected with a lentiviral particle. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen. In embodiments, the HIV antigen includes a gag antigen. In embodiments, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is provided. The method variously includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) and determining a first quantifiable measurement associated with at least one factor associated with the PBMC; contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent, and determining a second measurement associated with the at least one factor associated with the PBMC, whereby when the second quantifiable measurement is higher than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may include any of T cell proliferation or IFN gamma production.

The foregoing general description and following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following brief description of the drawings and detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts a flow diagram of an ex vivo treatment method of the present disclosure.

FIG. **2** depicts CD4+ T cell alteration and prevention of new infection in accordance with the present disclosure.

FIG. **3** depicts an exemplary lentiviral vector system comprised of a therapeutic vector, a helper plasmid, and an envelope plasmid. The therapeutic vector shown here is a preferred therapeutic vector, which is also referred to herein as AGT103, and contains miR30CCR5-miR21Vif-miR185-Tat.

FIGS. **4**A-**4**C depict an exemplary 3-vector lentiviral vector system in a circularized form.

FIGS. **5**A-**5**D depict an exemplary 4-vector lentiviral vector system in a circularized form.

FIG. **6** depicts exemplary vector sequences. Positive (i.e., genomic) strand sequences of the promoter and miR cluster were developed for inhibiting the spread of CCR5-tropic HIV strains. Sequences that are not underlined comprise the EF-1alpha promoter of transcription that was selected as being a preferable promoter for this miR cluster. Sequences that are underlined show the miR cluster consisting of miR30 CCR5, miR21 Vif, and miR185 Tat (as shown collectively in SEQ ID NO: 33).

FIG. **7** depicts exemplary lentiviral vector constructs according to various aspects of this disclosure.

FIGS. **8**A-**8**B show knockdown of CCR5 by an experimental vector and corresponding prevention of R5-tropic HIV infection in AGTc120 cells. FIG. **8**A shows CCR5 expression in AGTc120 cells with or without AGT103 lentivirus vector. FIG. **8**B shows the sensitivity of transduced AGTc120 cells to infection with a HIV BaL virus stock that was expressing green fluorescent protein (GFP) fused to the Nef gene of HIV.

FIGS. **9**A-**9**B depict data demonstrating regulation of CCR5 expression by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. FIG. **9**A shows screening data for potential candidates. FIG. **9**B shows CCR5 knock-down data following transduction with CCR5 shRNA-1 (SEQ ID NO: 16).

FIGS. **10**A-**10**B depict data demonstrating regulation of HIV components by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. FIG. **10**A shows knock-down data for the rev/tat target gene. FIG. **10**B shows knock-down data for the gag target gene.

FIG. **11** depicts data demonstrating that AGT103 reduces expression of Tat protein expression in cells transfected with an HIV expression plasmid, as described herein.

FIGS. **12**A-**12**B depict data demonstrating regulation of HIV components by synthetic microRNA sequences in a lentiviral vector of the present disclosure. In FIG. **12**A, tat knock-down data is shown. In FIG. **12**B, vif knock-down data is shown.

FIG. **13** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure.

FIG. **14** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure containing either a long or short WPRE sequence.

FIG. **15** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure with or without a WPRE sequence.

FIG. **16** depicts data demonstrating regulation of CCR5 expression by a CD4 promoter regulating synthetic microRNA sequences in a lentiviral vector of the present disclosure.

FIG. **17** depicts data demonstrating detection of HIV Gag-specific CD4 T cells.

FIGS. **18**A-**18**E depict data demonstrating HIV-specific CD4 T cell expansion and lentivirus transduction. FIG. **18**A shows an exemplary schedule of treatment. FIG. **18**B shows IFN-gamma production in CD4-gated T cells, as described herein. FIG. **18**C shows IFN-gamma production and GFP expression in CD4-gated T cells, as described herein. FIG. **18**D shows frequency of HIV-specific CD4+ T cells, as described herein. FIG. **18**E shows IFN-gamma production from PBMCs post-vaccination, as described herein.

FIGS. **19**A-**19**C depict data demonstrating a functional assay for a dose response of increasing AGT103-GFP and inhibition of CCR5 expression. FIG. **19**A shows dose response data for increasing amounts of AGT103-GFP. FIG. **19**B shows normally distributed populations in terms of CCR5 expression. FIG. **19**C shows percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP.

FIGS. **20**A-**20**B depict data demonstrating AGT103 transduction efficiency for primary human CD4+ T cells. FIG. **20**A shows frequency of transduced cells (GFP-positive) by FACS, as described herein. FIG. **20**B shows number of vector copies per cell, as described herein.

FIG. **21** depicts data demonstrating AGT103 inhibition of HIV replication in primary CD4+ T cells, as described herein.

FIG. **22** depicts data demonstrating AGT103 protection of primary human $CD4^+$ T cells from HIV-induced depletion.

FIGS. **23**A-D depict data demonstrating generation of a CD4+ T cell population that is highly enriched for HIV-specific, AGT103-transduced CD4 T cells. FIG. **23**A shows CD4 and CD8 expression profiles for cell populations, as described herein. FIG. **23**B shows CD4 and CD8 expression profiles for cell populations, as described herein. FIG. **23**C shows IFN-gamma and CD4 expression profiles for cell populations, as described herein. FIG. **23**D shows IFN-gamma and GFP expression profiles for cell populations, as described herein.

DETAILED DESCRIPTION

Overview

Disclosed herein are methods and compositions for treating and/or preventing human immunodeficiency virus (HIV) disease to achieve a functional cure. The methods and compositions include integrating lentivirus, non-integrating lentivirus, and related viral vector technology as described below.

Disclosed herein are therapeutic viral vectors (e.g., lentiviral vectors), immunotherapies, and methods for their use for treating HIV infection. In embodiments, methods and compositions for achieving a functional cure for HIV infection are provided. As depicted in FIG. **1** herein, the various aspects and embodiments include a first stimulation event, for example a first therapeutic immunization with vaccines intended to produce strong immune responses against HIV in HIV-infected patients, for example with stable suppression of viremia due to daily administration of HAART. In embodiments, the first stimulation event enriches the fraction of HIV-specific CD4 T cells. This is followed by (1) isolating peripheral leukocytes by leukapheresis or purifying PBMC from venous blood, (2) a second stimulating event, for example re-stimulating CD4 T cells ex vivo with a suitable stimulatory agent, such as any vaccine or protein, for example, HIV or HIV-related peptides, (3) performing therapeutic lentivirus transduction, ex vivo T cell culture, and (4) re-infusion back into the original patient.

The various methods and compositions can be used to prevent new cells, such as CD4+ T cells, from becoming infected with HIV. For example as illustrated in FIG. **2**, to prevent new cells from becoming infected, CCR5 expression can be targeted to prevent virus attachment. Further, destruction of any residual infecting viral RNA can also be targeted. In respect of the foregoing, and in reference to FIG. **2** herein, compositions and methods are provided to stop the HIV viral cycle in cells that have already become infected with HIV. To stop the HIV viral cycle, viral RNA produced by latently-infected cells, such as latently-infected CD4+ T cells, is targeted.

Previous efforts to achieve a cure for HIV have fallen short due to, among others, the failure to obtain sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications. When this number is below a critical threshold, a functional cure as described herein is not achieved. For example, upon termination of antiretroviral therapy HIV re-emergence generally follows. Thereafter, patients often experience rapid destruction of HIV-specific CD4 T cells, and also followed by return to progression of disease despite prior genetic therapy. By employing therapeutic immunization in accordance with the compositions and methods described herein, a new HIV treatment regimen has been developed including, in various embodiments, a functional cure.

Definitions and Interpretation

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g.: Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

As used herein, the term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

As used herein, the terms "administration of" or "administering" an active agent means providing an active agent of the invention to the subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

As used herein, the term "AGT103" refers to a particular embodiment of a lentiviral vector that contains a miR30-CCR5/miR21-Vif/miR185-Tat microRNA cluster sequence, as detailed herein.

As used herein, the term "AGT103T" refers to a cell that has been transduced with a lentivirus that contains the AGT103 lentiviral vector.

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other

integer or group of integers. Further, as used herein, the term "includes" means includes without limitation.

As used herein, the term "engraftment" refers to the ability for one skilled in the art to determine a quantitative level of sustained engraftment in a subject following infusion of a cellular source (see for e.g.: Rosenberg et al., *N. Engl. J. Med.* 323:570-578 (1990); Dudley et al., *J. Immunother.* 24:363-373 (2001); Yee et al., *Curr. Opin. Immunol.* 13:141-146 (2001); Rooney et al., *Blood* 92:1549-1555 (1998)).

The terms, "expression," "expressed," or "encodes" refer to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

The term "functional cure", as referenced above, and further defined herein, refers to a state or condition wherein HIV+ individuals who previously required ongoing HIV therapies such as cART or HAART, may survive with low or undetectable virus replication using lower doses, intermittent doses, or discontinued dosing of such HIV therapies. An individual may be said to have been "functionally cured" while still requiring adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of all or virtually all HIV such that no recurrence is detected within a specified time frame, for example, 1 month, 3 months, 6 months, 1 year, 3 years, and 5 years, and all other time frames as may be defined.

The term "HIV vaccine" encompasses immunogens plus vehicle plus adjuvant intended to elicit HIV-specific immune responses. The term "HIV vaccine" is within the meaning of the term "stimulatory agent" as described herein. A "HIV vaccine" may include purified or whole inactivated virus particles that may be HIV or a recombinant virus vectors capable of expressing HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides, in addition to recombinant bacterial vectors, plasmid DNA or RNA capable of directing cells to producing HIV proteins, glycoproteins or protein fragments able to elicit specific immunity. Alternately, specific methods for immune stimulation including anti-CD3/CD28 beads, T cell receptor-specific antibodies, mitogens, superantigens and other chemical or biological stimuli may be used to activate dendritic, T or B cells for the purposes of enriching HIV-specific CD4 T cells prior to transduction or for in vitro assay of lentivirus-transduced CD4 T cells. Activating substances may be soluble, polymeric assemblies, liposome or endosome-based or linked to beads. Cytokines including interleukin-2, 6, 7, 12, 15, 23 or others may be added to improve cellular responses to stimuli and/or improve the survival of CD4 T cells throughout the culture and transduction intervals. Alternately, and without limiting any of the foregoing, the term "HIV vaccine" encompasses the MVA/HIV62B vaccine and variants thereof. The MVA/HIV62B vaccine is a known highly attenuated double recombinant MVA vaccine. The MVA/HIV62B vaccine was constructed through the insertion of HIV-1 gag-pol and env sequences into the known MVA vector (see: for e.g.: Goepfert et al. (2014) J. Infect. Dis. 210(1): 99-110, and see WO2006026667, both of which are incorporated herein by reference). The term "HIV vaccine" also includes any one or more vaccines provided in Table 1, below.

TABLE 1

IAVI Clinical Trial ID*	Prime**
HVTN 704 AMP	VRC-HIVMAB060-00-AB
VAC89220HPX2004	Ad26.Mos.HIV Trivalent
01-I-0079	VRC4302
04/400-003-04	APL 400-003 GENEVAX-HIV
10-1074	10-1074
87 I-114	gp160 Vaccine (Immuno-AG)
96-I-0050	APL 400-003 GENEVAX-HIV
ACTG 326; PACTG 326	ALVAC vCP1452
Ad26.ENVA.01	Ad26.EnvA-01
Ad26.ENVA.01 Mucosal/IPCAVD003	Ad26.EnvA-01
Ad5HVR48.ENVA.01	Ad5HVR48.ENVA.01
ANRS VAC 01	ALVAC vCP125
ANRS VAC 02	rgp 160 + peptide V3 ANRS VAC 02
ANRS VAC 03	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 04	LIPO-6
ANRS VAC 04 bis	LIPO-6
ANRS VAC 05	ALVAC vCP125
ANRS VAC 06	ALVAC vCP125
ANRS VAC 07	ALVAC vCP300
ANRS VAC 08	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 09	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 09 bis	LIPO-6
ANRS VAC 10	ALVAC vCP1452
ANRS VAC 12	LPHIV1
ANRS VAC 14	gp160 MN/LAI
ANRS VAC 16	LPHIV1
ANRS VAC 17	LIPO-6
ANRS VAC 18	LIPO-5
APL 400-003RX101	APL 400-003 GENEVAX-HIV
AVEG 002	HIVAC-1e
AVEG 002A	HIVAC-1e
AVEG 002B	HIVAC-1e
AVEG 003	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 003A	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 003B	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 004	gp160 Vaccine (Immuno-AG)

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
AVEG 004A	gp160 Vaccine (Immuno-AG)
AVEG 004B	gp160 Vaccine (Immuno-AG)
AVEG 005A/B	Env 2-3
AVEG 005C	Env 2-3
AVEG 006X; VEU 006	MN rgp120
AVEG 007A/B	rgp120/HIV-1 SF-2
AVEG 007C	rgp120/HIV-1 SF-2
AVEG 008	HIVAC-1e
AVEG 009	MN rgp120
AVEG 010	HIVAC-1e
AVEG 011	UBI HIV-1 Peptide Immunogen, Multivalent
AVEG 012A/B	ALVAC vCP125
AVEG 013A	gp160 Vaccine (Immuno-AG)
AVEG 013B	gp160 Vaccine (Immuno-AG)
AVEG 014A/B	TBC-3B
AVEG 014C	TBC-3B
AVEG 015	rgp120/HIV-1 SF-2
AVEG 016	MN rgp120
AVEG 016A	MN rgp120
AVEG 016B	MN rgp120
AVEG 017	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
AVEG 018	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
AVEG 019	p17/p24:Ty-VLP
AVEG 020	gp120 C4-V3
AVEG 021	P3C541b Lipopeptide
AVEG 022	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 022A	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 023	UBI HIV-1 Peptide Immunogen, Multivalent
AVEG 024	rgp120/HIV-1 SF-2
AVEG 026	ALVAC vCP300
AVEG 027	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 028	Salmonella typhi CVD 908-HIV-1 LAI gp 120
AVEG 029	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 031	APL 400-047
AVEG 032	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 033	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 034/034A	ALVAC vCP1433
AVEG 036	MN rgp120
AVEG 038	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 201	rgp120/HIV-1 SF-2
AVEG 202/HIVNET 014	ALVAC-HIV MN120TMG strain (vCP205)
C060301	GTU-MultiHIV
C86P1	HIV gp140 ZM96
Cervico-vaginal CN54gp140-hsp70 Conjugate Vaccine (TL01)	CN54gp140
CM235 and SF2gp120	CM235 (ThaiE) gp120 plus SF2(B) gp120
CM235gp120 and SF2gp120	CM235 (ThaiE) gp120 plus SF2(B) gp120
CombiHIVvac (KombiVIChvak)	CombiHIVvac
CRC282	P2G12
CRO2049/CUT*HIVAC001	GTU-MultiHIV
CUTHIVAC002	DNA-C CN54ENV
DCVax-001	DCVax-001
DNA-4	DNA-4
DP6?001	DP6?001 DNA
DVP-1	EnvDNA
EN41-UGR7C	EN41-UGR7C
EnvDNA	EnvDNA
EnvPro	EnvPro
EuroNeut41	EN41-FPA2
EV01	NYVAC-C
EV02 (EuroVacc 02)	DNA-C
EV03/ANRSVAC20	DNA-C
Extention HVTN 073E/SAAVI 102	Sub C gp140
F4/AS01	F4/AS01
FIT Biotech	GTU-Nef
Guangxi CDC DNA vaccine	Chinese DNA
HGP-30 memory responses	HGP-30
HIV-CORE002	ChAdV63.HIVconsv
HIV-POL-001	MVA-mBN32
HIVIS 01	HIVIS-DNA
HIVIS 02	MVA-CMDR
HIVIS 03	HIVIS-DNA
HIVIS 05	HIVIS-DNA
HIVIS06	HIVIS-DNA
HIVIS07	HIVIS-DNA
HIVNET 007	ALVAC-HIV MN120TMG strain (vCP205)

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
HIVNET 026	ALVAC vCP1452
HPTN 027	ALVAC-HIV vCP1521
HVRF-380-131004	Vichrepol
HVTN 039	ALVAC vCP1452
HVTN 040	AVX101
HVTN 041	rgp120w61d
HVTN 042/ANRS VAC 19	ALVAC vCP1452
HVTN 044	VRC-HIVDNA009-00-VP
HVTN 045	pGA2/JS7 DNA
HVTN 048	EP HIV-1090
HVTN 049	Gag and Env DNA/PLG microparticles
HVTN 050/Merck 018	MRKAd5 HIV-1 gag
HVTN 052	VRC-HIVDNA009-00-VP
HVTN 054	VRC-HIVADV014-00-VP
HVTN 055	TBC-M335
HVTN 056	MEP
HVTN 057	VRC-HIVDNA009-00-VP
HVTN 059	AVX101
HVTN 060	HIV-1 gag DNA
HVTN 063	HIV-1 gag DNA
HVTN 064	EP HIV-1043
HVTN 065	pGA2/JS7 DNA
HVTN 067	EP-1233
HVTN 068	VRC-HIVADV014-00-VP
HVTN 069	VRC-HIVDNA009-00-VP
HVTN 070	PENNVAX-B
HVTN 071	MRKAd5 HIV-1 gag
HVTN 072	VRC-HIVDNA044-00-VP
HVTN 073	SAAVI DNA-C2
HVTN 076	VRC-HIVDNA016-00-VP
HVTN 077	VRC-HIVADV027-00-VP
HVTN 078	NYVAC-B
HVTN 080	PENNVAX-B
HVTN 082	VRC-HIVDNA016-00-VP
HVTN 083	VRC-HIVADV038-00-VP
HVTN 084	VRC-HIVADV054-00-VP
HVTN 085	VRC-HIVADV014-00-VP
HVTN 086, SAAVI 103	SAAVI MVA-C
HVTN 087	HIV-MAG
HVTN 088	Oligomeric gp140/MF59
HVTN 090	VSV-Indiana HIV gag vaccine
HVTN 092	DNA-HIV-PT123
HVTN 094	GEO-D03
HVTN 096	DNA-HIV-PT123
HVTN 097	ALVAC-HIV vCP1521
HVTN 098	PENNVAX-GP
HVTN 100	ALVAC-HIV-C (vCP2438)
HVTN 101	DNA-HIV-PT123
HVTN 102	DNA-HIV-PT123
HVTN 104	VRC-HIVMAB060-00-AB
HVTN 105	AIDSVAX B/E
HVTN 106	DNA Nat-B env
HVTN 110	Ad4-mgag
HVTN 112	HIV-1 nef/tat/vif, env pDNA vaccine
HVTN 114; GOVX-B11	AIDSVAX B/E
HVTN 116	VRC-HIVMAB060-00-AB
HVTN 203	ALVAC vCP1452
HVTN 204	VRC-HIVDNA016-00-VP
HVTN 205	pGA2/JS7 DNA
HVTN 502/Merck 023 (Step Study)	MRKAd5 HIV-1 gag/pol/nef
HVTN 503 (Phambili)	MRKAd5 HIV-1 gag/pol/nef
HVTN 505	VRC-HIVDNA016-00-VP
HVTN 702	ALVAC-HIV-C (vCP2438)
HVTN 703 AMP	VRC-HIVMAB060-00-AB
HVTN 908	pGA2/JS7 DNA
IAVI 001	DNA.HIVA
IAVI 002	DNA.HIVA
IAVI 003	MVA.HIVA
IAVI 004	MVA.HIVA
IAVI 005	DNA.HIVA
IAVI 006	DNA.HIVA
IAVI 008	MVA.HIVA
IAVI 009	DNA.HIVA
IAVI 010	DNA.HIVA
IAVI 011	MVA.HIVA
IAVI 016	MVA.HIVA
IAVI A001	tgAAC09
IAVI A002	tgAAC09

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
IAVI A003	AAV1-PG9
IAVI B001	Ad35-GRIN/ENV
IAVI B002	Adjuvanted GSK investigational HIV vaccine formulation 1
IAVI B003	Ad26.EnvA-01
IAVI B004	HIV-MAG
IAVI C001	ADVAX
IAVI C002	ADMVA
IAVI C003	ADMVA
IAVI C004/DHO-614	ADVAX
IAVI D001	TBC-M4
IAVI N004 HIV-CORE 004	Ad35-GRIN
IAVI P001	ADVAX
IAVI P002	ADVAX
IAVI R001	rcAd26.MOS1.HIVEnv
IAVI S001	SeV-G
IAVI V001	VRC-HIVDNA016-00-VP
IAVI V002	VRC-HIVDNA016-00-VP
IDEA EV06	DNA-HIV-PT123
IHV01	Full-Length Single Chain (FLSC)
IMPAACT P1112	VRC-HIVMAB060-00-AB
IPCAVD006	MVA mosaic
IPCAVD008	Trimeric gp140
IPCAVD009	Ad26.Mos.HIV Trivalent
IPCAVD010	Ad26.Mos.HIV Trivalent
ISS P-001	Tat vaccine
ISS P-002	Tat vaccine
LFn-p24 vaccine	LFn-p24
MCA-0835	3BNC117
Merck V520-007	Ad-5 HIV-1 gag (Merck)
MRC V001	rgp120w61d
MRK Ad5	Ad-5 HIV-1 gag (Merck)
MRKAd5 + ALVAC	MRKAd5 HIV-1 gag
Mucovac2	CN54gp140
MV1-F4	Measles Vector - GSK
MYM-V101	Virosome-Gp41
NCHECR-AE1	pHIS-HIV-AE
PACTG 230	AIDSVAX B/E
PAVE100	VRC-HIVDNA016-00-VP
PEACHI-04	ChAdV63.HIVconsv
PedVacc001 & PedVacc002	MVA.HIVA
PolyEnv1	PolyEnv1
PXVX-HIV-100-001	Ad4-mgag
RISVAC02	MVA-B
RisVac02 boost	MVA-B
RV 124	ALVAC-HIV MN120TMG strain (vCP205)
RV 132	ALVAC-HIV vCP1521
RV 135	ALVAC-HIV vCP1521
RV 138; B011	ALVAC-HIV MN120TMG strain (vCP205)
RV 144	ALVAC-HIV vCP1521
RV 151/WRAIR 984	LFn-p24
RV 156	VRC-HIVDNA009-00-VP
RV 156A	VRC-HIVDNA009-00-VP
RV 158	MVA-CMDR
RV 172	VRC-HIVDNA016-00-VP
RV 305	ALVAC-HIV vCP1521
RV 306	ALVAC-HIV vCP1521
RV 328	AIDSVAX B/E
RV 365	MVA-CMDR
RV262	Pennvax-G
SG06RS02	HIV gp140 ZM96
TAB9	TAB9
TaMoVac II	HIVIS-DNA
TAMOVAC-01-MZ	HIVIS-DNA
Tiantan vaccinia HIV Vaccine	Chinese DNA
Tiantan vaccinia HIV Vaccine and DNA	Chinese DNA
TMB-108	Ibalizumab
UBI HIV-1 MN China	UBI HIV-1 Peptide Immunogen, Multivalent
UBI HIV-1MN octameric - Australia study	UBI HIV-1 Peptide Immunogen, Multivalent
UBI V106	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
UCLA MIG-001	TBC-3B
UCLA MIG-003	ALVAC-HIV MN120TMG strain (vCP205)
UKHVCSpoke003	DNA - CN54ENV and ZM96GPN
V24P1	HIV p24/MF59 Vaccine
V3-MAPS	V3-MAPS
V520-016	MRKAd5 HIV-1 gag/pol/nef
V520-027	MRKAd5 HIV-1 gag/pol/nef

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
V526-001 MRKAd5 and MRKAd6 HIV-1 Trigene Vaccines	MRKAd5 HIV-1 gag/pol/nef
VAX 002	AIDSVAX B/B
VAX 003	AIDSVAX B/E
VAX 004	AIDSVAX B/B
VRC 004 (03-I-0022)	VRC-HIVDNA009-00-VP
VRC 006 (04-I-0172)	VRC-HIVADV014-00-VP
VRC 007 (04-I-0254)	VRC-HIVDNA016-00-VP
VRC 008 (05-I-0148)	VRC-HIVDNA016-00-VP
VRC 009 (05-I-0081)	VRC-HIVDNA009-00-VP
VRC 010 (05-I-0140)	VRC-HIVADV014-00-VP
VRC 011(06-I-0149)	VRC-HIVDNA016-00-VP
VRC 012 (07-I-0167)	VRC-HIVADV027-00-VP
VRC 015 (08-I-0171)	VRC-HIVADV014-00-VP
VRC 016	VRC-HIVDNA016-00-VP
VRC 602	VRC-HIVMAB060-00-AB
VRC 607	VRCHIVMAB080-00-AB
VRC01LS	VRCHIVMAB080-00-AB
VRI01	MVA-B
X001	CN54gp140

*IAVI is the International AIDS Vaccine Initiative, whose clinical trials database is publicly available at http://www.iavi.org/trials-database/trials.
**As used herein, the term "Prime" refers to the composition initially used as an immunological inoculant in a given clinical trial as referenced in Table 1 herein.

The term "in vivo" refers to processes that occur in a living organism. The term "ex vivo" refers to processes that occur outside of a living organism. For example, in vivo treatment refers to treatment that occurs within a patient's body, while ex vivo treatment is one that occurs outside of a patient's body, but still uses or accesses or interacts with tissues from that patient. Thereafter, an ex vivo treatment step may include a subsequent in vivo treatment step.

The term "miRNA" refers to a microRNA, and also may be referred to herein as "miR". The term "microRNA cluster" refers to at least two microRNAs that are situate on a vector in close proximity to each other and are co-expressed.

The term "packaging cell line" refers to any cell line that can be used to express a lentiviral particle.

The term "percent identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of ordinary skill in the art) or by visual inspection. Depending on the application, the "percent identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453(1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg-.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score =100, word length =12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score =50, wordlength =3 to obtain amino acid sequences homologous to the protein molecules

of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See ncbi.nlm-.nih.gov.

As used herein, "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

As used herein, a "pharmaceutically acceptable carrier" refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see, e.g., Berge et al. (1977) *J Pharm Sci* 66:1-19).

As used herein, the term "SEQ ID NO" is synonymous with the term "Sequence ID No."

As used herein, "small RNA" refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). "Small RNA" of the disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, for example through pathways that result in the destruction of the target gene mRNA.

As used herein, the term "stimulatory agent" refers to any exogenous agent that can stimulate an immune response, and includes, without limitation, a vaccine, a HIV vaccine, and HIV or HIV-related peptides. A stimulatory agent can preferably stimulate a T cell response.

As used herein, the term "subject" includes a human patient but also includes other mammals. The terms "subject," "individual," "host," and "patient" may be used interchangeably herein.

The term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present invention, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient's condition or its severity, and the age, weight, etc., of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, e.g., the route of administration, the condition of the subject, as well as other factors understood by those in the art.

As used herein, the term "therapeutic vector" is synonymous with a lentiviral vector such as the AGT103 vector.

The term "treatment" or "treating" generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

The term "vaccine", which is used interchangeably with the term "therapeutic vaccine" refers to an exogenous agent that can elicit an immune response in an individual and includes, without limitation, purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragents, or virus-like particles (VLPs).

Description of Aspects of the Disclosure

As detailed herein, in one aspect, a method of treating cells infected with HIV is provided. The method generally includes contacting peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting step is carried out ex vivo; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a period of time sufficient to achieve such transduction. In embodiments, the transduced PBMC are cultured from about 1 to about 35 days. The method may further include infusing the transduced PBMC into a subject. The subject may be a human. The stimulatory agent may include a peptide or mixture of peptides, and in a preferred embodiment includes a gag peptide. The stimulatory agent may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element may include a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In other embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or variants thereof. The at least one genetic element may include at least one of a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGT-GAGCGACTGTAAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACA-GTGAGCGACTGTAAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with CATCTCCATGGCTGTACCACCTT-GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT

CTCATGGAGTTCAGAAGAACACATCCGCACT-GACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with GGGCCTGGCTCGAGCAGGGGGC-GAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC-CCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTT-GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACT-GACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGAT-TCCGCTTCTTC CTGCCATAGCGTGGTCCCCTC-CCCTATGGCAGGCAGAAGCGGCACCTTCCCTC-CCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGT-GAGCGACTGTAAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTT-GTCGGGGGATGTGTACTTCTGAACTT GTGTT-GAATCTCATGGAGTTCAGAAGAACACATCCGCACT-GACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCA-GGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-GAAGCGGCACCTTCCCTCCCAATGA CCGCGTCT-TCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACA-GTGAGCGACTGTAAACTGAGCTTGCTCT ACTGT-GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTG-TACCACCTTGTCGGGGGATGTGTA CTTCTGAACTT-GTGTTGAATCTCATGGAGTTCAGAAGAACACATC-CGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTG-GCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTC-CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-GAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a subject is disclosed. The method generally includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC). The method further includes contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a period of time sufficient to achieve transduction. The method may further include further enrichment of the PBMC, for example, by preferably enriching the PBMC for CD4+ T cells. In embodiments, the transduced PBMC are cultured from about 1 to about 35 days. The method may further involve infusing the transduced PBMC into a subject. The subject may be a human. The first and second stimulatory agents may be the same or different from each other. The at least one of the first and second stimulatory agents may include a peptide or mixture of peptides. In embodiments, at least one of the first and second stimulatory agents includes a gag peptide. The at least one of the first and second stimulatory agents may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In embodiments, the first stimulatory agent is a HIV vaccine and the second stimulatory agent is a gag peptide.

In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or variants thereof. The at least one genetic element may include a microRNA or a shRNA, or a cluster thereof. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGT-GAGCGACTGTAAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACA-GTGAGCGACTGTAAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATG-GCTGTACCACCTTGTCGGGGGATGTGTACTTCT-GAACTTGTGTTGAAT CTCATGGAGTTCA-GAAGAACACATCCGCACTGACATTTTGGTATCTTT-CATCTGACCA (SEQ ID NO: 2); or at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with GGGCCTG-GCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT-GCCATAGCGTGG TCCCCTCCCCTATGGCAGGCA-GAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC-GT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTG-TACCACCTTGTCGGGGGATGTGTACTTCTGAACTT-GTGTTGAAT CTCATGGAGTTCAGAAGAACACATC-CGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGC-GAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTC-CCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC-CCTCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%,

at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector is disclosed. The lentiviral vector includes at least one encoded genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence. In another aspect a lentiviral vector is disclosed in the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one encoded genetic element may include a microRNA or a shRNA. The at least one encoded genetic element may include a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTC CGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector as described herein; at least one envelope plasmid for expressing an envelope protein preferably optimized for infecting a cell; and at least one helper plasmid for expressing a gene of interest, for example any of gag, pol, and rev genes, wherein when the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, wherein a lentiviral particle is produced by the packaging cell, wherein the lentiviral particle is capable of modulating a target sequence of interest, for example inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

In another aspect, a lentiviral particle capable of infecting a cell is disclosed. The lentiviral particle includes at least one envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. The envelope protein may be optimized for infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is disclosed. In embodiments, the modified cell is a CD4+ T cell. In embodiments, the CD4+ T cell is infected with a lentiviral particle as described herein. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen based on the prior immunization with a stimulatory agent. In a further preferred embodiment, the HIV antigen that is recognized by the CD4+ T cell includes a gag antigen. In a further preferred embodiment, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is disclosed. The method generally includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) and determining a first quantifiable measurement associated with at least one factor associated with the PBMC; contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent, and determining a second measurement associated with the at least one factor associated with the PBMC, whereby when the second quantifiable measurement is different (e.g., higher) than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may be T cell proliferation or IFN gamma production.

Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus, which is also commonly referred to as "HIV", is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS is a condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending upon the HIV subtype. Infection with HIV occurs by the transfer of bodily fluids, including but not limited to blood, semen, vaginal fluid, pre-ejaculate, saliva, tears, lymph or cerebro-spinal fluid, or breast milk. HIV may be present in an infected individual as both free virus particles and within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells, although tropism can vary among HIV subtypes. Immune cells that may be specifically susceptible to HIV infection include but are not limited to CD4+ T cells, macrophages, and dendritic cells. HIV infection leads to low levels of CD4+ T cells through a number of mechanisms, including but not limited to apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections and cancer.

Structurally, HIV is distinct from many other retroviruses. The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and at least nine genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth tev, which is a fusion of tat, env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles.

HIV replicates primarily in CD4 T cells, and causes cellular destruction or dysregulation to reduce host immunity. Because HIV establishes infection as an integrated provirus and may enter a state of latency wherein virus expression in a particular cell decreases below the level for cytopathology affecting that cell or detection by the host immune system, HIV is difficult to treat and has not been eradicated even after prolonged intervals of highly active antiretroviral therapy (HAART). In the vast majority of cases, HIV infection causes fatal disease although survival may be prolonged by HAART.

A major goal in the fight against HIV is to develop strategies for curing disease. Prolonged HAART has not accomplished this goal, so investigators have turned to alternative procedures. Early efforts to improve host immunity by therapeutic immunization (using a vaccine after infection has occurred) had marginal or no impact. Likewise, treatment intensification had moderate or no impact.

Some progress has been made using genetic therapy, but positive results are sporadic and found only among rare human beings carrying defects in one or both alleles of the gene encoding CCR5 (chemokine receptor), which plays a critical role in viral penetration of host cells. However, many investigators are optimistic that genetic therapy holds the best promise for eventually achieving an HIV cure.

As disclosed herein, the methods and compositions of the invention are able to achieve a functional cure that may or may not include complete eradication of all HIV from the body. As mentioned above, a functional cure is defined as a state or condition wherein HIV+ individuals who previously required HAART, may survive with low or undetectable virus replication and using lower or intermittent doses of HAART, or are potentially able to discontinue HAART altogether. As used herein, a functional cure may still possibly require adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of HIV to prevent all possibility of recurrence.

The primary obstacles to achieving a functional cure lie in the basic biology of HIV itself. Virus infection deletes CD4 T cells that are critical for nearly all immune functions. Most importantly, HIV infection and depletion of CD4 T cells requires activation of individual cells. Activation is a specific mechanism for individual CD4 T cell clones that recognize pathogens or other molecules, using a rearranged T cell receptor.

In the case of HIV, infection activates a population of HIV-specific T cells that become infected and are consequently depleted before other T cells that are less specific for the virus, which effectively cripples the immune system's defense against the virus. The capacity for HIV-specific T cell responses is rebuilt during prolonged HAART; however, when HAART is interrupted the rebounding virus infection repeats the process and again deletes the virus-specific cells, resetting the clock on disease progression.

Clearly, a functional cure is only possible if enough HIV-specific CD4 T cells are protected to allow for a host's native immunity to confront and control HIV once HAART is interrupted. In one embodiment, the present invention provides methods and compositions for improving the effectiveness of genetic therapy to provide a functional cure of HIV disease. In another embodiment, the present invention provides methods and compositions for enhancing host immunity against HIV to provide a functional cure. In yet another embodiment, the present invention provides methods and compositions for enriching HIV-specific CD4 T cells in a patient to achieve a functional cure.

In one embodiment of the invention, treatment results in enriching a subject's HIV-specific CD4 T cells by about 100%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1000%.

Gene Therapy

Viral vectors are used to deliver genetic constructs to host cells for the purposes of disease therapy or prevention.

Genetic constructs can include, but are not limited to, functional genes or portions of genes to correct or complement existing defects, DNA sequences encoding regulatory proteins, DNA sequences encoding regulatory RNA molecules including antisense, short homology RNA, long noncoding RNA, small interfering RNA or others, and decoy sequences encoding either RNA or proteins designed to compete for critical cellular factors to alter a disease state. Gene therapy involves delivering these therapeutic genetic constructs to target cells to provide treatment or alleviation of a particular disease.

There are multiple ongoing efforts to utilize genetic therapy in the treatment of HIV disease, but thus far, the results have been poor. A small number of treatment successes were obtained in rare HIV patients carrying a spontaneous deletion of the CCR5 gene (an allele known as CCR5delta32).

Lentivirus-delivered nucleases or other mechanisms for gene deletion/modification may be used to lower the overall expression of CCR5 and/or help to lower HIV replication. At least one study has reported having success in treating the disease when lentivirus was administered in patients with a genetic background of CCR5delta32. However, this was only one example of success, and many other patients without the CCR5delta32 genotype have not been treated as successfully. Consequently, there is a substantial need to improve the performance of viral genetic therapy against HIV, both in terms of performance for the individual viral vector construct and for improved use of the vector through a strategy for achieving functional HIV cure.

For example, some existing therapies rely on zinc finger nucleases to delete a portion of CCR5 in an attempt to render cells resistant to HIV infection. However, even after optimal treatment, only 30% of T cells had been modified by the nuclease at all, and of those that were modified, only 10% of the total CD4 T cell population had been modified in a way that would prevent HIV infection. In contrast, the disclosed methods result in virtually every cell carrying a lentivirus transgene having a reduction in CCR5 expression below the level needed to allow HIV infection.

For the purposes of the disclosed methods, gene therapy can include, but is not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIMS-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

Immunotherapy

Historically, vaccines have been a go-to weapon against deadly infectious diseases, including smallpox, polio, measles, and yellow fever. Unfortunately, there is no currently approved vaccine for HIV. The HIV virus has unique ways of evading the immune system, and the human body seems incapable of mounting an effective immune response against it. As a result, scientists do not have a clear picture of what is needed to provide protection against HIV.

However, immunotherapy may provide a solution that was previously unaddressed by conventional vaccine approaches. Immunotherapy, also called biologic therapy, is a type of treatment designed to boost the body's natural defenses to fight infections or cancer. It uses materials either made by the body or in a laboratory to improve, target, or restore immune system function.

In some embodiments of the disclosed invention, immunotherapeutic approaches may be used to enrich a population of HIV-specific CD4 T cells for the purpose of increasing the host's anti-HIV immunity. In some embodiments of the disclosed invention, integrating or non-integrating lentivirus vectors may be used to transduce a host's immune cells for the purposes of increasing the host's anti-HIV immunity. In yet another embodiment of the invention, a vaccine comprising HIV proteins including but not limited to a killed particle, a virus-like particle, HIV peptides or peptide fragments, a recombinant viral vector, a recombinant bacterial vector, a purified subunit or plasmid DNA combined with a suitable vehicle and/or biological or chemical adjuvants to increase a host's immune responses may be used to enrich the population of virus-specific T cells or antibodies, and these methods may be further enhanced through the use of HIV-targeted genetic therapy using lentivirus or other viral vector.

Methods

In one aspect, the disclosure provides methods for using viral vectors to achieve a functional cure for HIV disease. The methods generally include immunotherapy to enrich the proportion of HIV-specific CD4 T cells, followed by lentivirus transduction to deliver inhibitors of HIV and CCR5 and CXCR4 as required.

In one embodiment, the methods include a first stimulation event to enrich a proportion of HIV-specific CD4 T cells. The first stimulation can include administration of one or more of any agent suitable for enriching a patient's HIV-specific CD4+ T cells including but not limited to a vaccine.

Therapeutic vaccines can include one or more HIV protein with protein sequences representing the predominant viral types of the geographic region where treatment is occurring. Therapeutic vaccines will include purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for immunization. Vaccinations may be administered according to standard methods known in the art and HIV patients may continue antiretroviral therapy during the interval of immunization and subsequent ex vivo lymphocyte culture including lentivirus transduction.

In some embodiments, HIV+ patients are immunized with an HIV vaccine, increasing the frequency of HIV-specific CD4 T cells by about 2, about 25, about 250, about 500, about 750, about 1000, about 1250, or about 1500-fold (or any amount in between these values). The vaccine may be any clinically utilized or experimental HIV vaccine, including the disclosed lentiviral, other viral vectors or other bacterial vectors used as vaccine delivery systems. In another embodiment, the vectors encode virus-like particles (VLPs) to induce higher titers of neutralizing antibodies. In another embodiment, the vectors encode peptides or peptide fragments associated with HIV including but not limited to gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and tev, as well as LTR, TAR, RRE, PE, SLIP, CRS, and INS. Alternatively, the HIV vaccine used in the disclosed methods may comprise purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), or biological or chemical adjuvants including cytokines and/or chemokines.

In one embodiment, the methods include ex vivo re-stimulation of CD4 T cells from persons or patients previ-

ously immunized by therapeutic vaccination, using purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for re-stimulation. Ex vivo re-stimulation may be performed using the same vaccine or immune stimulating compound used for in vivo immunization, or it may be performed using a different vaccine or immune stimulating compound than those used for in vivo immunization. Moreover, in some embodiments, the patient does not require prior therapeutic vaccination or re-stimulation of CD4 T cells if the individual has sufficiently high antigen-specific CD4 T cell responses to HIV proteins. In these embodiments, such a patient may only require administration of the disclosed viral vectors to achieve a functional cure.

In embodiments, peripheral blood mononuclear cells (PBMCs) are obtained by leukapheresis and treated ex vivo to obtain about 1×10^{10} CD4 T cells of which about 0.1%, about 1%, about 5% or about 10% or about 30% are both HIV-specific in terms of antigen responses, and HIV-resistant by virtue of carrying the therapeutic transgene delivered by the disclosed lentivirus vector. Alternatively, about 1×10^{7}, about 1×10^{8}, about 1×10^{9}, about 1×10^{10}, about 1×10^{11}, or about 1×10^{12} CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells are isolated for ex vivo re-stimulation.

The isolated CD4 T cells can be cultured in appropriate medium throughout re-stimulation with HIV vaccine antigens, which may include antigens present in the prior therapeutic vaccination. Antiretroviral therapeutic drugs including inhibitors of reverse transcriptase, protease or integrase may be added to prevent virus re-emergence during prolonged ex vivo culture. CD4 T cell re-stimulation is used to enrich the proportion of HIV-specific CD4 T cells in culture. The same procedure may also be used for analytical objectives wherein smaller blood volumes with peripheral blood mononuclear cells obtained by purification, are used to identify HIV-specific T cells and measure the frequency of this sub-population.

The PBMC fraction may be enriched for HIV-specific CD4 T cells by contacting the cells with HIV proteins matching or complementary to the components of the vaccine previously used for in vivo immunization. Ex vivo re-stimulation can increase the relative frequency of HIV-specific CD4 T cells by about 5, about 10, 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold.

The methods additionally include combining in vivo therapeutic immunization and ex vivo re-stimulation of CD4 T cells with ex vivo lentiviral transduction and culturing.

Thus, in one embodiment, the re-stimulated PBMC fraction that has been enriched for HIV-specific CD4 T cells can be transduced with therapeutic anti-HIV lentivirus or other vectors and maintained in culture for a sufficient period of time for such transduction, for example from about 1 to about 21 days, including up to about 35 days. Alternatively, the cells may be cultured for about 1-about 18 days, about 1-about 15 days, about 1-about 12 days, about 1-about 9 days, or about 3-about 7 days. Thus, the transduced cells may be cultured for about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

In further embodiments, once the transduced cells have been cultured for a sufficient period of time, transduced CD4 T cells are infused back into the original patient. Infusion can be performed using various devices and methods known in the art. In some embodiments, infusion may be accompanied by pre-treatment with cyclophosphamide or similar compounds to increase the efficiency of re-engraftment.

In some embodiments, a CCR5-targeted therapy may be added to a subject's antiretroviral therapy regimen, which was continued throughout the treatment process. Examples of CCR5-targeted therapies include but are not limited to Maraviroc (a CCR5 antagonist) or Rapamycin (immunosuppressive agent that lowers CCR5). In some embodiments, the antiretroviral therapy may be ceased and the subject can be tested for virus rebound. If no rebound occurs, adjuvant therapy can also be removed and the subject can be tested again for virus rebound.

In various embodiments, continued virus suppression with reduced or no antiretroviral therapy including cART or HAART, and reduced or no adjuvant therapy for about 26 weeks can be considered a functional cure for HIV. Other definitions of a functional cure are described herein.

The lentiviral and other vectors used in the disclosed methods may encode at least one, at least two, at least three, at least four, or at least five genes, or at least six genes, or at least seven genes, or at least eight genes, or at least nine genes, or at least ten genes, or at least eleven genes, or at least twelve genes of interest. Given the versatility and therapeutic potential of HIV-targeted gene therapy, a viral vector of the invention may encode genes or nucleic acid sequences that include but are not limited to (i) an antibody directed to an antigen associated with an infectious disease or a toxin produced by the infectious pathogen, (ii) cytokines including interleukins that are required for immune cell growth or function and may be therapeutic for immune dysregulation encountered in HIV and other chronic or acute human viral or bacterial pathogens, (iii) factors that suppress the growth of HIV in vivo including CD8 suppressor factors, (iv) mutations or deletions of chemokine receptor CCR5, mutations or deletions of chemokine receptor CXCR4, or mutations or deletions of chemokine receptor CXCR5, (v) antisense DNA or RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, (vi) small interfering RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, or (vii) a variety of other therapeutically useful sequences that may be used to treat HIV or AIDS.

Additional examples of HIV-targeted gene therapy that can be used in the disclosed methods include, but are not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIMS-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

In some embodiments, a patient may be undergoing cART or HAART concurrently while being treated according to the methods of the invention. In other embodiments, a patient may undergo cART or HAART before or after being treated according to the methods of the invention.

In some embodiments, cART or HAART is maintained throughout treatment according to the methods of the invention and the patient may be monitored for HIV viral burden in blood and frequency of lentivirus-transduced CD4 T cells

in blood. Preferably, a patient receiving cART or HAART prior to being treated according to the methods of the invention is able to discontinue or reduce cART or HAART following treatment according to the methods of the invention.

For efficacy purposes, the frequency of transduced, HIV-specific CD4 T cells, which is a novel surrogate marker for gene therapy effects, may be determined, as discussed in more detail herein.

Compositions

In various aspects, the disclosure provides lentiviral vectors capable of delivering genetic constructs to inhibit HIV penetration of susceptible cells. For instance, one mechanism of action in accordance herein is to reduce mRNA levels for CCR5 and/or CXCR4 chemokine receptors for reducing the rates for viral entry into susceptible cells.

Alternatively, the disclosed lentiviral vectors are capable of inhibiting the formation of HIV-infected cells by reducing the stability of incoming HIV genomic RNA. And in yet another embodiment, the disclosed lentivirus vectors are capable of preventing HIV production from a latently infected cell, wherein the mechanism of action is to cause instability of viral RNA sequences through the action of inhibitory RNA including short-homology, small-interfering or other regulatory RNA species.

The therapeutic lentiviruses disclosed generally comprise at least one of two types of genetic cargo. First, the lentiviruses may encode genetic elements that direct expression of small RNA capable of inhibiting the production of chemokine receptors CCR5 and/or CXCR4 that are important for HIV penetration of susceptible cells. The second type of genetic cargo includes constructs capable of expressing small RNA molecules targeting HIV RNA sequences for the purpose of preventing reverse transcription, RNA splicing, RNA translation to produce proteins, or packaging of viral genomic RNA for particle production and spreading infection. An exemplary structure is diagrammed in FIG. **3**.

As shown in FIG. **3** (top panel), an exemplary construct may comprise numerous sections or components. For example, in one embodiment, an exemplary LV construct may comprise the following sections or components:

RSV—a Rous Sarcoma virus long terminal repeat;

5'LTR—a portion of an HIV long terminal repeat that can be truncated to prevent replication of the vector after chromosomal integration;

Psi—a packaging signal that allows for incorporation of the vector RNA genome into viral particles during packaging;

RRE—a Rev Responsive element can be added to improve expression from the transgene by mobilizing RNA out of the nucleus and into the cytoplasm of cells;

cPPT—a Poly purine tract that facilitates second strand DNA synthesis prior to integration of the transgene into the host cell chromosome;

Promoter—a promoter initiates RNA transcription from the integrated transgene to express micro-RNA clusters (or other genetic elements of the construct), and in some embodiments, the vectors may use an EF-1 promoter;

Anti-CCR5—a micro RNA targeting messenger RNA for the host cell factor CCR5 to reduce its expression on the cell surface;

Anti-Rev/Tat—a micro RNA targeting HIV genomic or messenger RNA at the junction between HIV Rev and Tat coding regions, which is sometimes designated miRNA Tat or given a similar description in this application;

Anti-Vif—a micro RNA targeting HIV genomic or messenger RNA within the Vif coding region;

WPRE—a woodchuck hepatitis virus post-transcriptional regulatory element is an additional vector component that can be used to facilitate RNA transport of the nucleus;

and

deltaU3 3'LTR—a modified version of a HIV 3' long terminal repeat where a portion of the U3 region has been deleted to improve safety of the vector.

One of ordinary skill in the art will recognize that the above components are merely examples, and that such components may be reorganized, substituted with other elements, or otherwise changed, so long as the construct is able to prevent expression of HIV genes and decrease the spread of infection.

Vectors of the invention may include either or both of the types of genetic cargo discussed above (i.e., genetic elements that direct expression of a gene or small RNAs, such as siRNA, shRNA, or miRNA that can prevent translation or transcription), and the vectors of the invention may also encode additionally useful products for the purpose of treatment or diagnosis of HIV. For instance, in some embodiments, these vectors may also encode green fluorescent protein (GFP) for the purpose of tracking the vectors or antibiotic resistance genes for the purposes of selectively maintaining genetically-modified cells in vivo.

The combination of genetic elements incorporated into the disclosed vectors is not particularly limited. For example, a vector herein may encode a single small RNA, two small RNAs, three small RNA, four small RNAs, five small RNAs, six small RNAs, seven small RNAs, eight small RNAs, nine small RNAs, or ten small RNAs, or eleven small RNAs, or twelve small RNAs. Such vectors may additionally encode other genetic elements to function in concert with the small RNAs to prevent expression and infection of HIV.

Those of ordinary skill in the art will understand that the therapeutic lentivirus may substitute alternate sequences for the promoter region, targeting of regulatory RNA, and types of regulatory RNA. Further, the therapeutic lentivirus of the disclosure may comprise changes in the plasmids used for packaging the lentivirus particles; these changes are required to increase levels of production in vitro.

Lentiviral Vector System

A lentiviral virion (particle) in accordance with various aspects and embodiments herein is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). In various embodiments, one vector containing a nucleic acid sequence encoding the lentiviral pol proteins is provided for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol proteins are expressed by multiple vectors. In other embodiments, vectors containing a nucleic acid sequence encoding the lentiviral Gag proteins for forming a viral capsid, operably linked to a promoter, are provided. In embodiments, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence. In other embodiments, the gag nucleic acid is on a separate vector from all the pol nucleic acid sequences that encode pot proteins.

Numerous modifications can be made to the vectors herein, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions. In embodiments, the gag, poi and env vector(s) do not

contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

The vector(s) forming the particle preferably do not contain a nucleic acid sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope protein.

In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelopes one can "infect" virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment such as that of the influenza virus, VSV-G, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia Virus such as MLV-E, MLV-A and GALV. These latter envelopes are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the desired host cell. For example, targeting specific receptors such as a dopamine receptor can be used for brain delivery. Another target can be vascular endothelium. These cells can be targeted using a filovirus envelope. For example, the GP of Ebola, which by post-transcriptional modification become the GP, and GP_2 glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Pat. No. 5,654,195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

Lentiviral vector systems as provided herein typically include at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (e.g., FIGS. **4**A-**4**B). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (e.g., FIGS. **5**A-**5**B). Accordingly, both 3-vector and 4-vector systems can be used to produce a lentivirus as described herein. In embodiments, the therapeutic vector, at least one envelope plasmid and at least one helper plasmid are transfected into a packaging cell, for example a packaging cell line. A non-limiting example of a packaging cell line is the 293T/17 HEK cell line. When the therapeutic vector, the envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, a lentiviral particle is ultimately produced.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line, a lentiviral particle is produced by the packaging cell line, wherein the lentiviral particle is capable of inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

In another aspect, the lentiviral vector, which is also referred to herein as a therapeutic vector, includes the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NOS: 32 or 80), and AU3 3' LTR (SEQ ID NO: 39). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, a helper plasmid includes the following elements: CAG promoter (SEQ ID NO: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO: 44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47). In another aspect, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, an envelope plasmid includes the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In various aspects, the plasmids used for lentiviral packaging are modified by substitution, addition, subtraction or mutation of various elements without loss of vector function. For example, and without limitation, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Various lentiviral packaging systems can be acquired commercially (e.g., Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, Md.), and can also be designed as described herein. Moreover, it is within the skill of a person ordinarily skilled in the art to substitute or modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the production efficiency of a lentiviral particle.

Bioassays

In various aspects, the present invention includes bioassays for determining the success of HIV treatment for achieving a functional cure. These assays provide a method for measuring the efficacy of the disclosed methods of immunization and treatment by measuring the frequency of transduced, HIV specific CD4 T cells in a patient. HIV-specific CD4 T cells are recognizable because, among others, they proliferate, change the composition of cell

surface markers, induce signaling pathways including phosphorylation, and/or express specific marker proteins that may be cytokines, chemokines, caspases, phosphorylated signaling molecules or other cytoplasmic and/or nuclear components. Specific responding CD4 T cells are recognized for example, using labeled monoclonal antibodies or specific in situ amplification of mRNA sequences, that allow sorting of HIV-specific cells using flow cytometry sorting, magnetic bead separation or other recognized methods for antigen-specific CD4 T cell isolation. The isolated CD4 T cells are tested to determine the frequency of cells carrying integrated therapeutic lentivirus. Single cell testing methods may also be used including microfluidic separation of individual cells that are coupled with mass spectrometry, PCR, ELISA or antibody staining to confirm responsiveness to HIV and presence of integrated therapeutic lentivirus.

Thus, in various embodiments, following application of a treatment according to the invention (e.g., (a) immunization, (b) ex vivo leukocyte/lymphocyte culture; (c) re-stimulation with purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles; and (d) infusion of the enriched, transduced T cells), a patient may be subsequently assayed to determine the efficacy of the treatment. A threshold value of target T cells in the body may be established to measure a functional cure at a determined value, for example, at about 1×10^8 HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus. Alternatively, the threshold value may be about 1×10^5, about 1×10^6, about 1×10^7, about 1×10^8, about 1×10^9, or about 1×10^{10} CD4 T cells in the body of the patient.

HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus can be determined using any suitable method, such as but not limited to flow cytometry, cell sorting, FACS analysis, DNA cloning, PCR, RT-PCR or Q-PCR, ELISA, FISH, western blotting, southern blotting, high throughput sequencing, RNA sequencing, oligonucleotide primer extension, or other methods known in the art.

While methods for defining antigen specific T cells with genetic modifications are known in the art, utilizing such methods to combine identifying HIV-specific T cells with integrated or non-integrated gene therapy constructs as a standard measure for efficacy is a novel concept in the field of HIV treatment, as described variously herein.

Doses and Dosage Forms

The disclosed methods and compositions can be used for treating HIV+ patients during various stages of their disease. Accordingly, dosing regimens may vary based upon the condition of the patient and the method of administration.

In various embodiments, HIV-specific vaccines for the initial in vivo immunization are administered to a subject in need in varying doses. In general, vaccines delivered by intramuscular injection include about 10 μg to about 300 μg, about 25 μg to about 275 μg, about 50 μg to about 250 μg, about 75 μg to about 225, or about 100 μg to about 200 μg of HIV protein, either total virus protein prepared from inactivated virus particles, virus-like particles or purified virus protein from recombinant systems or purified from virus preparations. Recombinant viral or bacterial vectors may be administered by any and all of the routes described. Intramuscular vaccines will include about 1 μg to about 100 μg, about 10 μg to about 90 μg, about 20 μg to about 80 μg, about 30 μg to about 70 μg, about 40 μg to about 60 μg, or about 50 μg of suitable adjuvant molecules and be suspended in oil, saline, buffer or water in volumes of 0.1 to 5 ml per injection dose, and may be soluble or emulsion preparations. Vaccines delivered orally, rectally, bucally, at genital mucosal or intranasally, including some virally-vectored or bacterially-vectored vaccines, fusion proteins, liposome formulations or similar preparations, may contain higher amounts of virus protein and adjuvant. Dermal, sub-dermal or subcutaneous vaccines utilize protein and adjuvant amounts more similar to oral, rectal or intranasal-delivered vaccines. Depending on responses to the initial immunization, vaccination may be repeated 1-5 times using the same or alternate routes for delivery. Intervals may be of 2-24 weeks between immunizations. Immune responses to vaccination are measured by testing HIV-specific antibodies in serum, plasma, vaginal secretions, rectal secretions, saliva or bronchoalveolar lavage fluids, using ELISA or similar methodology. Cellular immune responses are tested by in vitro stimulation with vaccine antigens followed by staining for intracellular cytokine accumulation followed by flow cytometry or similar methods including lymphoproliferation, expression of phosphorylated signaling proteins or changes in cell surface activation markers. Upper limits of dosing may be determined based on the individual patient and will depend on toxicity/safety profiles for each individual product or product lot.

Immunization may occur once, twice, three times, or repeatedly. For instance, an agent for HIV immunization may be administered to a subject in need once a week, once every other week, once every three weeks, once a month, every other month, every three months, every six months, every nine months, once a year, every eighteen months, every two years, every 36 months, or every three years.

Immunization will generally occur at least once before ex vivo expansion and enrichment of CD4 T cells, and immunization may occur once, twice, three times, or more after ex vivo leukocyte/lymphocyte culture/re-stimulation and infusion.

In one embodiment, HIV-vaccines for immunization are administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a wide variety of nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition comprising an HIV vaccine can also be formulated for injection.

HIV vaccine compositions for the purpose of immunization can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intracisternally, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, or via a buccal or nasal spray formulation.

Further, the HIV vaccine compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, and a suspension. Further, the composition may be a controlled release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the composition may be a transdermal delivery system.

In another embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a solid dosage form for oral administration, and the solid dosage form can

be powders, granules, capsules, tablets or pills. In yet another embodiment, the solid dosage form includes one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form is in immediate release or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

In a further embodiment, the pharmaceutical composition comprising a HIV vaccine is formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

In yet a further embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated as a nasal dosage form. Such dosage forms of the present invention comprise solution, suspension, and gel compositions for nasal delivery.

In one embodiment, the pharmaceutical composition is formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In other embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In particular embodiments, the composition comprising HIV vaccine or a pharmaceutically acceptable salt thereof is formulated to be suitable for administration to a pediatric patient.

In one embodiment, the pharmaceutical composition is formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emulsions, non-aqueous solutions or suppositories. In other embodiments, the non-aqueous solutions or suspensions includes propyleneglycol, polyethyleneglycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate. As a base for suppositories, witepsol, macrogol, tween 61, cacao oil, laurin oil or glycerinated gelatin can be used.

The dosage of the pharmaceutical composition can vary depending on the patient's weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

For the purposes of re-stimulation, lymphocytes, PBMCs, and/or CD4 T cells are generally removed from a patient and isolated for re-stimulation and culturing. The isolated cells may be contacted with the same HIV vaccine or activating agent used for immunization or a different HIV vaccine or activating agent. In one embodiment, the isolated cells are contacted with about 10 ng to 5 μg of an HIV vaccine or activating agent per about 10^6 cells in culture (or any other suitable amount). More specifically, the isolated cells may be contacted with about 50 ng, about 100 ng, about 200 ng, about 300 ng, about 400 ng, about 500 ng, about 600 ng, about 700 ng, about 800 ng, about 900 ng, about 1 μg, about 1.5 μg, about 2 μg, about 2.5 μg, about 3 μg, about 3.5 μg, about 4 μg, about 4.5 μg, or about 5 μg of an HIV vaccine or activating agent per about 10^6 cells in culture.

Activating agents or vaccines are generally used once for each in vitro cell culture but may be repeated after intervals of about 15 to about 35 days. For example, a repeat dosing could occur at about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

For transduction of the enriched, re-stimulated cells, the cells may be transduced with lentiviral vectors or with other known vector systems as disclosed, for example, in FIGS. **4**A-**4**C. The cells being transduced may be contacted with about 1-1,000 viral genomes (measured by RT-PCR assay of culture fluids containing lentivirus vector) per target cell in culture (or any other suitable amount). Lentivirus transduction may be repeated 1-5 times using the same range of 1-1,000 viral genomes per target cell in culture.

Cellular Enrichment

In various embodiments, cells such as T cells are obtained from an HIV infected patient and cultured. Culturing can occur in multiwell plates in a culture medium comprising conditioned media ("CM"). The levels of supernatant $p24^{gag}$ ("p24") and viral RNA levels may be assessed by standard means. Those patients whose CM-cultured cells have peak p24 supernatant levels of less than 1 ng/ml may be suitable patients for large-scale T-cell expansion in CM with or without the use of additional anti-viral agents. Additionally, different drugs or drug combinations of interest may be added to different wells and the impact on virus levels in the sample may be assessed by standard means. Those drug combinations providing adequate viral suppression are therapeutically useful combinations. It is within the capacity of a competent technician to determine what constitutes adequate viral suppression in relation to a particular subject. In order to test the effectiveness of drugs of interest in limiting viral expansion, additional factors such as anti-CD3 antibodies may be added to the culture to stimulate viral production. Unlike culture methods for HIV infected cell samples known in the art, CM allows the culture of T cells for periods of over two months, thereby providing an effective system in which to assay long term drug effectiveness.

This approach allows the inhibition of gene expression driven by the HIV LTR promoter region in a cell population by the culture of cells in a medium comprising the CM. Culture in CM4 likely inhibits HIV LTR driven gene expression by altering one or more interactions between transcription mediating proteins and HIV gene expression regulatory elements. Transcription-mediating proteins of interest include host cell encoded proteins such as AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp1, and the HIV encoded protein Tat. HIV gene expression regulatory elements of interest include binding sites for AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp1, as well as the transacting responsive element ("TAR") which interacts with Tat.

In a preferred embodiment, the HIV infected cells are obtained from a subject with susceptible transcription mediating protein sequences and susceptible HIV regulatory element sequences. In a more preferred embodiment, the HIV infected cells are obtained from a subject having wild-type transcription-mediating protein sequences and wild-type HIV regulatory sequences.

Another method of enriching T Cells utilizes immunoaffinity-based selection. This method includes the simultaneous enrichment or selection of a first and second population of cells, such as a CD4+ and CD8+ cell population. Cells containing primary human T cells are contacted with a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8 in an incubation composition, under conditions whereby the immunoaffinity reagents specifically bind to CD4 and CD8 molecules, respectively, on the surface of cells in the sample. Cells bound to the first and/or the second

immunoaffinity reagent are recovered, thereby generating an enriched composition comprising CD4+ cells and CD8+ cells. This approach may include incubation of the composition with a concentration of the first and/or second immunoaffinity reagent that is at a sub-optimal yield concentration. Notably, in some embodiments, transduced cells are a mixed T cell population, and in other embodiments transduced cells are not a mixed T cell population.

In some embodiments, immunoaffinity-based selection is used where the solid support is a sphere, such as a bead, such as a microbead or nanobead. In other embodiments, the bead can be a magnetic bead. In another embodiment, the antibody contains one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on the solid surface, such as a sphere or chromatography matrix, wherein the antibody is reversibly mobilized to the solid surface. In some embodiments, cells expressing a cell surface marker bound by the antibody on said solid surface are capable of being recovered from the matrix by disruption of the reversible binding between the binding reagent and binding partner. In some embodiments, the binding reagent is streptavidin or is a streptavidin analog or mutant.

Stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells may be obtained by contacting, in vitro or ex vivo, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface. The cells may be cultured in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation. In some embodiments, this approach may be used in conjunction with non-CD4+ T cell depletion and/or broad polyclonal expansion.

In another approach to T cell enrichment, PBMCs are stimulated with a peptide and enriched for cells secreting a cytokine, such as interferon-gamma. This approach generally involves stimulating a mixture of cells containing T cells with antigen, and effecting a separation of antigen-stimulated cells according to the degree to which they are labeled with the product. Antigen stimulation is achieved by exposing the cells to at least one antigen under conditions effective to elicit antigen-specific stimulation of at least one T cell. Labeling with the product is achieved by modifying the surface of the cells to contain at least one capture moiety, culturing the cells under conditions in which the product is secreted, released and specifically bound ("captured" or "entrapped") to said capture moiety; and labeling the captured product with a label moiety, where the labeled cells are not lysed as part of the labeling procedure or as part of the separation procedure. The capture moiety may incorporate detection of cell surface glycoproteins CD3 or CD4 to refine the enrichment step and increase the proportion of antigen-specific T cells in general, of CD4+ T cells in specific.

The following examples are given to illustrate aspects of the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

EXAMPLES

Example 1

Development of a Lentiviral Vector System

A lentiviral vector system was developed as summarized in FIG. **3** (linear form) and FIGS. **4**A-C (circularized form). Referring first to the top portion of FIG. **3**, a representative therapeutic vector has been designed and produced with the following elements being from left to right: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NOS: 32 or 80), and ΔU3 3' LTR (SEQ ID NO: 39). The therapeutic vector detailed in FIG. **3** is also referred to herein as AGT103.

Referring next to the middle portion of FIG. **3**, a helper plasmid has been designed and produced with the following elements being from left to right: CAG promoter (SEQ ID NO: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO: 44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47).

Referring next to the lower portion of FIG. **3**, an envelope plasmid has been designed and produced with the following elements being from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62).

Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, Va.) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid (as shown in FIG. **3**). The transfection of 293T/17 HEK cells, which produced functional viral particles, employed the reagent Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by anion-exchange chromatography. The concentration of lentiviral particles can be expressed in terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per cell by quantitative PCR, or by infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

As mentioned above, a 3-vector system (i.e., a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the 3-vector system is shown in FIGS. **4**A-**4**C. The schematic of FIGS. **4**A-**4**C is a circularized version of the linear system previously described in FIG. **3**. Briefly, and with reference to FIGS. **4**A-**4**C, FIG. **4**A depicts a helper plasmid, which, in this case, includes Rev. The vector appearing in FIG. **4**B is the envelope plasmid. The vector appearing in FIG. **4**C is the previously described therapeutic vector.

Referring more specifically to FIG. **4**A, the Helper plus Rev plasmid includes a CAG enhancer (SEQ ID NO: 40); a CAG promoter (SEQ ID NO: 41); a chicken beta actin intron (SEQ ID NO: 42); a HIV gag (SEQ ID NO: 43); a HIV Pol (SEQ ID NO: 44); a HIV Int (SEQ ID NO: 45); a HIV RRE (SEQ ID NO: 46); a HIV Rev (SEQ ID NO: 47); and a rabbit beta globin poly A (SEQ ID NO: 48).

The Envelope plasmid of FIG. **4**B includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

Synthesis of a 2-vector Lentiviral Packaging System Including Helper (Plus Rev) and Envelope Plasmids

Materials and Methods:

Construction of the helper plasmid: The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTC ATGAATTTGCCAG-GAAGAT-3') (SEQ ID NO: 81) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCAC-GAAT-3') (SEQ ID NO: 82). The sequence for the Gag, Pol, Integrase fragment was as follows:

(SEQ ID NO: 83)

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAAT

TGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT

GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC

ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCC

CATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATG

GCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTA

GTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGG

GCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACA

GTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACT

CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA

ACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAG

TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT

ATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA

GGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCT

TAGAGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATG

GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA

AATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAG

ACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTC

CATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAG

CTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAA

GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGG

GGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCT

AGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGT

ATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAA

GGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAAC

AGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAAT

TAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGA

AAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATG

GTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCA

ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATA

-continued

ATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAA

ATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC

CCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTA

GCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATA

TGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAG

TCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA

TGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATT

GGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGG

CCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT

GATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA

TAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCC

CAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTG

GTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGC

AGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGAT

GGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAGTACT

ACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCAT

TCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAAT

TAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA

GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGAT

TGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACA

TACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGG

GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT

CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAA

AAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAG

ATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA

Next, a DNA fragment containing the Rev, RRE, and rabbit beta globin poly A sequence with XbaI and XmaI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites The DNA sequence was as follows:

(SEQ ID NO: 84)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAAC

AGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCG

AGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGA

CAGAGACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGG

ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTA

CTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGA

AGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAA

AGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACT

ATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTC

TGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAAC

-continued

AGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGA

ATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT

TCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGA

CTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT

TTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA

ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCT

GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACA

GCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTT

AGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTA

AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACT

ACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGC

AGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTG

TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA

AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGC

TCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCAT

CTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCC

CCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTT

TTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAG

AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAAC

TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAA

TTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCA

AACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG

Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG enhancer/promoter plus a chicken beta actin intron sequence. A DNA fragment containing the CAG enhancer/promoter/intron sequence with MluI and EcoRI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows:

(SEQ ID NO: 85)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCC

CATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC

TGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC

CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT

TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT

ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC

CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTAT

TAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCAC

TCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT

TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCC

AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG

CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCG

-continued

AGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG

AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCC

GCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGG

GACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCT

CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCC

CTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT

GGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGG

GCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC

CGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTG

CGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGG

TCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGG

CCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG

TGCCGGGCGGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG

CCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCC

GGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATC

GTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAA

ATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTG

CGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGC

GCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACG

GCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG

ACCGGCGGGAATTC

Construction of the VSV-G Envelope Plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was synthesized by MWG Operon with flanking EcoRI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and the correct orientation was determined by sequencing using a CMV specific primer. The DNA sequence was as follows:

(SEQ ID NO: 86)

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAA

TTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAA

ATGTTCCTTCTAATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGG

CATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCA

CAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATGGGTCA

CTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATC

CGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAAC

GAAACAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT

ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCAC

CATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCACAGTTCAT

CAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTACAA

CCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATT

TCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGG

-continued

AAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAG

GCAAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCA

TCAGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAG

ATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCT

CAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTATTCC

CTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCC

AGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCCTGCTT

TCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGA

GTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGG

AACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGAAGACG

TGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTT

CCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAG

CTCAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGC

AACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAA

AATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTAT

TGCCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTC

TCCGAGTTGGTATCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGA

CAGATTTATACAGACATAGAGATGAGAATTC

A 4-vector system (i.e., a 3-vector lentiviral packaging system) has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in FIGS. **5**A-**5**D. Briefly, and with reference to FIG. **5**, the vector of FIG. **5**A is a helper plasmid, which, in this case, does not include Rev. The vector depicted in FIG. **5**B is a separate Rev plasmid. The vector depicted in FIG. **5**C is the envelope plasmid. The vector depicted in FIG. **5**D is the previously described therapeutic vector.

Referring, in part, to FIG. **5**A, the Helper plasmid includes a CAG enhancer (SEQ ID NO: 49); a CAG promoter (SEQ ID NO: 50); a chicken beta actin intron (SEQ ID NO: 51); a HIV gag (SEQ ID NO: 52); a HIV Pol (SEQ ID NO: 53); a HIV Int (SEQ ID NO: 54); a HIV RRE (SEQ ID NO: 55); and a rabbit beta globin poly A (SEQ ID NO: 56).

The Rev plasmid depicted in FIG. **5**B includes a RSV promoter (SEQ ID NO: 57); a HIV Rev (SEQ ID NO: 58); and a rabbit beta globin poly A (SEQ ID NO: 59).

The Envelope plasmid depicted in FIG. **5**C includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

Synthesis of a 3-vector Lentiviral Packaging System Including Helper, Rev, and Envelope Plasmids

Materials and Methods:

Construction of the Helper Plasmid without Rev:

The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by MWG Operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

(SEQ ID NO: 87)

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA

TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT

GGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACA

GCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA

TCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTT

CCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGAC

TTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATT

TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAA

CATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTG

GCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAG

CCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTA

GATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAA

AATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTA

CTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCA

GCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT

TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA

AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT

CACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATC

TCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCC

CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT

TTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGA

AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACT

TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAAT

TTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAA

ACTCATCAATGTATCTTATCACCCGGG

Construction of the Rev Plasmid:

The RSV promoter and HIV Rev sequence was synthesized as a single DNA fragment by MWG Operon with flanking MfeI and XbaI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA sequence was as follows:

(SEQ ID NO: 88)

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTG

TGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTC

AGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTAT

GCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGC

CTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGT

GGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATT

GGACGAACCACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCT

AGCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACC

TCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT

-continued

CCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC

CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG

CGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA

GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGA

AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG

GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGC

TACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACT

TCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTAC

AATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA

The plasmids for the 2-vector and 3-vector packaging systems could be modified with similar elements and the intron sequences could potentially be removed without loss of vector function. For example, the following elements could replace similar elements in the 2-vector and 3-vector packaging system:

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 64), phosphoglycerate kinase (PGK) (SEQ ID NO: 65), and ubiquitin C (UbC) (SEQ ID NO: 66) can replace the CMV (SEQ ID NO: 60) or CAG promoter (SEQ ID NO: 100). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 67) and bGH poly A (SEQ ID NO: 68) can replace the rabbit beta globin poly A (SEQ ID NO: 48). These sequences can also be further varied by addition, substitution, deletion or mutation.

HIV Gag, Pol, and Integrase sequences: The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 69); HIV Pol (SEQ ID NO: 70); and HIV Int (SEQ ID NO: 71) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 72), gibbon ape leukemia virus (GALV) (SEQ ID NO: 73), Rabies (FUG) (SEQ ID NO: 74), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 75), influenza A fowl plague virus (FPV) (SEQ ID NO: 76), Ross River alphavirus (RRV) (SEQ ID NO: 77), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 78), or Ebola virus (EboV) (SEQ ID NO: 79). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3' delta LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3' delta LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

Example 2

Development of an Anti-HIV Lentivirus Vector

The purpose of this example was to develop an anti-HIV lentivirus vector.

Inhibitory RNA Designs. The sequence of *Homo sapiens* chemokine C-C motif receptor 5 (CCR5) (GC03P046377) mRNA was used to search for potential siRNA or shRNA candidates to knockdown CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT RNAi Designer from Thermo Scientific. Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

The genomic sequence of Bal strain of human immunodeficiency virus type 1 (HIV-1 85US_ BaL, accession number AY713409) was used to search for potential siRNA or shRNA candidates to knockdown HIV replication levels in human cells. Based on sequence homology and experience, the search focused on regions of the Tat and Vif genes of HIV although an individual of skill in the art will understand that use of these regions is non-limiting and other potential targets might be selected. Importantly, highly conserved regions of gag or pol genes could not be targeted by shRNA because these same sequences were present in the packaging system complementation plasmids needed for vector manufacturing. As with the CCR5 (NM 000579.3, NM 001100168.1-specific) RNAs, potential HIV-specific RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Gene-E Software Suite hosted by the Broad Institute (broadinstitute.org/mai/public) or the BLOCK-iT RNAi Designer from Thermo Scientific (madesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=shrna&pid=67126273607 06061801). Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters

Vector Constructions. For CCR5, Tat or Vif shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested

lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered, purified and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Exemplary vector sequences that were determined to restrict HIV replication can be found in FIG. **6**. For example, the shRNA sequences with the highest activity against CCR5, Tat or Vif gene expression were then assembled into a microRNA (miR) cluster under control of the EF-1alpha promoter. The promoter and miR sequences are depicted in FIG. **6**.

Further, and using standard molecular biology techniques (e.g., Sambrook; Molecular Cloning: A Laboratory Manual, 4th Ed.) as well as the techniques described herein, a series of lentiviral vectors have been developed as depicted in FIG. **7** herein.

Vector 1 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24-Y); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 2 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 3 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shGag (SEQ ID NO: 12); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 4 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a 7SK element (SEQ ID NO: 103); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 5 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 6 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR155Tat (SEQ ID NO: 104); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 7 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 8 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 9 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a CD4 element (SEQ ID NO: 30); miR30CCR5 (SEQ ID NO: 1); miR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Development of Vectors

It should be noted that not all vectors developed for these experiments necessarily worked as might be predicted. More specifically, a lentivirus vector against HIV might include three main components: 1) inhibitory RNA to reduce the level of HIV binding proteins (receptors) on the target cell surface to block initial virus attachment and penetration; 2) overexpression of the HIV TAR sequence that will sequester viral Tat protein and decrease its ability to transactivate viral gene expression; and 3) inhibitory RNA that attack important and conserved sequences within the HIV genome.

With respect to the first point above, a key cell surface HIV binding protein is the chemokine receptor CCR5. HIV particles attach to susceptible T cells by binding to the CD4 and CCR5 cell surface proteins. Because CD4 is an essential glycoprotein on the cell surface that is important for the immunological function of T cells, this was not chosen as a target to manipulate its expression levels. However, people born homozygous for null mutations in the CCR5 gene and completely lacking receptor expression, live normal lives save for enhanced susceptibility to a few infectious diseases and the possibility of developing rare autoimmunity. Thus, modulating CCR5 was determined to be a relatively safe approach and was a primary target in the development of anti-HIV lentivirus vectors.

With respect to the second point above, the viral TAR sequence is a highly structured region of HIV genomic RNA that binds tightly to viral Tat protein. The Tat:TAR complex is important for efficient generation of viral RNA. Overexpression of the TAR region was envisioned as a decoy molecule that would sequester Tat protein and decrease the levels of viral RNA. However, TAR proved toxic to most mammalian cells including cells used for manufacturing lentivirus particles. Further, TAR was inefficient for inhibiting viral gene expression in other laboratories and has been discarded as a viable component in HIV gene therapy.

In various embodiments, viral gene sequences have been identified that meet 3 criteria: i) Sequences that are reasonably conserved across a range of HIV isolates representative of the epidemic in a geographic region of interest; ii) reduction in RNA levels due to the activity of an inhibitory

RNA in a viral vector will reduce the corresponding protein levels by an amount sufficient to meaningfully reduce HIV replication; and iii) the viral gene sequence(s) targeted by inhibitory RNA are not present in the genes required for packaging and assembling viral vector particles during manufacturing. In various embodiments, a sequence at the junction of HIV Tat and Rev genes and a second sequence within the HIV Vif gene have been targeted by inhibitory RNA. The Tat/Rev targeting has an additional benefit of reducing HIV envelope glycoprotein expression because this region overlaps with the envelope gene in the HIV genome.

Various methods for vector development and testing relies first on identifying suitable targets (as described herein) followed by constructing plasmid DNAs expressing individual or multiple inhibitory RNA species for testing in cell models, and finally constructing lentivirus vectors containing inhibitory RNA with proven anti-HIV function. The lentivirus vectors are tested for toxicity, yield during in vitro production, and effectiveness against HIV in terms of reducing CCR5 expression levels or lowering viral gene products to inhibit virus replication.

Table 2 below demonstrates progression through multiple versions of inhibitory constructs until arriving at a clinical candidate. Initially, shRNA (short homology RNA) molecules were designed and expressed from plasmid DNA constructs.

Plasmids 1-4, as detailed in Table 2 below, tested shRNA sequences against Gag, Pol and RT genes of HIV. While each shRNA was active for suppressing viral protein expression in a cell model, there were two important problems that prevented further development. First, the sequences were targeted to a laboratory isolate of HIV that was not representative of Clade B HIV strains currently circulating in North America and Europe. Second, these shRNA targeted critical components in the lentivirus vector packaging system and would severely reduce vector yield during manufacturing. Plasmid 5, as detailed in Table 2, was selected to target CCR5 and provided a lead candidate sequence. Plasmids 6, 7, 8, 9, 10, and 11, as detailed in Table 2, incorporated the TAR sequence and it was found they produced unacceptable toxicity for mammalian cells including cells used for lentivirus vector manufacturing. Plasmid 2, as detailed in Table 2, identified a lead shRNA sequence capable of reducing Tat RNA expression. Plasmid 12, as detailed in Table 2, demonstrated the effectiveness of shCCR5 expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 13, as detailed in Table 2, demonstrated the effectiveness of a shVif expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 14, as detailed in Table 2, demonstrated the effectiveness of shTat expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 15, as detailed in Table 2, contained the miR CCR5, miR Tat and miR Vif in the form of a miR cluster expressed from a single promoter. These miR do not target critical components in the lentivirus vector packaging system and proved to have negligible toxicity for mammalian cells. The miR within the cluster were equally effective to individual miR that were tested previously, and the overall impact was a substantial reduction in replication of a CCR5-tropic HIV BaL strain.

TABLE 2

Development of HIV Vectors

	Internal Code	Material	Description	Remarks	Decision
1	SIH-H1-shRT-1,3	Lentiviral vector	shRNA construct for RT of LAI strain	Wrong target, lab virus, no virus test	Abandon
2	SIH-H1-shRT43 (Tat/Rev NL4-3)	Lentiviral vector	H1 promoter shRNA Tat/Rev overlap	Tat protein knock-down >90%	Lead

Vector Construction: For Rev/Tat (RT) shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Two different Rev/Tat target sequences were tested for their ability to decrease Tat mRNA expression. The RT1,3 target sequence is (5'-ATGGCAGGAAGAAGCGGAG-3') (SEQ ID NO: 89) and shRNA sequence is (5'-ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCTTCTTCCTGCCATTTTTT-3') (SEQ ID NO: 90). The RT43 sequence is (5'-GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and shRNA sequence is (5'-GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 10). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).
Functional test for shRNA against Rev/Tat: The ability of the vector to reduce Tat expression was tested using a luciferase reporter plasmid which contained the Rev/Tat target sequences inserted into the 3'-UTR (untranslated region of the mRNA). Either the shRT1,3 or shRT43 plasmid was co-transfected with the plasmid containing luciferase and the Rev/Tar target sequence. There was a 90% reduction in light emission indicating strong function of the shRT43 shRNA sequence but less than 10% with the shRT1,3 plasmid.
Conclusion: The SIH-H1-shRT43 was superior to SIH-H1-shRT-1,3 in terms of reducing mRNA levels in the Luciferase assay system. This indicates potent inhibitory activity of the shRT43 sequence and it was selected as a lead candidate for further development.

3	SIH-H1-shGag-1	Lentiviral vector	shRNA construct for LAI Gag	Inhibits Gag expression but will inhibit packaging	Abandon

Vector Construction: For Gag shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. A Gag target sequence was tested for their ability to decrease Gag mRNA expression. The Gag target sequence is (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) and shRNA sequence is (5'-

TABLE 2-continued

Development of HIV Vectors

	Internal Code	Material	Description	Remarks	Decision
			GAAGAAATGATGACAGCATTTCAAGAGAATGCTGTCATCATTTCTTCTTTTT-3') (SEQ ID NO: 12). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences). Functional test for shRNA against Gag: The ability of the vector to reduce Gag expression was tested using a luciferase reporter plasmid which contained the Gag target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Gag plasmid was co-transfected with the plasmid containing luciferase and the Gag target sequence. There was nearly a 90% reduction in light emission indicating a strong effect of the shGag shRNA sequence. Conclusion: This shRNA sequence is potent against HIV Gag expression but was abandoned. The lentivirus packaging system requires production of Gag from the helper plasmid and shRNA inhibition of Gag will reduce lentivirus vector yield. This shRNA sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.		
4	SIH-H1-shPol-1	Lentiviral vector	shRNA construct for Pol	Inhibits Pol expression but will inhibit packaging	Abandon
			Vector Construction: A Pol shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. A Pol target sequence was tested for its ability to decrease Pol mRNA expression. The Pol target sequence is (5'-CAGGAGCAGATGATACAG-3') (SEQ ID NO: 13) and shRNA sequence is (5'-CAGGAGATGATACAGTTCAAGAGACTGTATCATCTGCTCCTGTTTTT-3') (SEQ ID NO: 14). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences). Functional tests for shRNA against HIV Pol: The ability of the vector to reduce Pol expression was tested using a luciferase reporter plasmid which contained the Pol target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Pol plasmid was co-transfected with the plasmid containing luciferase and the Pol target sequence. There was a 60% reduction in light emission indicating a strong effect of the shPol shRNA sequence. Conclusion: This shRNA sequence is potent against HIV Pol expression but was abandoned. The lentivirus packaging system requires production of Pol from the helper plasmid and shRNA inhibition of Pol will reduce lentivirus vector yield. This shRNA sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.		
5	SIH-H1-shCCR5-1	Lentiviral vector	shRNA construct for CCR5	Best of 5 candidates, Extracellular CCR5 protein reduction >90%	Lead
			Vector Construction: A CCR5 shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences). The CCR5 target sequence #1, which focuses on CCR5 gene sequence 1 (SEQ ID NO: 25), is (5'-GTGTCAAGTCCAATCTATG-3') (SEQ ID NO: 15) and the shRNA sequence is (5'-GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 16). The CCR5 target sequence #2, which focuses on CCR5 gene sequence 2 (SEQ ID NO: 26), is (5'-GAGCATGACTGACATCTAC-3') (SEQ ID NO: 17) and the shRNA sequence is (5'-GAGCATGACTGACATCTACTTCAAGAGAGTAGATGTCAGTCATGCTCTTTTT-3') (SEQ ID NO: 18). The CCR5 target sequence #3, which focuses on CCR5 gene sequence 3 (SEQ ID NO: 27), is (5'-GTAGCTCTAACAGGTTGGA-3') (SEQ ID NO: 19) and the shRNA sequence is (5'-GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCTGTTAGAGCTACTTTTT-3') (SEQ ID NO: 20). The CCR5 target sequence #4, which focuses on CCR5 gene sequence 4 (SEQ ID NO: 28, is (5'-GTTCAGAAACTACCTCTTA-3') (SEQ ID NO: 21) and the shRNA sequence is (5'-GTTCAGAAACTACCTCTTATTCAAGAGATAAGAGGTAGTTTCTGAACTTTTT-3') (SEQ ID NO: 22). The CCR5 target sequence #5, which focuses on CCR5 gene sequence 5 (SEQ ID NO: 29), is (5'-GAGCAAGCTCAGTTTACACC-3') (SEQ ID NO: 23) and the shRNA sequence is (5'-GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAAACTGAGCTTGCTCTTTTT-3') (SEQ ID NO: 24). Functional test for shRNA against CCR5: The ability of a CCR5 shRNA sequence to knock-down CCR5 RNA expression was initially tested by co-transfecting each of the lentiviral plasmids, in separate experiments for each plasmid, containing one of the five CCR5 target sequences with a plasmid expressing the human CCR5 gene. CCR5 mRNA expression was then assessed by qPCR analysis using CCR5-specific primers. Conclusion: Based on the reduction in CCR5 mRNA levels the shRNACCR5-1 was most potent for reducing CCR5 gene expression. This shRNA was selected as a lead candidate.		
6	SIH-U6-TAR	Lentiviral vector	U6 promoter-TAR	Toxic to cells	Abandon
7	SIH-U6-TAR-H1-shCCR5	Lentiviral vector	U6 promoter-TAR-H1-shCCR5	Toxic to cells	Abandon

TABLE 2-continued

Development of HIV Vectors

	Internal Code	Material	Description	Remarks	Decision
8	U6-TAR-H1-shRT	Lentiviral vector	U6 promoter-TAR-H1-RT	Suppress HIV, toxic to cells, poor packaging	Abandon
9	U6-TAR-7SK-shRT	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon
10	U6-TAR-H1-shRT-H1-shCCR5	Lentiviral vector	U6 promoter-TAR-H1-RT-H1-shCCR5	Toxic, poor packaging, H1 repeats	Abandon
11	U6-TAR-7SK-shRT-H1-CCR5	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon

Vector Construction: A TAR decoy sequence containing flanking KpnI restriction sites was synthesized by MWG operon and inserted into the pSIH lentiviral vector (System Biosciences) at the KpnI site. In this vector, TAR expression is regulated by the U6 promoter. The TAR decoy sequence is (5'-
CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTCTCGACAGCGACCAGATCTGAG
CCTGGGAGCTCTCTGGCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAATTCTTACT
GAGCAAGAAA-3') (SEQ ID NO: 8). Expression of the TAR decoy sequence was determined by qPCR analysis using specific primers for the TAR sequence. Additional vectors were constructed also containing the TAR sequence. The H1 promoter and shRT sequence was inserted in this vector in the XhoI site. The H1 shRT sequence is (5'-
GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGGCGG
GAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGT
GGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAA
ATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATCCGCGGAG
ACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 91). This vector could express TAR and knockdown RT. The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression. Another vector was constructed containing U6 TAR, H1 shRT, and H1 shCCR5. The H1 shCCR5 sequence was inserted into the SpeI site of the plasmid containing U6 TAR and H1 shRT. The H1 CCR5 sequence is (5'-
GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGGCGG
GAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGT
GGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAA
ATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATCCGTGTCA
AGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 92). The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression.
Functional test for TAR decoy activity: We tested the effect of SIH-U6-TAR on packaging efficiency. When TAR sequence was included, the yield of vector in the SIR packaging system was reduced substantially.
Conclusion: Lentivirus vectors expressing the TAR decoy sequence are unsuitable for commercial development due to low vector yields. These constructs were abandoned.

12	shCCR5	Lentiviral vector	microRNA sequence	Extracellular CCR5 protein reduction >90%	Lead

Vector Construction: A CCR5 microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences). The EF-1 promoter was substituted for a CMV promoter that was used in the plasmid construct Test Material 5. The EF-1 promoter was synthesized by MWG Operon containing flanking ClaI and BsrGI restriction sites and inserted into the pCDH vector containing shCCR5-1. The EF-1 promoter sequence is (5'-
CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGC
TCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGA
ACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTC
CCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG
CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAG
AGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGC
CTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGC
TTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCT
GGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGG
GGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGG
CCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC
TCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCC
CGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGG
AGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACA
AAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGG
GCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTT
GGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAG
TTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA
TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGT
GTCGTGA-3') (SEQ ID NO: 4).

TABLE 2-continued

Development of HIV Vectors

Internal Code	Material	Description	Remarks	Decision
Functional test for lentivirus CDH-shCCR5-1: The ability of the miR CCR5 sequences to knock-down CCR5 expression was determined by transducing CEM-CCR5 T cells and measuring cell surface CCR5 expression after staining with a fluorescently-labeled monoclonal antibody against CCR5 and measuring the intensity of staining, that is directly proportional to the number of cell surface CCR5 molecules, by analytical flow cytometry. The most effective shRNA sequence for targeting CCR5 was CCR5 shRNA sequence #1. However, the most effective CCR5 targeting sequence for constructing the synthetic microRNA sequence was overlapping with CCR5 sequence #5; this conclusion was based on sequence alignments and experience with miRNA construction. Finally, the miR30 hairpin sequence was used to construct the synthetic miR30 CCR5 sequence which is (5'-AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT-3') (SEQ ID NO: 1). The miR CCR5 target sequence is (5'-GAGCAAGCTCAGTTTACA-3') (SEQ ID NO: 5). At multiplicity of infection equal to 5, generating on average 1.25 genome copies of integrated lentivirus per cell, CCR5 expression levels were reduce by ≥90% indicating potent inhibition of CCR5 mRNA by the miR30CCR5 micro RNA construct in a lentivirus vector. Conclusion: The miR30CCR5 construct is potent for reducing CCR5 cell surface expression and is a lead candidate for a therapeutic lentivirus for HIV.				
13 shVif	Lentiviral vector	microRNA sequence	Vif protein reduction >80%	Lead
Vector Construction: A Vif microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience with constructing synthetic miRNA, the miR21 hairpin sequence was used to construct the synthetic miR21 Vif sequence which is (5'-CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA-3') (SEQ ID NO: 2). The miR Vif target sequence is (5'-GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6). Functional test for potency of miR21Vif: The ability of the miR Vif sequence to knock-down Vif expression was determined by measuring Vif protein expression by immunoblot analysis using an anti-Vif monoclonal antibody to identify the Vif protein. Conclusion: the miR21Vif reduced Vif protein expression by ≥10-fold as determined by quantitative image analysis of immunoblot data. This was sufficient to justify miR21Vif as a lead candidate for our therapeutic lentivirus.				
14 shTat	Lentiviral vector	microRNA sequence	Tat RNA reduction >80%	Lead
Vector Construction: A Tat microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. The microRNA cluster was inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience in the construction of synthetic miRNA, the miR185 hairpin sequence was selected for constructing a synthetic miR185 Tat sequence which is (5'-GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCC CTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG-3'). The miR Tat target sequence is (5'-TCCGCTTCTTCCTGCCATAG-3') (SEQ ID NO: 3). Functional test for potency of miR185Tat: The ability of miR Tat to knock-down Tat expression was determined by measuring Tat mRNA expression by RT-PCR analysis using Tat specific primers. We compared the miR185Tat with a similar miR155Tat on the basis of reducing the relative levels of Tat mRNA. Conclusion: The miR185Tat was approximately twice as potent for reducing Tat mRNA compare to miR155Tat, and was selected as the lead candidate for our therapeutic lentivirus.				
15 shCCR5-shVif-shTat	Lentiviral vector	microRNA cluster sequence	CCR5 reduction >90%, Vif protein reduction >80%, Tat RNA reduction >80%, >95% inhibition of HIV replication	Candidate
Vector Construction: A miR30CCR5 miR21Vif miR185Tat microRNA cluster sequence was constructed with a synthetic DNA fragment containing BsrGI and NotI restriction sites that was synthesized by MWG Operon. The DNA fragment was inserted into the pCDH lentiviral vector (System Biosciences) containing the EF-1 promoter. The miR cluster sequence is (5'-AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTT GTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC				

TABLE 2-continued

Development of HIV Vectors				
Internal Code	Material	Description	Remarks	Decision
ATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCT GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC-3') (SEQ ID NO: 31) and incorporates Test Material 12, Test Material 13 and Test Material 14 into a single cluster that can be expressed under control of the EF-1 promoter. Functional test for potency of the Lentivirus Vector AGT103 containing the microRNA cluster of miR30CCR5, miR21Vif and miR185Tat: The AGT103 vector was tested for potency against CCR5 using the assay for reduction in cell surface CCR5 expression (Test Material 12). The AGT103 vector was tested for potency against Vif using the assay for reduction in cell surface Vif expression (Test Material 13). The AGT103 vector was tested for potency against Tat using the assay for reduction in cell surface Tat expression (Test Material 14). Conclusion: Potency for reducing CCR5 expression by the miRNA cluster was similar to potency observed for the miR30CCR5 alone. Potency for reducing Vif expression by the miRNA cluster was similar to potency observed for the miR21Vif alone. Potency for reducing Tat expression by the miRNA cluster was similar to potency observed for the miR185Tat alone. The miRNA cluster is potent for reducing cell surface CCR5 levels and for inhibiting two HIV genes. Thus, AGT103 containing this miRNA cluster was selected as the therapeutic vector construct for our HIV functional cure program.				

Functional Assays. Individual lentivirus vectors containing CCR5, Tat or Vif shRNA sequences and, for experimental purposes, expressing green fluorescent protein (GFP) under control of the CMV Immediate Early Promoter, and designated AGT103/CMV-GFP were tested for their ability to knockdown CCR5, Tat or Vif expression. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days; protein and RNA were analyzed for CCR5, Tat or Vif expression. Protein levels were tested by Western blot assay or by labeling cells with specific fluorescent antibodies (CCR5 assay), followed by analytical flow cytometry comparing modified and unmodified cell fluorescence using either the CCR5-specific or isotype control antibodies.

Starting Testing of Lentivirus. T cell culture medium was made using RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin. Cytokine stocks of IL2 10,000 units/ml, IL-12 1 μg/ml, IL-7 1 μg/ml, IL-15 1 μg/ml were also prepared in advance.

Prior to transduction with the lentivirus, an infectious viral titer was determined and used to calculate the amount of virus to add for the proper multiplicity of infection (MOI).

Day 0-12: Antigen-specific enrichment. On day 0, cryopreserved PBMC were thawed, washed with 10 ml 37° C. medium at 1200 rpm for 10 minutes and resuspended at a concentration of 2×10^6/ml in 37° C. medium. The cells were cultured at 0.5 ml/well in a 24-well plate at 37° C. in 5% CO2. To define the optimal stimulation conditions, cells were stimulated with combinations of reagents as listed in Table 3 below:

TABLE 3

1	2	3	4	5	6
IL-2 + IL-12	IL-7 + IL-15	Peptides + IL-2 + IL-12	Peptides + IL-7 + IL-15	MVA + IL-2 + IL-12	MVA + IL-7 + IL-15

Final concentrations: IL-2=20 units/ml, IL-12=10 ng/ml, IL-7=10 ng/ml, IL-15=10 ng/ml, peptides=5 μg/ml individual peptide, MVA MOI=1.

On days 4 and 8, 0.5 ml fresh medium and cytokine at listed concentrations (all concentrations indicate the final concentration in the culture) were added to the stimulated cells.

Day 12-24: non-specific expansion and lentivirus transduction. On day 12, the stimulated cells were removed from the plate by pipetting and resuspended in fresh T cell culture medium at a concentration of 1×106/ml. The resuspended cells were transferred to T25 culture flasks and stimulated with DYNABEADS® Human T-Activator CD3/CD28 following the manufacturer's instruction plus cytokine as listed above; flasks were incubated in the vertical position.

On day 14, AGT103/CMV-GFP was added at MOI 20 and cultures were returned to the incubator for 2 days. At this time, cells were recovered by pipetting, collected by centrifugation at 1300 rpm for 10 minutes, resuspended in the same volume of fresh medium, and centrifuged again to form a loose cell pellet. That cell pellet was resuspended in fresh medium with the same cytokines used in previous steps, with cells at 0.5×10^6 viable cells per ml.

From days 14 to 23, the number of the cells was evaluated every 2 days and the cells were diluted to 0.5×10^6/ml with fresh media. Cytokines were added every time.

On day 24, the cells were collected and the beads were removed from the cells. To remove the beads, cells were transferred to a suitable tube that was placed in the sorting magnet for 2 minutes. Supernatant containing the cells was transferred to a new tube. Cells were then cultured for 1 day in fresh medium at 1×10^6/ml. Assays were performed to determine the frequencies of antigen-specific T cells and lentivirus transduced cells.

To prevent possible viral outgrowth, amprenavir (0.5 ng/ml) was added to the cultures on the first day of stimulation and every other day during the culture.

Examine antigen-specific T cells by intracellular cytokine staining for IFN-gamma. Cultured cells after peptide stimulation or after lentivirus transduction at 1×10^6 cells/ml were stimulated with medium alone (negative control), Gag peptides (5 μg/ml individual peptide), or PHA (5 μg/ml, positive control). After 4 hours, BD GolgiPlug™ (1:1000, BD Biosciences) was added to block Golgi transport. After 8 hours, cells were washed and stained with extracellular (CD3, CD4 or CD8; BD Biosciences) and intracellular (IFN-gamma; BD Biosciences) antibodies with BD Cytofix/Cytoperm™ kit following the manufacturer's instruction. Samples were analyzed on a BD FACSCalibur™ Flow Cytometer. Control samples labeled with appropriate isotype-matched antibodies were included in each experiment. Data were analyzed using Flowjo software.

Lentivirus transduction rate was determined by the frequency of GFP+ cells. The transduced antigen-specific T cells are determined by the frequency of CD3+CD4+GFP+ IFN gamma+cells; tests for CD3+CD8+GFP+IFN gamma+ cells are included as a control.

These results indicate that CD4 T cells, the target T cell population, can be transduced with lentiviruses that are designed to specifically knock down the expression of HIV-specific proteins, thus producing an expandable population of T cells that are immune to the virus. This example serves as a proof of concept indicating that the disclosed lentiviral constructs can be used in combination with vaccination to produce a functional cure in HIV patients.

Example 4

CCR5 Knockdown with Experimental Vectors

AGTc120 is a Hela cell line that stably expresses large amounts of CD4 and CCR5. AGTc120 was transduced with or without LV-CMV-mCherry (the red fluorescent protein mCherry expressed under control of the CMV Immediate Early Promoter) or AGT103/CMV-mCherry. Gene expression of the mCherry fluorescent protein was controlled by a CMV (cytomegalovirus immediate early promoter) expression cassette. The LV-CMV-mCherry vector lacked a microRNA cluster, while AGT103/CMV-mCherry expressed therapeutic miRNA against CCR5, Vif, and Tat.

As shown in FIG. **8**A, transduction efficiency was >90%. After 7 days, cells were collected and stained with fluorescent monoclonal antibody against CCR5 and subjected to analytical flow cytometry. Isotype controls are shown in gray on these histograms plotting Mean Fluorescence Intensity of CCR5 APC (x axis) versus cell number normalized to mode (y axis). After staining for cell surface CCR5, cells treated with no lentivirus or control lentivirus (expressing only the mCherry marker) showed no changes in CCR5 density while AGT103 (right section) reduced CCR5 staining intensity to nearly the levels of isotype control. After 7 days, cells were infected with or without R5-tropic HIV reporter virus Bal-GFP. 3 days later, cells were collected and analyzed by flow cytometry. More than 90% of cells were transduced. AGT103-CMV/CMVmCherry reduced CCR5 expression in transduced AGTc120 cells and blocked R5-tropic HIV infection compared with cells treated with the Control vector.

FIG. **8**B shows the relative insensitivity of transfected AGTc120 cells to infection with HIV. As above, the lentivirus vectors express mCherry protein and a transduced cell that was also infected with HIV (expressing GFP) would appear as a double positive cell in the upper right quadrant of the false color flow cytometry dot plots. In the absence of HIV (upper panels), there were no GFP+ cells under any condition. After HIV infection (lower panels), 56% of cells were infected in the absence of lentivirus transduction and 53.6% of cells became infected in AGTc120 cells transduced with the LV-CMV-mCherry. When cells were transduced with the therapeutic AGT103/CMV-mCherry vector, only 0.83% of cells appeared in the double positive quadrant indicating they were transduced and infected.

Dividing 53.62 (proportion of double positive cells with control vector) by 0.83 (the proportion of double positive cells with the therapeutic vector) shows that AGT103 provided greater than 65-fold protection against HIV in this experimental system.

Example 5

Regulation of CCR5 Expression by shRNA Inhibitor Sequences in a Lentiviral Vector

Inhibitory RNA Design. The sequence of *Homo sapiens* chemokine receptor CCR5 (CCR5, NC 000003.12) was used to search for potential siRNA or shRNA candidates to knockdown CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-IT RNA iDesigner from Thermo Scientific. A shRNA sequence may be inserted into a plasmid immediately after a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. The shRNA sequence may also be inserted into a lentiviral vector using similar promoters or embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and utilized independently of a plasmid or lentiviral vector.

Plasmid Construction. For CCR5 shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Oligonucleotide sequences were annealed by incubating at 70° C. then cooled to room temperature. Annealed oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37° C., then the enzymes were inactivated at 70° C. for 20 minutes. In parallel, plasmid DNA was digested with the restriction enzymes BamHI and EcoRI for one hour at 37° C. The digested plasmid DNA was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentration was determined and the plasma to oligonucleotide sequence was ligated in the ratio 3:1 insert to vector. The ligation reaction was done with T4 DNA ligase for 30 minutes at room temperature. 2.5 μL of the ligation mix were added to 25 μL of STBL3 competent bacterial cells. Transformation required heat shock at 42° C. Bacterial cells were spread on agar plates containing ampicillin and colonies were expanded in L broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacterial cultures using the Invitrogen DNA Miniprep kit and tested by restriction enzyme digestion. Insertion of the shRNA sequence into the plasmid was verified by DNA sequencing using a primer specific for the promoter used to regulate shRNA expression.

Functional Assay for CCR5 mRNA Reduction: The assay for inhibition of CCR5 expression required co-transfection of two plasmids. The first plasmid contains one of five different shRNA sequences directed against CCR5 mRNA. The second plasmid contains the cDNA sequence for human CCR5 gene. Plasmids were co-transfected into 293T cells. After 48 hours, cells were lysed and RNA was extracted using the RNeasy kit from Qiagen. cDNA was synthesized from RNA using a Super Script Kit from Invitrogen. The samples were then analyzed by quantitative RT-PCR using an Applied Biosystems Step One PCR machine. CCR5 expression was detected with SYBR Green from Invitrogen using the forward primer (5'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 93) and reverse primer (5'-CCCCAAAGAAGGTCAAGGTAATCA-3') (SEQ ID NO: 94) with standard conditions for polymerase chain reaction analysis. The samples were normalized to the mRNA for beta actin gene expression using the forward primer (5'-AGCGCGGCTACAGCTTCA-3') (SEQ ID NO: 95) and reverse primer (5'-GGCGACGTAGCACAGCTTCP-3')

(SEQ ID NO: 96) with standard conditions for polymerase chain reaction analysis. The relative expression of CCR5 mRNA was determined by its Ct value normalized to the level of actin messenger RNA for each sample. The results are shown in FIGS. **9**A-**9**B.

As shown in FIG. **9**A, CCR5 knock-down was tested in 293T cells by co-transfection of the CCR5 shRNA construct and a CCR5-expressing plasmid. Control samples were transfected with a scrambled shRNA sequence that did not target any human gene and the CCR5-expressing plasmid. After 60 hours post-transfection, samples were harvested and CCR5 mRNA levels were measured by quantitative PCR. Further, as shown in FIG. **9**B, CCR5 knock-down after transduction with lentivirus expressing CCR5 shRNA-1 (SEQ ID NO: 16).

Example 6

Regulation of HIV Components by shRNA Inhibitor Sequences in a Lentiviral Vector

Inhibitory RNA Design.

The sequences of HIV type 1 Rev/Tat (5'-GCGGAGACA-GCGACGAAGAGC-3') (SEQ ID NO: 9) and Gag (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) were used to design: Rev/Tat:

(5'GCGGAGACAGCGACGAAGAGCTTCAAGA-GAGCTCTTCGTCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 10) and

Gag:

(5'GAAGAAATGATGACAGCATTTCAAGAGAATGCT-GTCATCATTTCTTCTTTTT-3') (SEQ ID NO: 12) shRNA that were synthesized and cloned into plasmids as described above.

Plasmid Construction. The Rev/Tat or Gag target sequences were inserted into the 3'UTR (untranslated region) of the firefly luciferase gene used commonly as a reporter of gene expression in cells or tissues. Additionally, one plasmid was constructed to express the Rev/Tat shRNA and a second plasmid was constructed to express the Gag shRNA. Plasmid constructions were as described above.

Functional assay for shRNA targeting of Rev/Tat or Gag mRNA: Using plasmid co-transfection we tested whether a shRNA plasmid was capable of degrading luciferase messenger RNA and decreasing the intensity of light emission in co-transfected cells. A shRNA control (scrambled sequence) was used to establish the maximum yield of light from luciferase transfected cells. When the luciferase construct containing a Rev/Tat target sequence inserted into the 3'-UTR (untranslated region of the mRNA) was co-transfected with the Rev/Tat shRNA sequence there was nearly a 90% reduction in light emission indicating strong function of the shRNA sequence. A similar result was obtained when a luciferase construct containing a Gag target sequence in the 3'-UTR was co-transfected with the Gag shRNA sequence. These results indicate potent activity of the shRNA sequences.

As shown in FIG. **10**A, knock-down of the Rev/Tat target gene was measured by a reduction of luciferase activity, which was fused with the target mRNA sequence in the 3'UTR, by transient transfection in 293T cells. As shown in FIG. **10**B, knock-down of the Gag target gene sequence fused with the luciferase gene. The results are displayed as the mean±SD of three independent transfection experiments, each in triplicate.

Example 7

AGT103 Decreases Expression of Tat and Vif

Cells were transfected with exemplary vector AGT103/CMV-GFP. AGT103 and other exemplary vectors are defined in Table 3 below.

TABLE 3

Vector Designation	Composition
AGT103	EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE
Control-mCherry	CMV-mCherry
AGT103/CMV-mCherry	CMV-mCherry-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE-
Control-GFP	CMV-mCherry
AGT103/CMV-GFP	CMV-GFP-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE-

Abbreviations:
EF-1: elongation factor 1 transcriptional promoter
miR30CCR5—synthetic microRNA capable of reducing CCR5 protein on cell surfaces
miR21Vif—synthetic microRNA capable of reducing levels of HIV RNA and Vif protein expression
miR185Tat—synthetic micro RNA capable of reducing levels of HIV RNA and Tat protein expression
CMV—Immediate early transcriptional promoter from human cytomegalovirus
mCherry—coding region for the mCherry red fluorescent protein
GFP—coding region for the green fluorescent protein
WPRE—Woodchuck hepatitis virus post transcriptional regulatory element

A T lymphoblastoid cell line (CEM; CCRF-CEM; American Type Culture Collection Catalogue number CCL119) was transduced with AGT103/CMV-GFP. 48 hours later the cells were transfected with an HIV expression plasmid encoding the entire viral sequence. After 24 hours, RNA was extracted from cells and tested for levels of intact Tat sequences using reverse transcriptase polymerase chain reaction. Relative expression levels for intact Tat RNA were reduced from approximately 850 in the presence of control lentivirus vector, to approximately 200 in the presence of AGT103/CMV-GFP for a total reduction of >4 fold, as shown in FIG. **11**.

Example 8

Regulation of HIV Components by Synthetic MicroRNA Sequences in a Lentiviral Vector

Inhibitory RNA Design. The sequence of HIV-1 Tat and Vif genes were used to search for potential siRNA or shRNA candidates to knockdown Tat or Vif levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-IT RNA iDesigner from Thermo Scientific. The selected shRNA sequences most potent for Tat or Vif knockdown were embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-I alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and used independently of a plasmid or lentiviral vector.

Plasmid Construction. The Tat target sequence (5'-TC-CGCTTCTTCCTGCCATAG-3') (SEQ ID NO: 7) was incorporated into the miR185 backbone to create a Tat miRNA (5'-GGGCCTGGCTCGAGCAGGGGGC-GAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCC CTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTC-CCAATGACCGCGTCTTCGTCG-3') (SEQ ID NO: 3) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. Similarly, the Vif target

sequence (5'-GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6) was incorporated into the miR21 backbone to create a Vif miRNA (5'-CATCTCCATGGCTGTACCACCT-TGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACT-GACATTTTGGTATCTTTCATCTGACCA-3') (SEQ ID NO: 2) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. The resulting Vif/Tat miRNA-expressing lentivirus vectors were produced in 293T cells using a lentiviral vector packaging system. The Vif and Tat miRNA were embedded into a microRNA cluster consisting of miR CCR5, miR Vif, and miR Tat all expressed under control of the EF-1 promoter.

Functional assay for miR185Tat inhibition of Tat mRNA accumulation. A lentivirus vector expressing miR185 Tat (LV-EF1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000 under standard conditions. 24 hours later RNA was extracted and levels of Tat messenger RNA were tested by RT-PCR using Tat-specific primers and compared to actin mRNA levels for a control.

Functional assay for miR21 Vif inhibition of Vif protein accumulation. A lentivirus vector expressing miR21 Vif (LV-EF1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction, the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000. 24 hours later cells were lysed and total soluble protein was tested to measure the content of Vif protein. Cell lysates were separated by SDS-PAGE according to established techniques. The separated proteins were transferred to nylon membranes and probed with a Vif-specific monoclonal antibody or actin control antibody.

As shown in FIG. **12**A, Tat knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing either synthetic miR185 Tat or miR155 Tat microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000 for 24 hours and then RNA was extracted for qPCR analysis with primers for Tat. As shown in FIG. **12**B, Vif knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing a synthetic miR21 Vif microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000 for 24 hours and then protein was extracted for immunoblot analysis with an antibody for HIV Vif.

Example 9

Regulation of CCR5 Expression by Synthetic MicroRNA Sequences in a Lentiviral Vector

CEM-CCR5 cells were transduced with a lentiviral vector containing a synthetic miR30 sequence for CCR5 (AGT103: TGTAAACTGAGCTTGCTCTA (SEQ ID NO: 97), AGT103-R5-1: TGTAAACTGAGCTTGCTCGC (SEQ ID NO: 98), or AGT103-R5-2: CATAGATTGGACTTGACAC (SEQ ID NO: 99). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified by mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The target sequence of AGT103 and AGT103-R5-1 is in the same region as CCR5 target sequence #5. The target sequence of AGT103-R5-2 is the same as CCR5 target sequence #1. AGT103 (2% of total CCR5) is most effective at reducing CCR5 levels as compared with AGT103-R5-1 (39% of total CCR5) and AGT103-R5-2 which does not reduce CCR5 levels. The data is demonstrated in FIG. **13** herein.

Example 10

Regulation of CCR5 Expression by Synthetic MicroRNA Sequences in a Lentiviral Vector Containing Either a Long or Short WPRE Sequence

Vector Construction. Lentivirus vectors often require an RNA regulatory element for optimal expression of therapeutic genes or genetic constructs. A common choice is to use the Woodchuck hepatitis virus post transcriptional regulatory element (WPRE). We compared AGT103 that contains a full-length WPRE:

(SEQ ID NO: 32)
(5'AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT
AACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTT
GTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATA
AATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAA
CGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGG
CATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCC
CTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACA
GGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATC
ATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCG
GGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCT
TCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCG
CCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCT-3')

with a modified AGT103 vector containing a shortened WPRE element

(SEQ ID NO: 80)
(5'AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCT
TAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTAATGCCTC
TGTATCATGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTAT
AAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCCGTCA
ACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGG
GCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTC
CCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGAC
AGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTC-3').

Functional assay for modulating cell surface CCR5 expression as a function of long versus short WPRE element in the vector sequence. AGT103 containing long or short WPRE elements were used for transducing CEM-CCR5 T cells a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of detecting cell surface CCR5 protein. The antibody was conjugated to a fluorescent marker and the intensity of staining is directly proportional

to the level of CCR5 on the cell surface. A control lentivirus had no effect on cell surface CCR5 levels resulting in a single population with a mean fluorescence intensity of 73.6 units. The conventional AGT103 with a long WPRE element reduced CCR5 expression to a mean fluorescence intensity level of 11 units. AGT103 modified to incorporate a short WPRE element resulted in a single population of cells with mean fluorescence intensity of 13 units. Accordingly, substituting a short WPRE element had little or no effect on the capacity for AGT103 to reduce cell surface CCR5 expression.

As shown in FIG. **14**, CEM-CCR5 cells were transduced with AGT103 containing either a long or short WPRE sequence. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with either the short (5.5% of total CCR5) or long (2.3% of total CCR5) WPRE sequence.

Example 11

Regulation of CCR5 Expression by Synthetic MicroRNA Sequences in a Lentiviral Vector with or without a WPRE Sequence

Vector Construction. In order to test whether WPRE was required for AGT103 down regulation of CCR5 expression we constructed a modified vector without WPRE element sequences.

Functional assay for modulating cell surface CCR5 expression as a function of including or not including a long WPRE element in the AGT103 vector. In order to test whether WPRE was required for AGT103 modulation of CCR5 expression levels we transduced CEM-CCR5 T cells with AGT103 or a modified vector lacking WPRE using a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was directly conjugated to a fluorescent marker and the intensity of staining is directly proportional to the number of CCR5 molecules per cell surface. A lentivirus control vector had no effect on cell surface CCR5 levels resulting in a uniform population with mean fluorescence intensity of 164. The lentivirus vector (AGT103 with a long WPRE and also expressing GFP marker protein), AGT103 lacking GFP but containing a long WPRE element, or AGT103 lacking both GFP and WPRE all were similarly effective for modulating cell surface CCR5 expression. After removing GFP, AGT103 with or without WPRE elements were indistinguishable in terms of their capacity for modulating cell surface CCR5 expression.

CEM-CCR5 cells were transduced with AGT103 with or without GFP and WPRE. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with (0% of total CCR5) or without (0% of total CCR5) the WPRE sequence. This data is demonstrated in FIG. **15**.

Example 12

Regulation of CCR5 Expression by a CD4 Promoter Regulating Synthetic MicroRNA Sequences in a Lentiviral Vector

Vector Construction. A modified version of AGT103 was constructed to test the effect of substituting alternate promoters for expressing the microRNA cluster that suppresses CCR5, Vif and Tat gene expression. In place of the normal EF-1 promoter we substituted the T cell-specific promoter for CD4 glycoprotein expression using the sequence:

(SEQ ID NO: 30)

(5'TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCCTCTGCAAAGAA

AAAAAAAAAAAAAAAAGAACAAAGGGCCTAGATTTCCCTTCTGAGCCCCA

CCCTAAGATGAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGGAGGCG

GATCCTGTCAGCTTTGCTCTCTCTGTGGCTGGCAGTTTCTCCAAAGGGTA

ACAGGTGTCAGCTGGCTGAGCCTAGGCTGAACCCTGAGACATGCTACCTC

TGTCTTCTCATGGCTGGAGGCAGCCTTTGTAAGTCACAGAAAGTAGCTGA

GGGGCTCTGGAAAAAAGACAGCCAGGGTGGAGGTAGATTGGTCTTTGACT

CCTGATTTAAGCCTGATTCTGCTTAACTTTTTCCCTTGACTTTGGCATTT

TCACTTTGACATGTTCCCTGAGAGCCTGGGGGGTGGGGAACCCAGCTCCA

GCTGGTGACGTTTGGGGCCGGCCCAGGCCTAGGGTGTGGAGGAGCCTTGC

CATCGGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGCAGA-3').

Functional assay comparing EF-1 and CD4 gene promoters in terms of potency for reducing cell surface CCR5 protein expression. AGT103 Modified by Substituting the CD4 Gene promoter for the normal EF-1 promoter was used for transducing CEM-CCR5 T cells. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was conjugated to a fluorescent marker and staining intensity is directly proportional to the level of cell surface CCR5 protein. A control lentivirus transduction resulted in a population of CEM-CCR5 T cells that were stained with a CCR5-specific monoclonal antibody and produced a mean fluorescence intensity of 81.7 units. The modified AGT103 using a CD4 gene promoter in place of the EF-1 promoter for expressing microRNA showed a broad distribution of staining with a mean fluorescence intensity roughly equal to 17.3 units. Based on this result, the EF-1 promoter is at least similar and likely superior to the CD4 gene promoter for microRNA expression. Depending on the desired target cell population, the EF-1 promoter is universally active in all cell types and the CD4 promoter is only active in T-lymphocytes.

CEM-CCR5 cells were transduced with a lentiviral vector containing a CD4 promoter regulating a synthetic microRNA sequence for CCR5, Vif, and Tat (AGT103). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. In cells transduced with LV-CD4-AGT103, CCR5 levels were 11% of total CCR5. This is comparable to that observed for LV-AGT103 which contains the EF1 promoter. This data is demonstrated in FIG. **16**.

Example 13

Detecting HIV Gag-Specific CD4 T Cells

Cells and reagents. Viable frozen peripheral blood mononuclear cells (PBMC) were obtained from a vaccine company. Data were obtained with a representative specimen from an HIV+individual who was enrolled into an early

stage clinical trial (TRIAL REGISTRATION: clinicaltrials-.gov NCT01378156) testing a candidate HIV therapeutic vaccine. Two specimens were obtained for the "Before vaccination" and "After vaccination" studies. Cell culture products, supplements and cytokines were from commercial suppliers. Cells were tested for responses to recombinant Modified Vaccinia Ankara 62B from Geovax Corporation as described in Thompson, M., S. L. Heath, B. Sweeton, K. Williams, P. Cunningham, B. F. Keele, S. Sen, B. E. Palmer, N. Chomont, Y. Xu, R. Basu, M. S. Hellerstein, S. Kwa and H. L. Robinson (2016). "DNA/MVA Vaccination of HIV-1 Infected Participants with Viral Suppression on Antiretroviral Therapy, followed by Treatment Interruption: Elicitation of Immune Responses without Control of Re-Emergent Virus." PLoS One 11(10): e0163164. Synthetic peptides representing the entire HIV-1 Gag polyprotein were obtained from GeoVax the HIV (GAG) Ultra peptide sets were obtained from JPT Peptide Technologies GmbH (www.jpt.com), Berlin, Germany. HIV (GAG) Ultra contains 150 peptides each being 15 amino acids in length and overlapping by 11 amino acids. They were chemically synthesized then purified and analyzed by liquid chromatography—mass spectrometry. Collectively these peptides represent major immunogenic regions of the HIV Gag polyprotein and are designed for average coverage of 57.8% among known HIV strains. Peptide sequences are based on the HIV sequence database from the Los Alamos National Laboratory (www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html). Peptides are provided as dried trifluoroacetate salts, 25 micrograms per peptide, and are dissolved in approximately 40 microliters of DMSO then diluted with PBS to final concentration. Monoclonal antibodies for detecting CD4 and cytoplasmic IFN-gamma were obtained from commercial sources and intracellular staining was done with the BD Pharmingen Intracellular Staining Kit for interferon-gamma. Peptides were resuspended in DMSO and we include a DMSO only control condition.

Functional assay for detecting HIV-specific CD4+ T cells. Frozen PBMC were thawed, washed and resuspended in RPMI medium containing 10% fetal bovine serum, supplements and cytokines. Cultured PBMC collected before or after vaccination were treated with DMSO control, MVA GeoVax (multiplicity of infection equal to 1 plaque forming unit per cell), Peptides GeoVax (1 microgram/ml) or HIV (GAG) Ultra peptide mixture (1 microgram/ml) for 20 hours in the presence of Golgi Stop reagent. Cells were collected, washed, fixed, permeabilized and stained with monoclonal antibodies specific for cell surface CD4 or intracellular interferon-gamma. Stained cells were analyzed with a FACSCalibur analytical flow cytometer and data were gated on the CD4+ T cell subset. Cells highlighted within boxed regions are double-positive and designated HIV-specific CD4 T cells on the basis of interferon-gamma expression after MVA or peptide stimulation. Numbers within the boxed regions show the percentage of total CD4 that were identified as HIV-specific. We did not detect strong responses to DMSO or MVA. Peptides from GeoVax elicited fewer responding cells compared to HIV (GAG) Ultra peptide mixture from JPT but differences were small and not significant.

As shown in FIG. **17**, PBMCs from a HIV-positive patient before or after vaccination were stimulated with DMSO (control), recombinant MVA expressing HIV Gag from GeoVax (MVA GeoVax), Gag peptide from GeoVax (Pep GeoVax, also referred to herein as Gag peptide pool 1) or Gag peptides from JPT (HIV (GAG) Ultra, also referred to herein as Gag peptide pool 2) for 20 hours. IFNg production was detected by intracellular staining and flow cytometry using standard protocols. Flow cytometry data were gated on CD4 T cells. Numbers captured in boxes are the percentage of total CD4 T cells designated "HIV-specific" on the basis of cytokine response to antigen-specific stimulation.

Example 14

HIV-Specific CD4 T Cell Expansion and Lentivirus Transduction

Designing and testing methods for enriching PBMC to increase the proportion of HIV-specific CD4 T cells and transducing these cells with AGT103 to produce the cellular product AGT103T.

The protocol was designed for ex vivo culture of PBMC (peripheral blood mononuclear cells) from HIV-positive patients who had received a therapeutic HIV vaccine. In this example, the therapeutic vaccine consisted of three doses of plasmid DNA expressing HIV Gag, Pol and Env genes followed by two doses of MVA 62-B (modified vaccinia Ankara number 62-B) expressing the same HIV Gag, Pol, and Env genes. The protocol is not specific for a vaccine product and only requires a sufficient level of HIV-specific CD4+ T cells after immunization. Venous blood was collected and PBMC were purified by Ficoll-Paque density gradient centrifugation. Alternately, PBMC or defined cellular tractions can be prepared by positive or negative selection methods using antibody cocktails and fluorescence activated or magnetic bead sorting. The purified PBMC are washed and cultured in standard medium containing supplements, antibiotics and fetal bovine serum. To these cultures, a pool of synthetic peptides was added representing possible T cell epitopes within the HIV Gag polyprotein. Cultures are supplemented by adding cytokines interleukin-2 and interleukin-12 that were selected after testing combinations of interleukin-2 and interleukin-12, interleukin 2 and interleukin-7, interleukin 2 and interleukin-15. Peptide stimulation is followed by a culture interval of approximately 12 days. During the 12 days culture, fresh medium and fresh cytokine supplements were added approximately once every four days.

The peptide stimulation interval is designed to increase the frequency of HIV-specific CD4 T cells in the PBMC culture. These HIV-specific CD4 T cells were activated by prior therapeutic immunization and can be re-stimulated and caused to proliferate by synthetic peptide exposure. Our goal is to achieve greater than or equal to 1% of total CD4 T cells being HIV-specific by end of the peptide stimulation culture period.

On approximately day 12 of culture cells are washed to remove residual materials then stimulated with synthetic beads decorated with antibodies against CD4 T cell surface proteins CD3 and CD28. This well-established method for polyclonal stimulation of T cells will reactivate the cells and make them more susceptible for AGT103 lentivirus transduction. The lentivirus transduction is performed on approximately day 13 of culture and uses a multiplicity of infection between 1 and 5. After transduction cells are washed to remove residual lentivirus vector and cultured in media containing interleukin-2 and interleukin-12 with fresh medium and cytokines added approximately once every four days until approximately day 24 of culture.

Throughout the culture interval the antiretroviral drug Saquinavir is added at a concentration of approximately 100 nM to suppress any possible outgrowth of HIV.

On approximately day 24 of culture cells are harvested, washed, a sample is set aside for potency and release assay, then the remaining cells are suspended in cryopreservation medium before freezing in single aliquots of approximately 1×10^{10} cells per dose that will contain approximately 1×10^{8} HIV-specific CD4 T cells that are transduced with AGT103.

Potency of the cell product (AGT103T) is tested in one of two alternate potency assays. Potency assay 1 tests for the average number of genome copies (integrated AGT103 vector sequences) per CD4 T cell. The minimum potency is approximately 0.5 genome copies per CD4 T cell in order to release the product. The assay is performed by positive selection of CD3 positive/CD4 positive T cells using magnetic bead labeled monoclonal antibodies, extracting total cellular DNA and using a quantitative PCR reaction to detect sequences unique to the AGT103 vector. Potency assay 2 tests for the average number of genome copies of integrated AGT103 within the subpopulation of HIV-specific CD4 T cells. This essay is accomplished by first stimulating the PBMC with the pool of synthetic peptides representing HIV Gag protein. Cells are then stained with a specific antibody reagent capable of binding to the CD4 T cell and also capturing secreted interferon-gamma cytokine. The CD4 positive/interferon-gamma positive cells are captured by magnetic bead selection, total cellular DNA is prepared, and the number of genome copies of AGT103 per cell is determined with a quantitative PCR reaction. Release criterion based on potency using Assay 2 require that greater than or equal to 0.5 genome copies per HIV-specific CD4 T-cell are present in the AGT103 cell product.

Functional test for enriching and transducing HIV-specific CD4 T cells from PBMC of HIV-positive patients that received a therapeutic HIV vaccine. The impact of therapeutic vaccination on the frequency of HIV-specific CD4 T cells was tested by a peptide stimulation assay (FIG. **14** panel B). Before vaccination the frequency of HIV-specific CD4 T cells was 0.036% in this representative individual. After vaccination, the frequency of HIV-specific CD4 T cells was increased approximately 2-fold to the value of 0.076%. Responding cells (HIV-specific) identified by accumulation of cytoplasmic interferon-gamma, were only detected after specific peptide stimulation.

We also tested whether peptide stimulation to enrich for HIV-specific CD4 T cells followed by AGT103 transduction would reach our goal of generating approximately 1% of total CD4 T cells in culture that were both HIV-specific and transduced by AGT103. In this case, we used an experimental version of AGT103 that expresses green fluorescence protein (see GFP). In FIG. **14**, panel C the post-vaccination culture after peptide stimulation (HIV (GAG) Ultra) and AGT103 transduction demonstrated that 1.11% of total CD4 T cells were both HIV-specific (based on expressing interferon-gamma in response to peptide stimulation) and AGT103 transduced (based on expression of GFP).

Several patients from a therapeutic HIV vaccine study were tested to assess the range of responses to peptide stimulation and to begin defining eligibility criteria for entering a gene therapy arm in a future human clinical trial. FIG. **18**D shows the frequency of HIV-specific CD4 T cells in 4 vaccine trial participants comparing their pre- and post-vaccination specimens. In three cases the post-vaccination specimens show a value of HIV-specific CD4 T cells that was greater than or equal to 0.076% of total CD4 T cells. The ability to reach this value was not predicted by the pre-vaccination specimens as patient 001-004 and patient 001-006 both started with pre-vaccination values of 0.02% HIV-specific CD4 T cells but one reached an eventual post-vaccination value of 0.12% HIV-specific CD4 T cells while the other individual fail to increase this value after vaccination. The same three patients that responded well to vaccine, in terms of increasing the frequency of HIV-specific CD4 T cells, also showed substantial enrichment of HIV-specific CD4 T cells after peptide stimulation and culture. In the three cases shown in FIG. **18**E, peptide stimulation and subsequent culture generated samples where 2.07%, 0.72% or 1.54% respectively of total CD4 T cells were HIV-specific. These values indicate that a majority of individuals responding to a therapeutic HIV vaccine will have a sufficiently large ex vivo response to peptide stimulation in order to enable our goal of achieving approximately 1% of total CD4 T cells that are HIV-specific and transduced with AGT103 in the final cell product.

FIG. **18**A describes the schedule of treatment. FIG. **18**B demonstrates that PBMCs were stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production was detected by intracellular staining by FACS. $CD4^+$ T cells were gated for analysis. FIG. **18**C demonstrates $CD4^+$ T cells were expanded and transduced with AGT103-GFP using the method as shown in FIG. **18**A. Expanded $CD4^+$ T cells were rested in fresh medium without any cytokine for 2 days and re-stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production and GFP expression was detected by FACS. $CD4^+$ T cells were gated for analysis. FIG. **18**D demonstrates frequency of HIV-specific $CD4^+$ T cells (IFN gamma positive, pre- and post-vaccination) were detected from 4 patients. Panel E demonstrates Post-vaccination PBMCs from 4 patients were expanded and HIV-specific $CD4^+$ T cells were examined.

Example 15

Dose Response

Vector Construction. A modified version of AGT103 was constructed to test the dose response for increasing AGT103 and its effects on cell surface CCR5 levels. The AGT103 was modified to include a green fluorescent protein (GFP) expression cassette under control of the CMV promoter. Transduced cells expression the miR30CCR5 miR21Vif miR185Tat micro RNA cluster and emit green light due to expressing GFP.

Functional assay for dose response of increasing AGT103-GFP and inhibition of CCR5 expression. CEM-CCR5 T cells were transduced with AGT103-GFP using multiplicity of infection per cell from 0 to 5. Transduced cells were stained with a fluorescently conjugated (APC) monoclonal antibody specific for cell surface CCR5. The intensity of staining is proportional to the number of CCR5 molecules per cell surface. The intensity of green fluorescence is proportional to the number of integrated AGT103-GFP copies per cell.

FIG. **19**A demonstrates the dose response for increasing AGT103-GFP and its effects on cell surface CCR5 expression. At multiplicity of infection equal to 0.4 only 1.04% of cells are both green (indicating transduction) and showing significantly reduced CCR5 expression. At multiplicity of infection equal to 1 the number of CCR5low, GFP+ cells increases to 68.1%! At multiplicity of infection equal to 5 the number of CCR5low, GFP+ cells increased to 95.7%. These data are presented in histogram form in FIG. **19**B that shows a normally distribution population in terms of CCR5 staining, moving toward lower mean fluorescence intensity with increasing doses of AGT103-GFP. The potency of AGT103-GFP is presented in graphical form in FIG. **19**C

showing the percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP. At multiplicity of infection equal to 5, there was greater than 99% reduction in CCR5 expression levels.

Example 16

AGT103 Efficiently Transduces Primary Human CD4+ T Cells

Transducing primary CD4 T cells with AGT103 lentivirus vector. A modified AGT103 vector containing the green fluorescence protein marker (GFP) was used at multiplicities of infection between 0.2 and 5 for transducing purified, primary human CD4 T cells.

Functional assay for transduction efficiency of AGT103 in primary human CD4 T cells. CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media containing interleukin-2 for 1 day before AGT103 transduction. The relationship between lentivirus vector dose (the multiplicity of infection) and transduction efficiency is demonstrated in FIG. **20**A showing that multiplicity of infection equal to 0.2 resulted in 9.27% of CD4 positive T cells being transduced by AGT103 and that value was increased to 63.1% of CD4 positive T cells being transduced by AGT103 with a multiplicity of infection equal to 5. In addition to achieving efficient transduction of primary CD4 positive T cells it is also necessary to quantify the number of genome copies per cell. In FIG. **20**B total cellular DNA from primary human CD4 T cells transduced at several multiplicities of infection were tested by quantitative PCR to determine the number of genome copies per cell. In a multiplicity of infection equal to 0.2 we measured 0.096 genome copies per cell that was in good agreement with 9.27% GFP positive CD4 T cells in FIG. **20**A. Multiplicity of infection equal to 1 generated 0.691 genome copies per cell and multiplicity of infection equal to 5 generated 1.245 genome copies per cell.

As shown in FIGS. **20**A-**20**B, CD4+ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations. After 2 days, beads were removed and CD4+ T cells were collected. As shown in FIG. **20**A, frequency of transduced cells (GFP positive) were detected by FACS. As shown in FIG. **20**B, the number of vector copies per cell was determined by qPCR. At a multiplicity of infection (MOI) of 5, 63% of CD4+ T cells were transduced with an average of 1 vector copy per cell.

Example 17

AGT103 Inhibits HIV Replication in Primary CD4+ T Cells

Protecting primary human CD4 positive T cells from hiv infection by transducing cells with AGT103. Therapeutic lentivirus AGT103 was used for transducing primary human CD4 positive T cells at multiplicities of infection between 0.2 and 5 per cell. The transduced cells were then challenged with a CXCR4-tropic HIV strain NL4.3 that does not require cell surface CCR5 for penetration. This assay tests the potency of microRNA against Vif and Tat genes of HIV in terms of preventing productive infection in primary CD4 positive T cells, but uses an indirect method to detect the amount of HIV released from infected, primary human CD4 T cells.

Functional assay for AGT103 protection against CXCR4-tropic HIV infection of primary human CD4 positive T cells. CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media containing interleukin-2 for 1 day before AGT103 transduction using multiplicities of infection between 0.2 and 5. Two days after transduction the CD4 positive T cell cultures were challenged with HIV strain NL4.3 that was engineered to express the green fluorescent protein (GFP). The transduced and HIV-exposed primary CD4 T cell cultures were maintained for 7 days before collecting cell-free culture fluids containing HIV. The cell-free culture fluids were used to infect a highly permissive T cell line C8166 for 2 days. The proportion of HIV-infected C8166 cells was determined by flow cytometry detecting GFP fluorescence. With a mock lentivirus infection, the dose of 0.1 multiplicity of infection for NL4.3 HIV resulted in an amount of HIV being released into culture fluids that was capable of establishing productive infection in 15.4% of C8166 T cells. With the dose 0.2 multiplicity of infection for AGT103, this value for HIV infection of C8166 cells is reduced to 5.3% and multiplicity of infection equal to 1 for AGT103 resulted in only 3.19% of C8166 T cells being infected by HIV. C8166 infection was reduced further to 0.62% after AGT103 transduction using a multiplicity of infection equal to 5. There is a clear dose response relationship between the amount of AGT103 used for transduction and the amount of HIV released into the culture medium.

As shown in FIG. **21**, CD4+ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and CD4+ T cells were infected with 0.1 MOI of HIV NL4.3-GFP. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30 U/ml) for 7 days. At the end of the culture, supernatant was collected to infect the HIV permissive cell line C8166 for 2 days. HIV-infected C8166 cells (GFP positive) were detected by FACS. There was a reduction in viable HIV with an increase in the multiplicity of infection of AGT103 as observed by less infection of C8166 cells MOI 0.2=65.6%, MOI 1=79.3%, and MOI 5=96%).

Example 18

AGT103 Protects Primary Human CD4+ T Cells from HIV-Induced Depletion

AGT103 transduction of primary human CD4 T cells to protect against HIV-mediated cytopathology and cell depletion. PBMC were obtained from healthy, HIV-negative donors and stimulated with CD3/CD28 beads then cultured for 1 day in medium containing interleukin-2 before AGT103 transduction using multiplicities of infection between 0.2 and 5.

Functional assay for AGT103 protection of primary human CD4 T cells against HIV-mediated cytopathology. AGT103-transduced primary human CD4 T cells were infected with HIV NL 4.3 strain (CXCR4-tropic) that does not require CCR5 for cellular entry. When using the CXCR4-tropic NL 4.3, only the effect of Vif and Tat microRNA on HIV replication is being tested. The dose of HIV NL 4.3 was 0.1 multiplicity of infection. One day after

HIV infection, cells were washed to remove residual virus and cultured in medium plus interleukin-2. Cells were collected every three days during a 14-day culture then stained with a monoclonal antibody that was specific for CD4 and directly conjugated to a fluorescent marker to allow measurement of the proportion of CD4 positive T cells in PBMC. Untreated CD4 T cells or CD4 T cells transduced with the control lentivirus vector were highly susceptible to HIV challenge and the proportion of CD4 positive T cells in PBMC fell below 10% by day 14 culture. In contrast, there was a dose-dependent effect of AGT103 on preventing cell depletion by HIV challenge. With a AGT103 dose of 0.2 multiplicity of infection more than 20% of PBMC were CD4 T cells by day 14 of culture and this value increased to more than 50% of PBMC being CD4 positive T cells by day 14 of culture with a AGT103 dose of multiplicity of infection equal to 5. Again, there is a clear dose response effect of AGT103 on HIV cytopathogenicity in human PBMC.

As shown in FIG. **22**, PBMCs were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and cells were infected with 0.1 MOI of HIV NL4.3. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30 U/ml). Cells were collected every 3 days and the frequency of $CD4^+$ T cells were analyzed by FACS. After 14 days of exposure to HIV, there was an 87% reduction in $CD4^+$ T cells transduced with LV-Control, a 60% reduction with AGT103 MOI 0.2, a 37% reduction with AGT103 MOI 1, and a 17% reduction with AGT103 MOI 5.

Example 19

Generating a Population of CD4+ T Cells Enriched for HIV-Specificity and Transduced with AGT103/CMV-GFP

Therapeutic vaccination against HIV had minimal effect on the distribution of CD4+, CD8+ and CD4+/CD8+ T cells. As shown in FIG. **23**A, the CD4 T cell population is shown in the upper left quadrant of the analytical flow cytometry dot plots, and changes from 52% to 57% of total T cells after the vaccination series. These are representative data.

Peripheral blood mononuclear cells from a participant in an HIV therapeutic vaccine trial were cultured for 12 days in medium+/−interleukin-2/interleukin-12 or +/−interleukin-7/interleukin-15. Some cultures were stimulated with overlapping peptides representing the entire p55 Gag protein of HIV-1 (HIV (GAG) Ultra peptide mixture) as a source of epitope peptides for T cell stimulation. These peptides are 10-20 amino acids in length and overlap by 20-50% of their length to represent the entire Gag precursor protein (p55) from HIV-1 BaL strain. The composition and sequence of individual peptides can be adjusted to compensate for regional variations in the predominant circulating HIV sequences or when detailed sequence information is available for an individual patient receiving this therapy. At culture end, cells were recovered and stained with anti-CD4 or anti-CD8 monoclonal antibodies and the CD3+ population was gated and displayed here. The HIV (GAG) Ultra peptide mixture stimulation for either pre- or post-vaccination samples was similar to the medium control indicating that HIV (GAG) Ultra peptide mixture was not toxic to cells and was not acting as a polyclonal mitogen. The results of this analysis can be found in FIG. **23**B.

HIV (GAG) Ultra peptide mixture and interleukin-2/interleukin-12 provided for optimal expansion of antigen-specific CD4 T cells. As shown in the upper panels of FIG. **23**C, there was an increase in cytokine (interferon-gamma) secreting cells in post-vaccination specimens exposed to HIV (GAG) Ultra peptide mixture. In the pre-vaccination sample, cytokine secreting cells increased from 0.43 to 0.69% as a result of exposure to antigenic peptides. In contrast, the post-vaccination samples showed an increase of cytokine secreting cells from 0.62 to 1.76% of total CD4 T cells as a result of peptide stimulation. These data demonstrate the strong impact of vaccination on the CD4 T cell responses to HIV antigen.

Finally, AGT103/CMV-GFP transduction of antigen-expanded CD4 T cells produced HIV-specific and HIV-resistant helper CD4 T cells that are needed for infusion into patients as part of a functional cure for HIV (in accordance with other various aspects and embodiments, AGT103 alone is used; for example, clinical embodiments may not include the CMV-GFP segment). The upper panels of FIG. **23**C show the results of analyzing the CD4+ T cell population in culture. The x axis of FIG. **23**C shows Green Fluorescent Protein (GFP) emission indicating that individual cells were transduced with the AGT103/CMV-GFP. In the post-vaccination samples 1.11% of total CD4 T cells that were both cytokine secreting was recovered, indicating that the cells are responding specifically to HIV antigen, and transduced with AGT103/CMV-GFP. This is the target cell population and the clinical product intended for infusion and functional cure of HIV. With the efficiency of cell expansion during the antigen stimulation and subsequent polyclonal expansion phases of ex vivo culture, 4×10^8 antigen-specific, lentivirus transduced CD4 T cells can be produced. This exceeds the target for cell production by 4-fold and will allow achievement of a count of antigen-specific and HIV-resistant CD4 T cells of approximately 40 cells/microliter of blood or around 5.7% of total circulating CD4 T cells.

Table 4 below shows the results of the ex vivo production of HIV-specific and HIV-resistant CD4 T cells using the disclosed vectors and methods.

TABLE 4

Material/manipulation	Total CD4 T cells	Percentage HIV-specific	Percentage HIV-specific and HIV-resistant
Leukapheresis pack from HIV+ patient	~7×10^8	~0.12	N/A
Peptide expansion ex vivo	~8×10^8	~2.4	N/A
Mitogen expansion	~1.5×10^{10}	~2.4	N/A
Lentivirus transduction	~1.5×10^{10}	~2.4	~1.6

Example 20

Clinical Study for Treatment of HIV

AGT103T is a genetically modified autologous PBMC containing $>5\times10^7$ HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector.

A Phase I clinical trial will test the safety and feasibility of infusing ex vivo modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 cells per mm^3 of blood and stable virus suppression below 200 copies per ml of plasma while on cART. All study participants will continue receiving their standard antiretroviral medications through the Phase I clinical trial. Up to 40 study participants

receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro testing to measure the frequency of CD4+ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polyprotein. Subjects in the upper half of vaccine responders, based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not continue in the study. We anticipate that the cut-off for higher responders is a HIV-specific CD4+ T cell frequency ≥0.065% of total CD4 T cells. Subjects enrolled into the gene therapy arm of our trial undergo leukapheresis followed by purification of PBMC (using Ficoll density gradient centrifugation or negative selection with antibodies) that are cultured ex vivo and stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during ex vivo culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1\times10^{10}$ autologous PBMC. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include the product must include $\geq 0.5\times10^{7}$ HIV-specific CD4 T cells that are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103T subjects receive one dose of busulfuram (or Cytoxan) conditioning regimen followed by infusion of $\leq 1\times10^{10}$ PBMC containing genetically modified CD4 T cells.

A Phase II study will evaluate efficacy of AGT103T cell therapy. Phase II study participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws. Persistently high viremia requires participants to return to their original antiretroviral drug regimen with or without Maraviroc according to recommendations of their HIV care physician.

If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

Patient Selection

Inclusion Criteria:

- Aged between 18 and 60 years.
- Documented HIV infection prior to study entry.
- Must be willing to comply with study-mandated evaluations; including not changing their antiretroviral regimen (unless medically indicated) during the study period.
- CD4+ T-cell count >600 cell per millimeter cubed (cells/mm3)
- CD4+ T-cell nadir of >400 cells/mm3
- HIV viral load <1,000 copies per milliliter (mL)

Exclusion Criteria:

- Any viral hepatitis
- Acute HIV infection
- HIV viral load >1,000 copies/mL
- Active or recent (prior 6 months) AIDS defining complication
- Any change in HIV medications within 12 weeks of entering the study
- Cancer or malignancy that has not been in remission for at least 5 years with the exception of successfully treated basal cell carcinoma of the skin
- Current diagnosis of NYHA grade 3 or 4 congestive heart failure or uncontrolled angina or arrhythmias
- History of bleeding problems
- Use of chronic steroids in past 30 days
- Pregnant or breast feeding
- Active drug or alcohol abuse
- Serious illness in past 30 days
- Currently participating in another clinical trial or any prior gene therapy

Safety Assessments

- Acute infusion reaction
- Post-infusion safety follow-up

Efficacy Assessments—Phase I

- Number and frequency of modified CD4 T cells.
- Durability of modified CD4 T cells.
- In vitro response to Gag peptide restimulation (ICS assay) as a measure of memory T cell function.
- Polyfunctional anti-HIV CD8 T cell responses compare to pre- and post-vaccination time points.
- Frequency of CD4 T cells making doubly spliced HIV mRNA after in vitro stimulation.

Efficacy assessments—Phase II

- Number and frequency of genetically modified CD4 T cells.
- Maintenance of viral suppression (<2,000 vRNA copies per ml but 2 consecutive weekly draws not exceeding 5×10^{4} vRNA copies per ml are permitted) with Maraviroc monotherapy.
- Continued virus suppression during and after Maraviroc withdrawal.
- Stable CD4 T cell count.

AGT103T Consists of Up to 1×10^{10} Genetically Modified, Autologous CD4+ T Cells Containing $\geq 5\times10^{7}$ HIV-Specific CD4 T Cells that are Also Transduced with AGT103 Lentivirus Vector.

A Phase I clinical trial will test the safety and feasibility of infusing ex vivo modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 cells per mm^3 of blood and stable virus suppression below 200 copies per ml of plasma while on cART. Up to 40 study participants

receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro testing to measure the frequency of CD4+ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polyprotein. Subjects in the upper half of vaccine responders, based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not continue in the study. We anticipate that the cut-off for higher responders is a HIV-specific CD4+ T cell frequency ≥0.065% of total CD4 T cells. Subjects enrolled into the gene therapy arm of our trial undergo leukapheresis and the CD4+ T cells are enriched by negative selection. The enriched CD4 subset is admixed with 10% the number of cells from the CD4-negative subset to provide a source and antigen-presenting cells. The enriched CD4 T cells are stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during ex vivo culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1\times10^{10}$ autologous cells enriched for the CD4+ T cell subset. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include that the product must include $\geq 0.5\times10^{7}$ HIV-specific CD4 T cells that are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103T subjects receive one dose of busulfuram (or Cytoxan) conditioning regimen followed by infusion of $\leq 1\times10^{10}$ enriched and genetically modified CD4 T cell.

A Phase II study will evaluate efficacy of AGT103T cell therapy. Phase II study participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws. Persistently high viremia requires participants to return to their original antiretroviral drug regimen with or without Maraviroc according to recommendations of their HIV care physician.

If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

Sequences

The following sequences are referred to herein:

SEQ ID NO:	Description	Sequence
1	miR30 CCR5	AGGTATATTGCTGTTGACAGTGAGCGACTGTAAAC TGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAG AGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGA CTTCAAGGGGCTT
2	miR21 Vif	CATCTCCATGGCTGTACCACCTTGTCGGGGGATGT GTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCA GAAGAACACATCCGCACTGACATTTTGGTATCTTT CATCTGACCA
3	miR185 Tat	GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT TCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTC CCTCCCAATGACCGCGTCTTCGTCG
4	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTG GGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCC CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACGCCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG GTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCC CTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGG GCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGT TCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC CCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGC GCTCGGGAGAGCGGGCGGGTGAGTCACCCACACA AAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG TCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGA GTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT TTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCAT TTCAGGTGTCGTGA
5	CCR5 target sequence	GAGCAAGCTCAGTTTACA
6	Vif target sequence	GGGATGTGTACTTCTGAACTT
7	Tat target sequence	TCCGCTTCTTCCTGCCATAG
8	TAR decoy sequence	CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTT CTCGACAGCGACCAGATCTGAGCCTGGGAGCTCTC TGGCTGTCAGTAAGCTGGTACAGAAGGTTGACGAA AATTCTTACTGAGCAAGAAA
9	Rev/Tat target sequence	GCGGAGACAGCGACGAAGAGC

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SEQ ID NO:	Description	Sequence
10	Rev/Tat shRNA sequence	GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTC TTCGTCGCTGTCTCCGCTTTTT
11	Gag target sequence	GAAGAAATGATGACAGCAT
12	Gag shRNA sequence	GAAGAAATGATGACAGCATTTCAAGAGAATGCTGT CATCATTTCTTCTTTTT
13	Pol target sequence	CAGGAGCAGATGATACAG
14	Pol shRNA sequence	CAGGAGATGATACAGTTCAAGAGACTGTATCATCT GCTCCTGTTTTT
15	CCR5 target sequence #1	GTGTCAAGTCCAATCTATG
16	CCR5 shRNA sequence #1	GTGTCAAGTCCAATCTATGTTCAAGAGACATAGAT TGGACTTGACACTTTTT
17	CCR5 target sequence #2	GAGCATGACTGACATCTAC
18	CCR5 shRNA sequence #2	GAGCATGACTGACATCTACTTCAAGAGAGTAGATG TCAGTCATGCTCTTTTT
19	CCR5 target sequence #3	GTAGCTCTAACAGGTTGGA
20	CCR5 shRNA sequence #3	GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACC TGTTAGAGCTACTTTTT
21	CCR5 target sequence #4	GTTCAGAAACTACCTCTTA
22	CCR5 shRNA sequence #4	GTTCAGAAACTACCTCTTATTCAAGAGATAAGAGG TAGTTTCTGAACTTTTT
23	CCR5 target sequence #5	GAGCAAGCTCAGTTTACACC
24	CCR5 shRNA sequence #5	GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTA AACTGAGCTTGCTCTTTTT

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SEQ ID NO:	Description	Sequence
25	*Homo sapiens* CCR5 gene, sequence 1	ATGGATTATCAAGTGTCAAGTCCAATCTATGACAT CAATTATTATACATCGGAGCCCTGCCAAAAAATCA ATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCG CTCTACTCACTGGTGTTCATCTTTGGTTTTGTGGGC
26	*Homo sapiens* CCR5 gene, sequence 2	AACATGCTGGTCATCCTCATCCTGATAAACTGCAA AAGGCTGAAGAGCATGACTGACATCTACCTGCTCA ACCTGGCCATCTCTGACCTGTTTTTCCTTCTTACTG TCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGG ACTTTGGAAATACAATGTGTCAACTCTTGACAGGG CTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTC ATCATCCTCCTGACAATCGATAGGTACCTGGCTGTC GTCCATGCTGTGTTTGCTTTAAAAGCCAGGACGGT CACCTTTGGGGTGGTGACAAGTGTGATCACTTGGG TGGTGGCTGTGTTTGCGTCTCTCCCAGGAATCATCT TTACCAGATCTCAAAAAGAAGGTCTTCATTACACC TGCAGCTCTCATTTTCCATACAGTCAGTATCAATTC TGGAAGAATTTCCAGACATTAAAGATAGTCATCTT GGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTG CTACTCGGGAATCCTAAAAACTCTGCTTCGGTGTC GAAATGAGAAGAAGAGGCACAGGGCTGTGAGGCT TATCTTCACCATCATGATTGTTTATTTTCTCTTCTG GGCTCCCTACAACATTGTCCTTCTCCTGAAC
27	*Homo sapiens* CCR5 gene, sequence 3	ACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGT AGCTCTAACAGGTTGGACCAAGCTATGCAGGTGA
28	*Homo sapiens* CCR5 gene, sequence 4	CAGAGACTCTTGGGATGACGCACTGCTGCATCAAC CCCATCATCTATGCCTTTGTCGGGGAGAAGTTCAG AAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGC CAAACGCTTCTGCAAATGCTGTTCTATTTTCCAG
29	*Homo sapiens* CCR5 gene, sequence 5	CAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACAC CCGATCCACTGGGGAGCAGGAAATATCTGTGGGCT TGTGA
30	CD4 promoter sequence	TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCC CTCTGCAAAGAAAAAAAAAAAAAAAAAAGAACAA AGGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAG ATGAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGG TGGAGGCGGATCCTGTCAGCTTTGCTCTCTCTGTGG CTGGCAGTTTCTCCAAAGGGTAACAGGTGTCAGCT GGCTGAGCCTAGGCTGAACCCTGAGACATGCTACC TCTGTCTTCTCATGGCTGGAGGCAGCCTTTGTAAGT CACAGAAAGTAGCTGAGGGGCTCTGGAAAAAAGA CAGCCAGGGTGGAGGTAGATTGGTCTTTGACTCCT GATTTAAGCCTGATTCTGCTTAACTTTTTCCCTTGA CTTTGGCATTTTCACTTTGACATGTTCCCTGAGAGC CTGGGGGGTGGGGAACCCAGCTCCAGCTGGTGACG TTTGGGGCCGGCCCAGGCCTAGGGTGTGGAGGAGC CTTGCCATCGGGCTTCCTGTCTCTCTTCATTTAAGC ACGACTCTGCAGA
31	miR30-CCR5/miR21-Vif/miR185 Tat microRNA	AGGTATATTGCTGTTGACAGTGAGCGACTGTAAAC TGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAG AGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGA CTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTA CCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTG TTGAATCTCATGGAGTTCAGAAGAACACATCCGCA CTGACATTTTGGTATCTTTCATCTGACCAGCTAGCG

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SEQ ID NO:	Description	Sequence
	cluster sequence	GGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTT CTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAG GCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTC TTCGTC
32	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAAGATTG ACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTA TGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT ATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG TATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTG TGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACT GTGTTTGCTGACGCAACCCCCACTGGTTGGGGCAT TGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGC TTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGC CGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGC TGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGG AAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTT GCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTA CGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTC CCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGC GTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCC TTTGGGCCGCCTCCCCGCCT
33	Elongation Factor-1 alpha (EF1-alpha) promoter; miR30CCR5; miR21Vif; miR185 Tat	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTG GGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCC CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACGCCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG GTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCC CTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGG GCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGT TCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC CCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGC GCTCGGGAGAGCGGGCGGGTGAGTCACCCACACA AAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG TCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGA GTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT TTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCAT TTCAGGTGTCGTGATGTACAAGGTATATTGCTGTTG ACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTG TGAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC CGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCC GGGCATCTCCATGGCTGTACCACCTTGTCGGGGGA TGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGT TCAGAAGAACACATCCGCACTGACATTTTGGTATC TTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCA GGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCG TGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACC TTCCCTCCCAATGACCGCGTCTTCGTC
34	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACAT GGTAACGATGAGTTAGCAACATGCCTTACAAGGAG AGAAAAAGCACCGTGCATGCCGATTGGTGGAAGT AAGGTGGTACGATCGTGCCTTATTAGGAAGGCAAC AGACGGGTCTGACATGGATTGGACGAACCACTGAA TTGCCGCATTGCAGAGATATTGTATTTAAGTGCCTA GCTCGATACAATAAACG

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SEQ ID NO:	Description	Sequence
35	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAG CTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCC TCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGT GTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGA TCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCT AGCA
36	Psi Packaging signal	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGG AGAGAG
37	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG GAAGCACTATGGGCGCAGCCTCAATGACGCTGACG GTACAGGCCAGACAATTATTGTCTGGTATAGTGCA GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC AAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA GATACCTAAAGGATCAACAGCTCC
38	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGT GCAGGGGAAAGAATAGTAGACATAATAGCAACAG ACATACAAACTAAAGAATTACAAAAACAAATTAC AAAATTCAAAATTTTA
39	3' delta LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGAC CAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG GAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTT GAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGT CAGTGTGGAAAATCTCTAGCAGTAGTAGTTCATGT CA
40	Helper/ Rev; CMV early (CAG) enhancer; Enhance Trans-cription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAA CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA CGACCCCCGCCCATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCCAGTACATGACCTTATGGGA CTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT C
41	Helper/ Rev; Chicken beta actin (CAG) promoter; Trans-cription	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCC CAATTTTGTATTTATTTATTTTTTAATTATTTTGTG CAGCGATGGGGGCGGGGGGGGGGGGCGCGCGCC AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGG GCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGG CGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG CGCGGCGGGCG
42	Helper/ Rev; Chicken beta actin intron; Enhance gene expres-sion	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC CGCGCCGCCTCGCGCCGCCCCGCCCCGGCTCTGACT GACCGCGTTACTCCCACAGGTGAGCGGGCGGGACG GCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTT AATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAA GCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGG GGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGT GCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTT TGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCC GGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGA GGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTG GGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCG GGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGT TGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCC GTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGC GGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGG CGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGA GGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTC

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SEQ ID NO:	Description	Sequence
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		TAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC
		CCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC
		GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGT
		GCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGG
		CCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCA
		TCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTG
		CCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC
		TTCTGGCGTGTGACCGGCGG
43	Helper/ Rev; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAG
		AATTAGATCGATGGGAAAAAATTCGGTTAAGGCCA
		GGGGGAAAGAAAAAATATAAATTAAAACATATAG
		TATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTT
		AATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAG
		ACAAATACTGGGACAGCTACAACCATCCCTTCAGA
		CAGGATCAGAAGAACTTAGATCATTATATAATACA
		GTAGCAACCCTCTATTGTGTGCATCAAAGGATAGA
		GATAAAAGACACCAAGGAAGCTTTAGACAAGATA
		GAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCAC
		AGCAAGCAGCAGCTGACACAGGACACAGCAATCA
		GGTCAGCCAAAATTACCCTATAGTGCAGAACATCC
		AGGGGCAAATGGTACATCAGGCCATATCACCTAGA
		ACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGA
		AGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCA
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		CACCATGCTAAACACAGTGGGGGGACATCAAGCA
		GCCATGCAAATGTTAAAAGAGACCATCAATGAGG
		AAGCTGCAGAATGGGATAGAGTGCATCCAGTGCAT
		GCAGGGCCTATTGCACCAGGCCAGATGAGAGAAC
		CAAGGGGAAGTGACATAGCAGGAACTACTAGTAC
		CCTTCAGGAACAAATAGGATGGATGACACATAATC
		CACCTATCCCAGTAGGAGAAATCTATAAAAGATGG
		ATAATCCTGGGATTAAATAAAATAGTAAGAATGTA
		TAGCCCTACCAGCATTCTGGACATAAGACAAGGAC
		CAAAGGAACCCTTTAGAGACTATGTAGACCGATTC
		TATAAAACTCTAAGAGCCGAGCAAGCTTCACAAGA
		GGTAAAAAATTGGATGACAGAAACCTTGTTGGTCC
		AAAATGCGAACCCAGATTGTAAGACTATTTTAAAA
		GCATTGGGACCAGGAGCGACACTAGAAGAAATGA
		TGACAGCATGTCAGGGAGTGGGGGGACCCGGCCA
		TAAAGCAAGAGTTTTGGCTGAAGCAATGAGCCAAG
		TAACAAATCCAGCTACCATAATGATACAGAAAGGC
		AATTTTAGGAACCAAAGAAAGACTGTTAAGTGTTT
		CAATTGTGGCAAAGAAGGGCACATAGCCAAAAAT
		TGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAAT
		GTGGAAAGGAAGGACACCAAATGAAAGATTGTAC
		TGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGC
		CTTCCCACAAGGGAAGGCCAGGGAATTTTCTTCAG
		AGCAGACCAGAGCCAACAGCCCCACCAGAAGAGA
		GCTTCAGGTTTGGGGAAGAGACAACAACTCCCTCT
		CAGAAGCAGGAGCCGATAGACAAGGAACTGTATC
		CTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACC
		CCTCGTCACAATAA
44	Helper/ Rev; HIV Pol; Protease and reverse trans-criptase	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGA
		TAGGGGGAATTGGAGGTTTTATCAAAGTAGGACAG
		TATGATCAGATACTCATAGAAATCTGCGGACATAA
		AGCTATAGGTACAGTATTAGTAGGACCTACACCTG
		TCAACATAATTGGAAGAAATCTGTTGACTCAGATT
		GGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAG
		ACTGTACCAGTAAAATTAAAGCCAGGAATGGATGG
		CCCAAAAGTTAAACAATGGCCATTGACAGAAGAA
		AAAATAAAAGCATTAGTAGAAATTTGTACAGAAAT
		GGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT
		GAAAATCCATACAATACTCCAGTATTTGCCATAAA
		GAAAAAAGACAGTACTAAATGGAGAAAATTAGTA
		GATTTCAGAGAACTTAATAAGAGAACTCAAGATTT
		CTGGGAAGTTCAATTAGGAATACCACATCCTGCAG
		GGTTAAAACAGAAAAAATCAGTAACAGTACTGGA
		TGTGGGCGATGCATATTTTTCAGTTCCCTTAGATAA
		AGACTTCAGGAAGTATACTGCATTTACCATACCTA
		GTATAAACAATGAGACACCAGGGATTAGATATCAG
		TACAATGTGCTTCCACAGGGATGGAAAGGATCACC
		AGCAATATTCCAGTGTAGCATGACAAAAATCTTAG

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SEQ ID NO:	Description	Sequence
		AGCCTTTTAGAAAACAAAATCCAGACATAGTCATC
		TATCAATACATGGATGATTTGTATGTAGGATCTGA
		CTTAGAAATAGGGCAGCATAGAACAAAAATAGAG
		GAACTGAGACAACATCTGTTGAGGTGGGGATTTAC
		CACACCAGACAAAAAACATCAGAAAGAACCTCCA
		TTCCTTTGGATGGGTTATGAACTCCATCCTGATAAA
		TGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGA
		CAGCTGGACTGTCAATGACATACAGAAATTAGTGG
		GAAAATTGAATTGGGCAAGTCAGATTTATGCAGGG
		ATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGG
		AACCAAAGCACTAACAGAAGTAGTACCACTAACA
		GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGG
		AGATTCTAAAAGAACCGGTACATGGAGTGTATTAT
		GACCCATCAAAAGACTTAATAGCAGAAATACAGA
		AGCAGGGGCAAGGCCAATGGACATATCAAATTTAT
		CAAGAGCCATTTAAAAATCTGAAAACAGGAAAAT
		ATGCAAGAATGAAGGGTGCCCACACTAATGATGTG
		AAACAATTAACAGAGGCAGTACAAAAAATAGCCA
		CAGAAAGCATAGTAATATGGGGAAAGACTCCTAA
		ATTTAAATTACCCATACAAAAGGAAACATGGGAAG
		CATGGTGGACAGAGTATTGGCAAGCCACCTGGATT
		CCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTG
		AAGTTATGGTACCAGTTAGAGAAAGAACCCATAAT
		AGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCA
		ATAGGGAAACTAAATTAGGAAAAGCAGGATATGT
		AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTA
		ACGGACACAACAAATCAGAAGACTGAGTTACAAG
		CAATTCATCTAGCTTTGCAGGATTCGGGATTAGAA
		GTAAACATAGTGACAGACTCACAATATGCATTGGG
		AATCATTCAAGCACAACCAGATAAGAGTGAATCAG
		AGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAA
		AAGGAAAAAGTCTACCTGGCATGGGTACCAGCAC
		ACAAAGGAATTGGAGGAAATGAACAAGTAGATGG
		GTTGGTCAGTGCTGGAATCAGGAAAGTACTA
45	Helper Rev; HIV Integrase; Integration of viral RNA	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA
		TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA
		GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAA
		ATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGG
		GGAAGCCATGCATGGACAAGTAGACTGTAGCCCA
		GGAATATGGCAGCTAGATTGTACACATTTAGAAGG
		AAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG
		GATATATAGAAGCAGAAGTAATTCCAGCAGAGAC
		AGGGCAAGAAACAGCATACTTCCTCTTAAAATTAG
		CAGGAAGATGGCCAGTAAAAACAGTACATACAGA
		CAATGGCAGCAATTTCACCAGTACTACAGTTAAGG
		CCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTT
		GGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
		AGAATCTATGAATAAAGAATTAAAGAAAATTATAG
		GACAGGTAAGAGATCAGGCTGAACATCTTAAGAC
		AGCAGTACAAATGGCAGTATTCATCCACAATTTTA
		AAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG
		GGAAAGAATAGTAGACATAATAGCAACAGACATA
		CAAACTAAAGAATTACAAAAACAAATTACAAAAA
		TTCAAAATTTTCGGGTTTATTACAGGGACAGCAGA
		GATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTG
		GAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT
		AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAA
		AGATCATCAGGGATTATGGAAAACAGATGGCAGG
		TGATGATTGTGTGGCAAGTAGACAGGATGAGGATT
		AA
46	Helper/ Rev; HIV RRE; Binds Rev element	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG
		GAAGCACTATGGGCGCAGCGTCAATGACGCTGACG
		GTACAGGCCAGACAATTATTGTCTGGTATAGTGCA
		GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC
		AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC
		AAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA
		GATACCTAAAGGATCAACAGCTCCT
47	Helper/ Rev; HIV Rev; Nuclear export	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC
		TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC
		AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
		ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGA
		GAGAGACAGAGACAGATCCATTCGATTAGTGAAC

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SEQ ID NO:	Description	Sequence
	and stabilize viral mRNA	GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT ACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA ATAG
48	Helper/ Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAAATCATTTAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGGC TGCCATGAACAAAGGTGGCTATAAAGAGGTCATCA GTATATGAAACAGCCCCCTGCTGTCCATTCCTTATT CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT TATATTTTGTTTTGTGTTATTTTTTTCTTTAACATC CCTAAAATTTTCCTTACATGTTTTACTAGCCAGATT TTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGT CCCTCTTCTCTTATGAAGATC
49	Helper; CMV early (CAG) enhancer; Enhance trans-cription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAA CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA CGACCCCCGCCCATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCCAGTACATGACCTTATGGGA CTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT C
50	Helper; Chicken beta actin (CAG) promoter; Trans-cription	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCT GCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCC CAATTTTGTATTTATTTATTTTTTAATTATTTTGTG CAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCC AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGG GCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGG CGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG CGCGGCGGGCG
51	Helper; Chicken beta actin intron; Enhance gene expres-sion	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACT GACCGCGTTACTCCCACAGGTGAGCGGGCGGGACG GCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTT AATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAA GCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGG GGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGT GCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTT TGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCC GGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGA GGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTG GGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCG GGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGT TGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCC GTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGC GGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGG CGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGA GGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTC GAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGG TAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTC CCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCC GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGT GCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGG CCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCA TCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTG CCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC TTCTGGCGTGTGACCGGCGG

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SEQ ID NO:	Description	Sequence
52	Helper; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAG AATTAGATCGATGGGAAAAAATTCGGTTAAGGCCA GGGGGAAAGAAAAAATATAAATTAAAACATATAG TATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTT AATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAG ACAAATACTGGGACAGCTACAACCATCCCTTCAGA CAGGATCAGAAGAACTTAGATCATTATATAATACA GTAGCAACCCTCTATTGTGTGCATCAAAGGATAGA GATAAAAGACACCAAGGAAGCTTTAGACAAGATA GAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCAC AGCAAGCAGCAGCTGACACAGGACACAGCAATCA GGTCAGCCAAAATTACCCTATAGTGCAGAACATCC AGGGGCAAATGGTACATCAGGCCATATCACCTAGA ACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGA AGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCA GCATTATCAGAAGGAGCCACCCCACAAGATTTAAA CACCATGCTAAACACAGTGGGGGGACATCAAGCA GCCATGCAAATGTTAAAAGAGACCATCAATGAGG AAGCTGCAGAATGGGATAGAGTGCATCCAGTGCAT GCAGGGCCTATTGCACCAGGCCAGATGAGAGAAC CAAGGGGAAGTGACATAGCAGGAACTACTAGTAC CCTTCAGGAACAAATAGGATGGATGACACATAATC CACCTATCCCAGTAGGAGAAATCTATAAAAGATGG ATAATCCTGGGATTAAATAAAATAGTAAGAATGTA TAGCCCTACCAGCATTCTGGACATAAGACAAGGAC CAAAGGAACCCTTTAGAGACTATGTAGACCGATTC TATAAAACTCTAAGAGCCGAGCAAGCTTCACAAGA GGTAAAAAATTGGATGACAGAAACCTTGTTGGTCC AAAATGCGAACCCAGATTGTAAGACTATTTTAAAA GCATTGGGACCAGGAGCGACACTAGAAGAAATGA TGACAGCATGTCAGGGAGTGGGGGGACCCGGCCA TAAAGCAAGAGTTTTGGCTGAAGCAATGAGCCAAG TAACAAATCCAGCTACCATAATGATACAGAAAGGC AATTTTAGGAACCAAAGAAAGACTGTTAAGTGTTT CAATTGTGGCAAAGAAGGGCACATAGCCAAAAAT TGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAAT GTGGAAAGGAAGGACACCAAATGAAAGATTGTAC TGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGC CTTCCCACAAGGGAAGGCCAGGGAATTTTCTTCAG AGCAGACCAGAGCCAACAGCCCCACCAGAAGAGA GCTTCAGGTTTGGGGAAGAGACAACAACTCCCTCT CAGAAGCAGGAGCCGATAGACAAGGAACTGTATC CTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACC CCTCGTCACAATAA
53	Helper; HIV Pol; Protease and reverse trans-criptase	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGA TAGGGGGAATTGGAGGTTTTATCAAAGTAGGACAG TATGATCAGATACTCATAGAAATCTGCGGACATAA AGCTATAGGTACAGTATTAGTAGGACCTACACCTG TCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAG ACTGTACCAGTAAAATTAAAGCCAGGAATGGATGG CCCAAAAGTTAAACAATGGCCATTGACAGAAGAA AAAATAAAAGCATTAGTAGAAATTTGTACAGAAAT GGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT GAAAATCCATACAATACTCCAGTATTTGCCATAAA GAAAAAAGACAGTACTAAATGGAGAAAATTAGTA GATTTCAGAGAACTTAATAAGAGAACTCAAGATTT CTGGGAAGTTCAATTAGGAATACCACATCCTGCAG GGTTAAAACAGAAAAAATCAGTAACAGTACTGGA TGTGGGCGATGCATATTTTTCAGTTCCCTTAGATAA AGACTTCAGGAAGTATACTGCATTTACCATACCTA GTATAAACAATGAGACACCAGGGATTAGATATCAG TACAATGTGCTTCCACAGGGATGGAAAGGATCACC AGCAATATTCCAGTGTAGCATGACAAAAATCTTAG AGCCTTTTAGAAAACAAAATCCAGACATAGTCATC TATCAATACATGGATGATTTGTATGTAGGATCTGA CTTAGAAATAGGGCAGCATAGAACAAAAATAGAG GAACTGAGACAACATCTGTTGAGGTGGGGATTTAC CACACCAGACAAAAAACATCAGAAAGAACCTCCA TTCCTTTGGATGGGTTATGAACTCCATCCTGATAAA TGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGA CAGCTGGACTGTCAATGACATACAGAAATTAGTGG GAAAATTGAATTGGGCAAGTCAGATTTATGCAGGG

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SEQ ID NO:	Description	Sequence
		ATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGG
		AACCAAAGCACTAACAGAAGTAGTACCACTAACA
		GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGG
		AGATTCTAAAAGAACCGGTACATGGAGTGTATTAT
		GACCCATCAAAAGACTTAATAGCAGAAATACAGA
		AGCAGGGGCAAGGCCAATGGACATATCAAATTTAT
		CAAGAGCCATTTAAAAATCTGAAAACAGGAAAAT
		ATGCAAGAATGAAGGGTGCCCACACTAATGATGTG
		AAACAATTAACAGAGGCAGTACAAAAAATAGCCA
		CAGAAAGCATAGTAATATGGGGAAAGACTCCTAA
		ATTTAAATTACCCATACAAAAGGAAACATGGGAAG
		CATGGTGGACAGAGTATTGGCAAGCCACCTGGATT
		CCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTG
		AAGTTATGGTACCAGTTAGAGAAAGAACCCATAAT
		AGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCA
		ATAGGGAAACTAAATTAGGAAAAGCAGGATATGT
		AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTA
		ACGGACACAACAAATCAGAAGACTGAGTTACAAG
		CAATTCATCTAGCTTTGCAGGATTCGGGATTAGAA
		GTAAACATAGTGACAGACTCACAATATGCATTGGG
		AATCATTCAAGCACAACCAGATAAGAGTGAATCAG
		AGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAA
		AAGGAAAAAGTCTACCTGGCATGGGTACCAGCAC
		ACAAAGGAATTGGAGGAAATGAACAAGTAGATGG
		GTTGGTCAGTGCTGGAATCAGGAAAGTACTA
54	Helper; HIV Integrase; Integration of viral RNA	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA
		TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA
		GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAA
		ATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGG
		GGAAGCCATGCATGGACAAGTAGACTGTAGCCCA
		GGAATATGGCAGCTAGATTGTACACATTTAGAAGG
		AAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG
		GATATATAGAAGCAGAAGTAATTCCAGCAGAGAC
		AGGGCAAGAAACAGCATACTTCCTCTTAAAATTAG
		CAGGAAGATGGCCAGTAAAAACAGTACATACAGA
		CAATGGCAGCAATTTCACCAGTACTACAGTTAAGG
		CCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTT
		GGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
		AGAATCTATGAATAAAGAATTAAAGAAAATTATAG
		GACAGGTAAGAGATCAGGCTGAACATCTTAAGAC
		AGCAGTACAAATGGCAGTATTCATCCACAATTTTA
		AAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG
		GGAAAGAATAGTAGACATAATAGCAACAGACATA
		CAAACTAAAGAATTACAAAAACAAATTACAAAAA
		TTCAAAATTTTCGGGTTTATTACAGGGACAGCAGA
		GATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTG
		GAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT
		AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAA
		AGATCATCAGGGATTATGGAAAACAGATGGCAGG
		TGATGATTGTGTGGCAAGTAGACAGGATGAGGATT
		AA
55	Helper; HIV RRE; Binds Rev element	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG
		GAAGCACTATGGGCGCAGCGTCAATGACGCTGACG
		GTACAGGCCAGACAATTATTGTCTGGTATAGTGCA
		GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC
		AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC
		AAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA
		GATACCTAAAGGATCAACAGCTCCT
56	Helper; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT
		CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT
		AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG
		AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG
		AGGGCAAATCATTTAAAACATCAGAATGAGTATTT
		GGTTTAGAGTTTGGCAACATATGCCATATGCTGGC
		TGCCATGAACAAAGGTGGCTATAAAGAGGTCATCA
		GTATATGAAACAGCCCCCTGCTGTCCATTCCTTATT
		CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT
		TATATTTTGTTTTGTGTTATTTTTTTCTTTAACATC
		CCTAAAATTTTCCTTACATGTTTTACTAGCCAGATT
		TTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGT
		CCCTCTTCTCTTATGAAGATC

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SEQ ID NO:	Description	Sequence
57	Rev; RSV promoter; Transcription	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC
		TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC
		AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
		ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGA
		GAGAGACAGAGACAGATCCATTCGATTAGTGAAC
		GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG
		CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT
		ACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG
		GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG
		GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA
		ATAG
58	Rev; HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC
		TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC
		AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
		ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGA
		GAGAGACAGAGACAGATCCATTCGATTAGTGAAC
		GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG
		CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT
		ACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG
		GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG
		GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA
		ATAG
59	Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT
		CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT
		AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG
		AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG
		AGGGCAAATCATTTAAAACATCAGAATGAGTATTT
		GGTTTAGAGTTTGGCAACATATGCCCATATGCTGG
		CTGCCATGAACAAAGGTTGGCTATAAAGAGGTCAT
		CAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTA
		TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTT
		TTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACA
		TCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGA
		TTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCT
		GTCCCTCTTCTCTTATGGAGATC
60	Envelope; CMV promoter; Transcription	ACATTGATTATTGACTAGTTATTAATAGTAATCAAT
		TACGGGGTCATTAGTTCATAGCCCATATATGGAGT
		TCCGCGTTACATAACTTACGGTAAATGGCCCGCCT
		GGCTGACCGCCCAACGACCCCCGCCCATTGACGTC
		AATAATGACGTATGTTCCCATAGTAACGCCAATAG
		GGACTTTCCATTGACGTCAATGGGTGGAGTATTTA
		CGGTAAACTGCCCACTTGGCAGTACATCAAGTGTA
		TCATATGCCAAGTACGCCCCCTATTGACGTCAATG
		ACGGTAAATGGCCCGCCTGGCATTATGCCCAGTAC
		ATGACCTTATGGGACTTTCCTACTTGGCAGTACATC
		TACGTATTAGTCATCGCTATTACCATGGTGATGCG
		GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGT
		TTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG
		ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAA
		CGGGACTTTCCAAAATGTCGTAACAACTCCGCCCC
		ATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGG
		AGGTCTATATAAGC
61	Envelope; Beta globin intron; Enhance gene expression	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTC
		GCTATTGTAAAATTCATGTTATATGGAGGGGGCAA
		AGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTC
		CCTTGTATCACCATGGACCCTCATGATAATTTTGTT
		TCTTTCACTTTCTACTCTGTTGACAACCATTGTCTC
		CTCTTATTTTCTTTTCATTTTCTGTAACTTTTTCGT
		TAAACTTTAGCTTGCATTTGTAACGAATTTTTAAAT
		TCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTC
		TCTAATCACTTTTTTTTCAAGGCAATCAGGGTATAT
		TATATTGTACTTCAGCACAGTTTTAGAGAACAATTG
		TTATAATTAAATGATAAGGTAGAATATTTCTGCAT
		ATAAATTCTGGCTGGCGTGGAAATATTCTTATTGGT
		AGAAACAACTACACCCTGGTCATCATCCTGCCTTT
		CTCTTTATGGTTACAATGATATACACTGTTTGAGAT
		GAGGATAAAATACTCTGAGTCCAAACCGGGCCCCT
		CTGCTAACCATGTTCATGCCTTCTTCTCTTTCCTAC
		AG

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SEQ ID NO:	Description	Sequence
62	Envelope; VSV-G; Glycoprotein envelope-cell entry	ATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATT GGGGTGAATTGCAAGTTCACCATAGTTTTTCCACA CAACCAAAAAGGAAACTGGAAAAATGTTCCTTCTA ATTACCATTATTGCCCGTCAAGCTCAGATTTAAATT GGCATAATGACTTAATAGGCACAGCCTTACAAGTC AAAATGCCCAAGAGTCACAAGGCTATTCAAGCAG ACGGTTGGATGTGTCATGCTTCCAAATGGGTCACT ACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT AACACATTCCATCCGATCCTTCACTCCATCTGTAGA ACAATGCAAGGAAAGCATTGAACAAACGAAACAA GGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAG TTGTGGATATGCAACTGTGACGGATGCCGAAGCAG TGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTG ATGAATACACAGGAGAATGGGTTGATTCACAGTTC ATCAACGGAAAATGCAGCAATTACATATGCCCCAC TGTCCATAACTCTACAACCTGGCATTCTGACTATAA GGTCAAAGGGCTATGTGATTCTAACCTCATTTCCAT GGACATCACCTTCTTCTCAGAGGACGGAGAGCTAT CATCCCTGGGAAAGGAGGGCACAGGGTTCAGAAG TAACTACTTTGCTTATGAAACTGGAGGCAAGGCCT GCAAAATGCAATACTGCAAGCATTGGGGAGTCAG ACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATA AGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGC CCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGAC CTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGA GGATCTTGGATTATTCCCTCTGCCAAGAAACCTGG AGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGT GGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAA CCGGTCCTGCTTTCACCATAATCAATGGTACCCTAA AATACTTTGAGACCAGATACATCAGAGTCGATATT GCTGCTCCAATCCTCTCAAGAATGGTCGGAATGAT CAGTGGAACTACCACAGAAAGGGAACTGTGGGAT GACTGGGCACCATATGAAGACGTGGAAATTGGACC CAATGGAGTTCTGAGGACCAGTTCAGGATATAAGT TTCCTTTATACATGATTGGACATGGTATGTTGGACT CCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCG AACATCCTCACATTCAAGACGCTGCTTCGCAACTT CCTGATGATGAGAGTTTATTTTTTGGTGATACTGGG CTATCCAAAAATCCAATCGAGCTTGTAGAAGGTTG GTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTT CTTTATCATAGGGTTAATCATTGGACTATTCTTGGT TCTCCGAGTTGGTATCCATCTTTGCATTAAATTAAA GCACACCAAGAAAAGACAGATTTATACAGACATA GAGATGA
63	Envelope; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAAATCATTTAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCCATATGCTGG CTGCCATGAACAAAGGTTGGCTATAAAGAGGTCAT CAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTA TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTT TTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACA TCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGA TTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCT GTCCCTCTTCTCTTATGGAGATC
64	Promoter; EF-1	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTG GGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCC CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACGCCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG GTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCC CTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGG GCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGT TCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC CCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGC GCTCGGGAGAGCGGGCGGGTGAGTCACCCACACA AAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG TCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGA GTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT TTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCAT TTCAGGTGTCGTGA
65	Promoter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTG GGTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGT TCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCG CACATTCTTCACGTCCGTTCGCAGCGTCACCCGGAT CTTCGCCGCTACCCTTGTGGGCCCCCCGGCGACGC TTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGC GGTTCGCGGCGTGCCGGACGTGACAAACGGAAGC CGCACGTCTCACTAGTACCCTCGCAGACGGACAGC GCCAGGGAGCAATGGCAGCGCGCCGACCGCGATG GGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCG CCGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGA GGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCC TGCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCG GAGCGCACGTCGGCAGTCGGCTCCCTCGTTGACCG AATCACCGACCTCTCTCCCCAG
66	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCC TCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGG CGCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCT CAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCT TAGAACCCCAGTATCAGCAGAAGGACATTTTAGGA CGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTT CCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCC TTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGC GGTGAACGCCGATGATTATATAAGGACGCGCCGGG TGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTG GGTCGCGGTTCTTGTTTGTGGATCGCTGTGATCGTC ACTTGGTGAGTTGCGGGCTGCTGGGCTGGCCGGGG CTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAA GCGTGTGGAGAGACCGCCAAGGGCTGTAGTCTGGG TCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGGG GGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTT GAATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTC GTTGAAACAAGGTGGGGGGCATGGTGGGCGGCAA GAACCCAAGGTCTTGAGGCCTTCGCTAATGCGGGA AAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCA TCTGGGGACCCTGACGTGAAGTTTGTCACTGACTG GAGAACTCGGGTTTGTCGTCTGGTTGCGGGGGCGG CAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTAC CTTTGGGAGCGCGCGCCTCGTCGTGTCGTGACGTC ACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCC ACCTGCCGGTAGGTGTGCGGTAGGCTTTTCTCCGTC GCAGGACGCAGGGTTCGGGCCTAGGGTAGGCTCTC CTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGA GGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTT ATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTT TTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTT GTGAAGTTTTTTAGGCACCTTTTGAAATGTAATCAT TTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTA AA
67	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCA ATAGCATCACAAATTTCACAAATAAAGCATTTTTTT CACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCA ATGTATCTTATCA

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SEQ ID NO:	Descrip-tion	Sequence
68	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTG CCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGC CACTCCCACTGTCCTTTCCTAATAAAATGAGGAAA TTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGA GGATTGGGAAGACAATAGCAGGCATGCTGGGGAT GCGGTGGGCTCTATGG
69	HIV Gag; Bal	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAG AATTAGATAGGTGGGAAAAAATTCGGTTAAGGCCA GGGGGAAAGAAAAAATATAGATTAAAACATATAG TATGGGCAAGCAGGGAACTAGAAAGATTCGCAGT CAATCCTGGCCTGTTAGAAACATCAGAAGGCTGCA GACAAATACTGGGACAGCTACAACCATCCCTTCAG ACAGGATCAGAAGAACTTAGATCATTATATAATAC AGTAGCAACCCTCTATTGTGTACATCAAAAGATAG AGGTAAAAGACACCAAGGAAGCTTTAGACAAAAT AGAGGAAGAGCAAAACAAATGTAAGAAAAAGGCA CAGCAAGCAGCAGCTGACACAGGAAACAGCGGTC AGGTCAGCCAAAATTTCCCTATAGTGCAGAACCTC CAGGGGCAAATGGTACATCAGGCCATATCACCTAG AACTTTAAATGCATGGGTAAAAGTAATAGAAGAG AAAGCTTTCAGCCCAGAAGTAATACCCATGTTTTC AGCATTATCAGAAGGAGCCACCCCACAAGATTTAA ACACCATGCTAAACACAGTGGGGGGACATCAAGC AGCCATGCAAATGTTAAAAGAACCCATCAATGAGG AAGCTGCAAGATGGGATAGATTGCATCCCGTGCAG GCAGGGCCTGTTGCACCAGGCCAGATAAGAGATCC AAGGGGAAGTGACATAGCAGGAACTACCAGTACC CTTCAGGAACAAATAGGATGGATGACAAGTAATCC ACCTATCCCAGTAGGAGAAATCTATAAAAGATGGA TAATCCTGGGATTAAATAAAATAGTAAGGATGTAT AGCCCTACCAGCATTTTGGACATAAGACAAGGACC AAAGGAACCCTTTAGAGACTATGTAGACCGGTTCT ATAAAACTCTAAGAGCCGAGCAAGCTTCACAGGA GGTAAAAAAATTGGATGACAGAAACCTTGTTGGTCC AAAATGCGAACCCAGATTGTAAGACTATTTTAAAA GCATTGGGACCAGCAGCTACACTAGAAGAAATGAT GACAGCATGTCAGGGAGTGGGAGGACCCAGCCAT AAAGCAAGAATTTTGGCAGAAGCAATGAGCCAAG TAACAAATTCAGCTACCATAATGATGCAGAAAGGC AATTTTAGGAACCAAAGAAAGATTGTTAAATGTTT CAATTGTGGCAAAGAAGGGCACATAGCCAGAAAC TGCAGGGCCCCTAGGAAAAGGGGCTGTTGGAAAT GTGGAAAGGAAGGACACCAAATGAAAGACTGTAC TGAGAGACAGGCTAATTTTTTAGGGAAAATCTGGC CTTCCCACAAAGGAAGGCCAGGGAATTTCCTTCAG AGCAGACCAGAGCCAACAGCCCCACCAGCCCCAC CAGAAGAGAGCTTCAGGTTTGGGGAAGAGACAAC AACTCCCTCTCAGAAGCAGGAGCTGATAGACAAGG AACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTG GCAACGACCCCTCGTCACAATAA
70	HIV Pol; Bal	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGA TAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAG TATGATCAGATACTCATAGAAATCTGTGGACATAA AGCTATAGGTACAGTATTAATAGGACCTACACCTG TCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGTTGCACTTTAAATTTTCCCATTAGTCCTATTGAA ACTGTACCAGTAAAATTAAAACCAGGAATGGATGG CCCAAAAGTTAAACAATGGCCACTGACAGAAGAA AAAATAAAAGCATTAATGGAAATCTGTACAGAAAT GGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCT GAAAATCCATACAATACTCCAGTATTTGCCATAAA GAAAAAAGACAGTACTAAATGGAGAAAATTAGTA GATTTCAGAGAACTTAATAAGAAAACTCAAGACTT CTGGGAAGTACAATTAGGAATACACATCCCGCAGG GGTTAAAAAAGAAAAAATCAGTAACAGTACTGGA TGTGGGTGATGCATATTTTTCAGTTCCCTTAGATAA AGAATTCAGGAAGTATACTGCATTTACCATACCTA GTATAAACAATGAAACACCAGGGATCAGATATCA GTACAATGTACTTCCACAGGGATGGAAAGGATCAC CAGCAATATTTCAAAGTAGCATGACAAGAATCTTA GAGCCTTTTAGAAAACAAAATCCAGAAATAGTGAT
71	HIV Inte-grase; Bal	CTATCAATACATGGATGATTTGTATGTAGGATCTG ACTTAGAAATAGGGCAGCATAGAACAAAAATAGA GGAACTGAGACAACATCTGTTGAGGTGGGGATTTA CCACACCAGACAAAAAACATCAGAAAGAACCTCC ATTCCTTTGGATGGGTTATGAACTCCATCCTGATAA ATGGACAGTACAGCCTATAGTGCTGCCAGAAAAAG ACAGCTGGACTGTCAATGACATACAGAAGTTAGTG GGAAAATTGAATTGGGCAAGTCAGATTTACCCAGG AATTAAAGTAAAGCAATTATGTAGGCTCCTTAGGG GAACCAAGGCATTAACAGAAGTAATACCACTAAC AAAAGAAACAGAGCTAGAACTGGCAGAGAACAGG GAAATTCTAAAAGAACCAGTACATGGGGTGTATTA TGACCCATCAAAAGACTTAATAGCAGAAATACAGA AGCAGGGGCAAGGCCAATGGACATATCAAATTTAT CAAGAGCCATTTAAAAATCTGAAAACAGGAAAAT ATGCAAGAATGAGGGGTGCCCACACTAATGATGTA AAACAATTAACAGAGGCAGTGCAAAAAATAACCA CAGAAAGCATAGTAATATGGGGAAAGACTCCTAA ATTTAAACTACCCATACAAAAAGAAACATGGGAA ACATGGTGGACAGAGTATTGGCAAGCCACCTGGAT TCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGT GAAATTATGGTACCAGTTAGAGAAAGAACCCATAA TAGGAGCAGAAACATTCTATGTAGATGGAGCAGCT AACCGGGAGACTAAATTAGGAAAAGCAGGATATG TTACTAACAGAGGAAGACAAAAAGTTGTCTCCCTA ACTGACACAACAAATCAGAAGACTGAGTTACAAG CAATTCATCTAGCTTTACAAGATTCAGGATTAGAA GTAAACATAGTAACAGACTCACAATATGCATTAGG AATCATTCAAGCACAACCAGATAAAAGTGAATCAG AGTTAGTCAGTCAAATAATAGAACAGTTAATAAAA AAGGAAAAGGTCTACCTGGCATGGGTACCAGCGC ACAAAGGAATTGGAGGAAATGAACAAGTAGATAA ATTAGTCAGTACTGGAATCAGGAAAGTACTA
72	Envelope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAG CCTAATAATAGTTCGGGCAGGGTTTGACGACCCCC GCAAGGCTATCGCATTAGTACAAAAACAACATGGT AAACCATGCGAATGCAGCGGAGGGCAGGTATCCG AGGCCCCACCGAACTCCATCCAACAGGTAACTTGC CCAGGCAAGACGGCCTACTTAATGACCAACCAAAA ATGGAAATGCAGAGTCACTCCAAAAAATCTCACCC CTAGCGGGGGAGAACTCCAGAACTGCCCCTGTAAC ACTTTCCAGGACTCGATGCACAGTTCTTGTTATACT GAATACCGGCAATGCAGGGCGAATAATAAGACAT ACTACACGGCCACCTTGCTTAAAATACGGTCTGGG AGCCTCAACGAGGTACAGATATTACAAAACCCCAA TCAGCTCCTACAGTCCCCTTGTAGGGGCTCTATAA ATCAGCCCGTTTGCTGGAGTGCCACAGCCCCCATC CATATCTCCGATGGTGGAGGACCCCTCGATACTAA

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SEQ ID NO:	Description	Sequence
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		ACTTTTAGGTTACTCCAGATGTCCAATTTTAGCCTT
		GCCCAAGATTGTTGGCTCTGTTTAAAACTAGGTAC
		CCCTACCCCTCTTGCGATACCCACTCCCTCTTTAAC
		CTACTCCCTAGCAGACTCCCTAGCGAATGCCTCCT
		GTCAGATTATACCTCCCCTCTTGGTTCAACCGATGC
		AGTTCTCCAACTCGTCCTGTTTATCTTCCCCTTTCA
		TTAACGATACGGAACAAATAGACTTAGGTGCAGTCA
		CCTTTACTAACTGCACCTCTGTAGCCAATGTCAGTA
		GTCCTTTATGTGCCCTAAACGGGTCAGTCTTCCTCT
		GTGGAAATAACATGGCATACACCTATTTACCCCAA
		AACTGGACAGGACTTTGCGTCCAAGCCTCCCTCCT
		CCCCGACATTGACATCATCCCGGGGGATGAGCCAG
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		GACTGGGAATCACCGCAGCATTCACCACCGGAGCT
		ACAGGCCTAGGTGTCTCCGTCACCCAGTATACAAA
		ATTATCCCATCAGTTAATATCTGATGTCCAAGTCTT
		ATCCGGTACCATACAAGATTTACAAGACCAGGTAG
		ACTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAGG
		GGACTGGACCTACTAACGGCAGAACAAGGAGGAA
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		CTAACAAGTCAGGAATTGTGAGAAACAAAATAAG
		AACCCTACAAGAAGAATTACAAAAACGCAGGGAA
		AGCCTGGCATCCAACCCTCTCTGGACCGGGCTGCA
		GGGCTTTCTTCCGTACCTCCTACCTCTCCTGGGACC
		CCTACTCACCCTCCTACTCATACTAACCATTGGGCC
		ATGCGTTTTCAATCGATTGGTCCAATTTGTTAAAGA
		CAGGATCTCAGTGGTCCAGGCTCTGGTTTTGACTC
		AGCAATATCACCAGCTAAAACCCATAGAGTACGAG
		CCATGA
73	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCA
		CCAGATGAGTCCTGGGAGCTGGAAAAGACTGATCA
		TCCTCTTAAGCTGCGTATTCGGAGACGGCAAAACG
		AGTCTGCAGAATAAGAACCCCCACCAGCCTGTGAC
		CCTCACCTGGCAGGTACTGTCCCAAACTGGGGACG
		TTGTCTGGGACAAAAAGGCAGTCCAGCCCCTTTGG
		ACTTGGTGGCCCTCTCTTACACCTGATGTATGTGCC
		CTGGCGGCCGGTCTTGAGTCCTGGGATATCCCGGG
		ATCCGATGTATCGTCCTCTAAAAGAGTTAGACCTC
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		TAGGACCAGGATGGCAAATTCCCCCTTCTACGTGT
		GTCCCCGAGCTGGCCGAACCCATTCAGAAGCTAGG
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		ATGGAGTTGTGAGACCACGGGTACCGTTTATTGGC
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		TGGGACCAAAATGTGAAATGGGAGCAAAAATTTC
		AAAAGTGTGAACAAACCGGCTGGTGTAACCCCCTC
		AAGATAGACTTCACAGAAAAAGGAAAACTCTCCA
		GAGATTGGATAACGGAAAAAACCTGGGAATTAAG
		GTTCTATGTATATGGACACCCAGGCATACAGTTGA
		CTATCCGCTTAGAGGTCACTAACATGCCGGTTGTG
		GCAGTGGGCCCAGACCCTGTCCTTGCGGAACAGGG
		ACCTCCTAGCAAGCCCCTCACTCTCCCTCTCTCCCC
		ACGGAAAGCGCCGCCCACCCCTCTACCCCCGGCGG
		CTAGTGAGCAAACCCCTGCGGTGCATGGAGAAACT
		GTTACCCTAAACTCTCCGCCTCCCACCAGTGGCGA
		CCGACTCTTTGGCCTTGTGCAGGGGGCCTTCCTAAC
		CTTGAATGCTACCAACCCAGGGGCCACTAAGTCTT
		GCTGGCTCTGTTTGGGCATGAGCCCCCCTTATTATG
		AAGGGATAGCCTCTTCAGGAGAGGTCGCTTATACC
		TCCAACCATACCCGATGCCACTGGGGGGCCCAAGG
		AAAGCTTACCCTCACTGAGGTCTCCGGACTCGGGT
		CATGCATAGGGAAGGTGCCTCTTACCCATCAACAT
		CTTTGCAACCAGACCTTACCCATCAATTCCTCTAAA
		AACCATCAGTATCTGCTCCCCTCAAACCATAGCTG
		GTGGGCCTGCAGCACTGGCCTCACCCCCTGCCTCT
		CCACCTCAGTTTTTAATCAGTCTAAAGACTTCTGTG
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SEQ ID NO:	Description	Sequence
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		CCCATAGACCTCCAGCAAGGCCTAACCAGCCTCCA
		AATCGCCATTGACGCTGACCTCCGGGCCCTTCAGG
		ACTCAATCAGCAAGCTAGAGGACTCACTGACTTCC
		CTATCTGAGGTAGTACTCCAAAATAGGAGAGGCCT
		TGACTTACTATTCCTTAAAGAAGGAGGCCTCTGCG
		CGGCCCTAAAAGAAGAGTGCTGTTTTTATGTAGAC
		CACTCAGGTGCAGTACGAGACTCCATGAAAAAACT
		TAAAGAAAGACTAGATAAAAGACAGTTAGAGCGC
		CAGAAAAACCAAAACTGGTATGAAGGGTGGTTCA
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		TCGCTGGGCCCCTATTGCTCCTCCTTTTGTTACTCA
		CTCTTGGGCCCTGCATCATCAATAAATTAATCCAAT
		TCATCAATGATAGGATAAGTGCAGTCAAAATTTTA
		GTCCTTAGACAGAAATATCAGACCCTAGATAACGA
		GGAAAACCTTTAA
74	Envelope; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTG
		GGTTTTTCGTTGTGTTTCGGGAAGTTCCCCATTTAC
		ACGATACCAGACGAACTTGGTCCCTGGAGCCCTAT
		TGACATACACCATCTCAGCTGTCCAAATAACCTGG
		TTGTGGAGGATGAAGGATGTACCAACCTGTCCGAG
		TTCTCCTACATGGAACTCAAAGTGGGATACATCTC
		AGCCATCAAAGTGAACGGGTTCACTTGCACAGGTG
		TTGTGACAGAGGCAGAGACCTACACCAACTTTGTT
		GGTTATGTCACAACCACATTCAAGAGAAAGCATTT
		CCGCCCCACCCCAGACGCATGTAGAGCCGCGTATA
		ACTGGAAGATGGCCGGTGACCCCAGATATGAAGA
		GTCCCTACACAATCCATACCCCGACTACCACTGGC
		TTCGAACTGTAAGAACCACCAAAGAGTCCCTCATT
		ATCATATCCCCAAGTGTGACAGATTTGGACCCATA
		TGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCG
		GAAAGTGCTCAGGAATAACGGTGTCCTCTACCTAC
		TGCTCAACTAACCATGATTACACCATTTGGATGCC
		CGAGAATCCGAGACCAAGGACACCTTGTGACATTT
		TTACCAATAGCAGAGGGAAGAGAGCATCCAACGG
		GAACAAGACTTGCGGCTTTGTGGATGAAAGAGGCC
		TGTATAAGTCTCTAAAAGGAGCATGCAGGCTCAAG
		TTATGTGGAGTTCTTGGACTTAGACTTATGGATGG
		AACATGGGTCGCGATGCAAACATCAGATGAGACC
		AAATGGTGCCCTCCAGATCAGTTGGTGAATTTGCA
		CGACTTTCGCTCAGACGAGATCGAGCATCTCGTTG
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		GGATGCATTAGAGTCCATCATGACCACCAAGTCAG
		TAAGTTTCAGACGTCTCAGTCACCTGAGAAAACTT
		GTCCCAGGGTTTGGAAAAGCATATACCATATTCAA
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		AGCACACCAAGAAAAGACAGATTTATACAGACAT
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75	Envelope; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCC
		TCACATCATCGATGAGGTGATCAACATTGTCATTA
		TTGTGCTTATCGTGATCACGGGTATCAAGGCTGTCT
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		GTTTCCTACTTCTGGCTGGCAGGTCCTGTGGCATGT
		ACGGTCTTAAGGGACCCGACATTTACAAAGGAGTT
		TACCAATTTAAGTCAGTGGAGTTTGATATGTCACA
		TCTGAACCTGACCATGCCCAACGCATGTTCAGCCA
		ACAACTCCCACCATTACATCAGTATGGGGACTTCT
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SEQ ID NO:	Description	Sequence
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		GGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGG
		GGGGAAATACATGAGGAGTGGCTGGGGCTGGACA
		GGCTCAGATGGCAAGACCACCTGGTGTAGCCAGAC
		GAGTTACCAATACCTGATTATACAAAATAGAACCT
		GGGAAAACCACTGCACATATGCAGGTCCTTTTGGG
		ATGTCCAGGATTCTCCTTTCCCAAGAGAAGACTAA
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		GGACTTTGTCAGACTCTTCAGGGGTGGAGAATCCA
		GGTGGTTATTGCCTGACCAAATGGATGATTCTTGCT
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		AGATCAACTACTGATGAGGAACCACTTGAGAGATC
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		TGGTACCTAGAACATGCAAAGACCGGCGAAACTA
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		GTAGTTGTGGTGCATTTAAGGTGCCTGGTGTAAAA
		ACCGTCTGGAAAAGACGCTGA
76	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCA
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		CTTGAGGGGAAAGTCCATGGGGATCCAGAGCGAT
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		TGGCAATGGAAAACCAGCACACTATTGATTTGGCT
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SEQ ID NO:	Description	Sequence
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		GCATCATGCTTTTTGCTTCTTGCCATTGCAATGGGC
		CTTGTTTTCATATGTGTGAAGAACGGAAACATGCG
		GTGCACTATTTGTATATAA
77	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTAC
		TAGACCATACCTAGCACATTGCGCCGATTGCGGGG
		ACGGGTACTTCTGCTATAGCCCAGTTGCTATCGAG
		GAGATCCGAGATGAGGCGTCTGATGGCATGCTTAA
		GATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGG
		CAGGCACCCACGCCCACACGAAGCTCCGATATATG
		GCTGGTCATGATGTTCAGGAATCTAAGAGAGATTC
		CTTGAGGGTGTACACGTCCGCAGCGTGCTCCATAC
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		CCACCAGGCGACTACCTCAAGGTTTCGTTCGAGGA
		CGCAGATTCGCACGTGAAGGCATGTAAGGTCCAAT
		ACAAGCACAATCCATTGCCGGTGGGTAGAGAGAA
		GTTCGTGGTTAGACCACACTTTGGCGTAGAGCTGC
		CATGCACCTCATACCAGCTGACAACGGCTCCCACC
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		CGTTCCCTCTGACTAACGTCACCTGCCGAGTGCCGT
		TGGCTCGAGCGCCGGATGCCACCTATGGTAAGAAG
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		GCTCTTCTCCTATAGGAGTTTAGGAGCCGAACCGC
		ACCCGTACGAGGAATGGGTTGACAAGTTCTCTGAG
		CGCATCATCCCAGTGACGGAAGAAGGGATTGAGTA
		CCAGTGGGGCAACAACCCGCCGGTCTGCCTGTGGG
		CGCAACTGACGACCGAGGGCAAACCCCATGGCTG
		GCCACATGAAATCATTCAGTACTATTATGGACTAT
		ACCCCGCCGCCACTATTGCCGCAGTATCCGGGGCG
		AGTCTGATGGCCCTCCTAACTCTGGCGGCCACATG
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		TTGACACTGGGGCTGCTTTGCTGCGCACCGAGGGC
		GAATGCA
78	Envelope; MLV 10A1	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTAC
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		ACGGGTACTTCTGCTATAGCCCAGTTGCTATCGAG
		GAGATCCGAGATGAGGCGTCTGATGGCATGCTTAA
		GATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGG
		CAGGCACCCACGCCCACACGAAGCTCCGATATATG
		GCTGGTCATGATGTTCAGGAATCTAAGAGAGATTC
		CTTGAGGGTGTACACGTCCGCAGCGTGCTCCATAC
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		CCACCAGGCGACTACCTCAAGGTTTCGTTCGAGGA
		CGCAGATTCGCACGTGAAGGCATGTAAGGTCCAAT
		ACAAGCACAATCCATTGCCGGTGGGTAGAGAGAA
		GTTCGTGGTTAGACCACACTTTGGCGTAGAGCTGC
		CATGCACCTCATACCAGCTGACAACGGCTCCCACC
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		ACCGGATCGCACCCTGCTATCACAGACGGCGGGCA
		ACGTCAAAATAACAGCAGGCGGCAGGACTATCAG
		GTACAACTGTACCTGCGGCCGTGACAACGTAAGCA
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		GCTCTTCTCCTATAGGAGTTTAGGAGCCGAACCGC
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SEQ ID NO:	Descrip-tion	Sequence
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		AGTCTGATGGCCCTCCTAACTCTGGCGGCCACATG
		CTGCATGCTGGCCACCGCGAGGAGAAAGTGCCTAA
		CACCGTACGCCCTGACGCCAGGAGCGGTGGTACCG
		TTGACACTGGGGCTGCTTTGCTGCGCACCGAGGGC
		GAATGCA
79	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGA
		TCGATTCAAGAGGACATCATTCTTTCTTTGGGTAAT
		TATCCTTTTCCAAAGAACATTTTCCATCCCACTTGG
		AGTCATCCACAATAGCACATTACAGGTTAGTGATG
		TCGACAAACTGGTTTGCCGTGACAAACTGTCATCC
		ACAAATCAATTGAGATCAGTTGGACTGAATCTCGA
		AGGGAATGGAGTGGCAACTGACGTGCCATCTGCAA
		CTAAAAGATGGGGCTTCAGGTCCGGTGTCCCACCA
		AAGGTGGTCAATTATGAAGCTGGTGAATGGGCTGA
		AAACTGCTACAATCTTGAAATCAAAAAACCTGACG
		GGAGTGAGTGTCTACCAGCAGCGCCAGACGGGATT
		CGGGGCTTCCCCCGGTGCCGGTATGTGCACAAAGT
		ATCAGGAACGGGACCGTGTGCCGGAGACTTTGCCT
		TCCACAAAGAGGGTGCTTTCTTCCTGTATGACCGA
		CTTGCTTCCACAGTTATCTACCGAGGAACGACTTTC
		GCTGAAGGTGTCGTTGCATTTCTGATACTGCCCCA
		AGCTAAGAAGGACTTCTTCAGCTCACACCCCTTGA
		GAGAGCCGGTCAATGCAACGGAGGACCCGTCTAGT
		GGCTACTATTCTACCACAATTAGATATCAAGCTAC
		CGGTTTTGGAACCAATGAGACAGAGTATTTGTTCG
		AGGTTGACAATTTGACCTACGTCCAACTTGAATCA
		AGATTCACACCACAGTTTCTGCTCCAGCTGAATGA
		GACAATATATACAAGTGGGAAAAGGAGCAATACC
		ACGGGAAAACTAATTTGGAAGGTCAACCCCGAAAT
		TGATACAACAATCGGGGAGTGGGCCTTCTGGGAAA
		CTAAAAAAACCTCACTAGAAAAATTCGCAGTGAAG
		AGTTGTCTTTCACAGCTGTATCAAACAGAGCCAAA
		AACATCAGTGGTCAGAGTCCGGCGCGAACTTCTTC
		CGACCCAGGGACCAACACAACAACTGAAGACCAC
		AAAATCATGGCTTCAGAAAATTCCTCTGCAATGGT
		TCAAGTGCACAGTCAAGGAAGGGAAGCTGCAGTG
		TCGCATCTGACAACCCTTGCCACAATCTCCACGAG
		TCCTCAACCCCCCACAACCAAACCAGGTCCGGACA
		ACAGCACCCACAATACACCCGTGTATAAACTTGAC
		ATCTCTGAGGCAACTCAAGTTGAACAACATCACCG
		CAGAACAGACAACGACAGCACAGCCTCCGACACT
		CCCCCCGCCACGACCGCAGCCGGACCCCTAAAAGC
		AGAGAACACCAACACGAGCAAGGGTACCGACCTC
		CTGGACCCCGCCACCACAACAAGTCCCCAAAACCA
		CAGCGAGACCGCTGGCAACAACAACACTCATCACC
		AAGATACCGGAGAAGAGAGTGCCAGCAGCGGGAA
		GCTAGGCTTAATTACCAATACTATTGCTGGAGTCG
		CAGGACTGATCACAGGCGGGAGGAGAGCTCGAAG
		AGAAGCAATTGTCAATGCTCAACCCAAATGCAACC
		CTAATTTACATTACTGGACTACTCAGGATGAAGGT
		GCTGCAATCGGACTGGCCTGGATACCATATTTCGG
		GCCAGCAGCCGAGGGAATTTACATAGAGGGGCTG
		ATGCACAATCAAGATGGTTTAATCTGTGGGTTGAG
		ACAGCTGGCCAACGAGACGACTCAAGCTCTTCAAC
		TGTTCCTGAGAGCCACAACCGAGCTACGCACCTTT
		TCAATCCTCAACCGTAAGGCAATTGATTTCTTGCTG
		CAGCGATGGGGCGGCACATGCCACATTTTGGGACC
		GGACTGCTGTATCGAACCACATGATTGGACCAAGA
		ACATAACAGACAAAATTGATCAGATTATTCATGAT
		TTTGTTGATAAAACCCTTCCGGACCAGGGGGACAA
		TGACAATTGGTGGACAGGATGGAGACAATGGATA
		CCGGCAGGTATTGGAGTTACAGGCGTTATAATTGC
		AGTTATCGCTTTATTCTGTATATGCAAATTTGTCTT
		TTAG
80	Short WPRE sequence	AATCAACCTCTGGATTACAAAATTTGTGAAAGATT
		GACTGATATTCTTAACTATGTTGCTCCTTTTACGCT
		GTGTGGATATGCTGCTTTAATGCCTCTGTATCATGC
		TATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTT
		GTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTT
		GTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTC

-continued

SEQ ID NO:	Descrip-tion	Sequence
		TGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCAT
		TGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGC
		TTTCCCCCTCCCGATCGCCACGGCAGAACTCATCG
		CCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGG
		TTGCTGGGCACTGATAATTCCGTGGTGTTGTC
81	Primer	TAAGCAGAATTC ATGAATTTGCCAGGAAGAT
82	Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGA AT
83	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAA
		AATGATAGGGGGAATTGGAGGTTTTATCAAAGTAA
		GACAGTATGATCAGATACTCATAGAAATCTGCGGA
		CATAAAGCTATAGGTACAGTATTAGTAGGACCTAC
		ACCTGTCAACATAATTGGAAGAAATCTGTTGACTC
		AGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTA
		TTGAGACTGTACCAGTAAAATTAAAGCCAGGAATG
		GATGGCCCAAAAGTTAAACAATGGCCATTGACAGA
		AGAAAAAATAAAAGCATTAGTAGAAATTTGTACA
		GAAATGGAAAAGGAAGGAAAAATTTCAAAAATTG
		GGCCTGAAAATCCATACAATACTCCAGTATTTGCC
		ATAAAGAAAAAAGACAGTACTAAATGGAGAAAAT
		TAGTAGATTTCAGAGAACTTAATAAGAGAACTCAA
		GATTTCTGGGAAGTTCAATTAGGAATACCACATCC
		TGCAGGGTTAAAACAGAAAAAATCAGTAACAGTA
		CTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTA
		GATAAAGACTTCAGGAAGTATACTGCATTTACCAT
		ACCTAGTATAAACAATGAGACACCAGGGATTAGAT
		ATCAGTACAATGTGCTTCCACAGGGATGGAAAGGA
		TCACCAGCAATATTCCAGTGTAGCATGACAAAAAT
		CTTAGAGCCTTTTAGAAAACAAAATCCAGACATAG
		TCATCTATCAATACATGGATGATTTGTATGTAGGAT
		CTGACTTAGAAATAGGGCAGCATAGAACAAAAAT
		AGAGGAACTGAGACAACATCTGTTGAGGTGGGGA
		TTTACCACACCAGACAAAAAACATCAGAAAGAAC
		CTCCATTCCTTTGGATGGGTTATGAACTCCATCCTG
		ATAAATGGACAGTACAGCCTATAGTGCTGCCAGAA
		AAGGACAGCTGGACTGTCAATGACATACAGAAATT
		AGTGGGAAAATTGAATTGGGCAAGTCAGATTTATG
		CAGGGATTAAAGTAAGGCAATTATGTAAACTTCTT
		AGGGGAACCAAAGCACTAACAGAAGTAGTACCAC
		TAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAA
		CAGGGAGATTCTAAAAGAACCGGTACATGGAGTGT
		ATTATGACCCATCAAAAGACTTAATAGCAGAAATA
		CAGAAGCAGGGGCAAGGCCAATGGACATATCAAA
		TTTATCAAGAGCCATTTAAAAATCTGAAAACAGGA
		AAGTATGCAAGAATGAAGGGTGCCCACACTAATG
		ATGTGAAACAATTAACAGAGGCAGTACAAAAAAT
		AGCCACAGAAAGCATAGTAATATGGGGAAAGACT
		CCTAAATTTAAATTACCCATACAAAAGGAAACATG
		GGAAGCATGGTGGACAGAGTATTGGCAAGCCACCT
		GGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCT
		TAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCC
		ATAATAGGAGCAGAAACTTTCTATGTAGATGGGGC
		AGCCAATAGGGAAACTAAATTAGGAAAAGCAGGA
		TATGTAACTGACAGAGGAAGACAAAAAGTTGTCCC
		CCTAACGGACACAACAAATCAGAAGACTGAGTTAC
		AAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA
		GAAGTAAACATAGTGACAGACTCACAATATGCATT
		GGGAATCATTCAAGCACAACCAGATAAGAGTGAA
		TCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAAT
		AAAAAAGGAAAAAGTCTACCTGGCATGGGTACCA
		GCACACAAAGGAATTGGAGGAAATGAACAAGTAG
		ATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTA
		TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA
		TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA
		GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAA
		ATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGG
		GGAAGCCATGCATGGACAAGTAGACTGTAGCCCA
		GGAATATGGCAGCTAGATTGTACACATTTAGAAGG
		AAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG
		GATATATAGAAGCAGAAGTAATTCCAGCAGAGAC
		AGGGCAAGAAACAGCATACTTCCTCTTAAAATTAG
		CAGGAAGATGGCCAGTAAAAACAGTACATACAGA

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SEQ ID NO:	Description	Sequence
		CAATGGCAGCAATTTCACCAGTACTACAGTTAAGG
		CCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTT
		GGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
		AGAATCTATGAATAAAGAATTAAAGAAAATTATAG
		GACAGGTAAGAGATCAGGCTGAACATCTTAAGAC
		AGCAGTACAAATGGCAGTATTCATCCACAATTTTA
		AAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG
		GGAAAGAATAGTAGACATAATAGCAACAGACATA
		CAAACTAAAGAATTACAAAAACAAATTACAAAAA
		TTCAAAATTTTCGGGTTTATTACAGGGACAGCAGA
		GATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTG
		GAAAGGTGAAGGGGCAGTAGTAATACAAGATAAT
		AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAA
		AGATCATCAGGGATTATGGAAAACAGATGGCAGG
		TGATGATTGTGTGGCAAGTAGACAGGATGAGGATT
		AA
84	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACG
		AAGAGCTCATCAGAACAGTCAGACTCATCAAGCTT
		CTCTATCAAAGCAACCCACCTCCCAATCCCGAGGG
		GACCCGACAGGCCCGAAGGAATAGAAGAAGAAGG
		TGGAGAGAGAGACAGAGACAGATCCATTCGATTA
		GTGAACGGATCCTTGGCACTTATCTGGGACGATCT
		GCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGA
		GAGACTTACTCTTGATTGTAACGAGGATTGTGGAA
		CTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATA
		TTGGTGGAATCTCCTACAATATTGGAGTCAGGAGC
		TAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTG
		GGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAA
		TGACGCTGACGGTACAGGCCAGACAATTATTGTCT
		GGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGC
		TATTGAGGCGCAACAGCATCTGTTGCAACTCACAG
		TCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTG
		GCTGTGGAAAGATACCTAAAGGATCAACAGCTCCT
		AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT
		CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT
		AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG
		AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG
		AGGGCAAATCATTTAAAACATCAGAATGAGTATTT
		GGTTTAGAGTTTGGCAACATATGCCATATGCTGGC
		TGCCATGAACAAAGGTGGCTATAAAGAGGTCATCA
		GTATATGAAACAGCCCCCTGCTGTCCATTCCTTATT
		CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT
		TATATTTTGTTTTGTGTTATTTTTTTCTTTAACATC
		CCTAAAATTTTCCTTACATGTTTTACTAGCCAGATT
		TTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGT
		CCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCC
		CAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTG
		TGTGAAATTGTTATCCGCTCACAATTCCACACAACA
		TACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGG
		TGCCTAATGAGTGAGCTAACTCACATTAATTGCGT
		TGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTG
		TCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCA
		ACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCC
		CCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCA
		TGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA
		GGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGT
		GAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAA
		AAGCTAACTTGTTTATTGCAGCTTATAATGGTTACA
		AATAAAGCAATAGCATCACAAATTTCACAAATAAA
		GCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCC
		AAACTCATCAATGTATCTTATCAGCGGCCGCCCCG
		GG
85	DNA fragment containing the CAG enhancer/ promoter/ intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGT
		CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA
		CATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
		CCCAACGACCCCCGCCCATTGACGTCAATAATGAC
		GTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
		ATTGACGTCAATGGGTGGACTATTTACGGTAAACT
		GCCCACTTGGCAGTACATCAAGTGTATCATATGCC
		AAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
		GGCCCGCCTGGCATTATGCCCAGTACATGACCTTA
		TGGGACTTTCCTACTTGGCAGTACATCTACGTATTA
		GTCATCGCTATTACCATGGGTCGAGGTGAGCCCCA

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SEQ ID NO:	Description	Sequence
		CGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCC
		CACCCCCAATTTTGTATTTATTTATTTTTTAATTAT
		TTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCG
		CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG
		GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCA
		ATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATG
		GCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGC
		GAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCT
		TCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCG
		CCCGCCCCGGCTCTGACTGACCGCGTTACTCCCAC
		AGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGC
		TGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCT
		TTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCG
		GGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGG
		GGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCG
		CGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCT
		GCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTG
		TGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCC
		GCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCT
		GCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGG
		GGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCC
		CTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCC
		GGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCG
		CGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCA
		GGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGG
		GCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC
		CCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGC
		CGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAG
		GGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGA
		GCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTA
		GCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGG
		AAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGC
		CGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGG
		GCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA
		CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGAC
		CGGCGGGAATTC
86	DNA fragment containing VSV-G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTA
		TTCATTGGGGTGAATTGCAAGTTCACCATAGTTTTT
		CCACACAACCAAAAAGGAAACTGGAAAAATGTTC
		CTTCTAATTACCATTATTGCCCGTCAAGCTCAGATT
		TAAATTGGCATAATGACTTAATAGGCACAGCCTTA
		CAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCA
		AGCAGACGGTTGGATGTGTCATGCTTCCAAATGGG
		TCACTACTTGTGATTTCCGCTGGTATGGACCGAAGT
		ATATAACACATTCCATCCGATCCTTCACTCCATCTG
		TAGAACAATGCAAGGAAAGCATTGAACAAACGAA
		ACAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTC
		AAAGTTGTGGATATGCAACTGTGACGGATGCCGAA
		GCAGTGATTGTCCAGGTGACTCCTCACCATGTGCT
		GGTTGATGAATACACAGGAGAATGGGTTGATTCAC
		AGTTCATCAACGGAAAATGCAGCAATTACATATGC
		CCCACTGTCCATAACTCTACAACCTGGCATTCTGAC
		TATAAGGTCAAAGGGCTATGTGATTCTAACCTCAT
		TTCCATGGACATCACCTTCTTCTCAGAGGACGGAG
		AGCTATCATCCCTGGGAAAGGAGGGCACAGGGTTC
		AGAAGTAACTACTTTGCTTATGAAACTGGAGGCAA
		GGCCTGCAAAATGCAATACTGCAAGCATTGGGGAG
		TCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCT
		GATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGA
		ATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTC
		AGACCTCAGTGGATGTAAGTCTAATTCAGGACGTT
		GAGAGGATCTTGGATTATTCCCTCTGCCAAGAAAC
		CTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTC
		CAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCA
		GGAACCGGTCCTGCTTTCACCATAATCAATGGTAC
		CCTAAAATACTTTGAGACCAGATACATCAGAGTCG
		ATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGA
		ATGATCAGTGGAACTACCACAGAAAGGGAACTGT
		GGGATGACTGGGCACCATATGAAGACGTGGAAATT
		GGACCCAATGGAGTTCTGAGGACCAGTTCAGGATA
		TAAGTTTCCTTTATACATGATTGGACATGGTATGTT
		GGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGG
		TGTTCGAACATCCTCACATTCAAGACGCTGCTTCGC
		AACTTCCTGATGATGAGAGTTTATTTTTTGGTGATA

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SEQ ID NO:	Description	Sequence
		CTGGGCTATCCAAAAATCCAATCGAGCTTGTAGAA
		GGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCT
		TTTTTCTTTATCATAGGGTTAATCATTGGACTATTC
		TTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAA
		TTAAAGCACACCAAGAAAAGACAGATTTATACAG
		ACATAGAGATGAGAATTC
87	Helper plasmid containing RRE and rabbit beta globin poly A	TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGC
		AGCAGGAAGCACTATGGGCGCAGCGTCAATGACG
		CTGACGGTACAGGCCAGACAATTATTGTCTGGTAT
		AGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTG
		AGGCGCAACAGCATCTGTTGCAACTCACAGTCTGG
		GGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGT
		GGAAAGATACCTAAAGGATCAACAGCTCCTAGATC
		TTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA
		AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGG
		AAATTTATTTTCATTGCAATAGTGTGTTGGAATTTT
		TTGTGTCTCTCACTCGGAAGGACATATGGGAGGGC
		AAATCATTTAAAACATCAGAATGAGTATTTGGTTT
		AGAGTTTGGCAACATATGCCATATGCTGGCTGCCA
		TGAACAAAGGTGGCTATAAAGAGGTCATCAGTATA
		TGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATA
		GAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATAT
		TTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAA
		AATTTTCCTTACATGTTTTACTAGCCAGATTTTTCC
		TCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTC
		TTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGC
		TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGA
		AATTGTTATCCGCTCACAATTCCACACAACATACGA
		GCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTA
		ATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT
		CACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGC
		CAGCGGATCCGCATCTCAATTAGTCAGCAACCATA
		GTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACT
		CCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTG
		ACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGC
		CTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAG
		GCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTA
		ACTTGTTTATTGCAGCTTATAATGGTTACAAATAAA
		GCAATAGCATCACAAATTTCACAAATAAAGCATTT
		TTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTC
		ATCAATGTATCTTATCACCCGGG
88	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCT
		GAGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGG
		GCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGA
		TATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAA
		TGTAGTCTTATGCAATACACTTGTAGTCTTGCAACA
		TGGTAACGATGAGTTAGCAACATGCCTTACAAGGA
		GAGAAAAAGCACCGTGCATGCCGATTGGTGGAAG
		TAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAA
		CAGACAGGTCTGACATGGATTGGACGAACCACTGA
		ATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCT
		AGCTCGATACAATAAACGCCATTTGACCATTCACC
		ACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTA
		GTGAACCGTCAGATCGCCTGGAGACGCCATCCACG
		CTGTTTTGACCTCCATAGAAGACACCGGGACCGAT
		CCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTC
		CTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
		ACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCT
		ATCAAAGCAACCCACCTCCCAATCCCGAGGGGACC
		CGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGA
		GAGAGAGACAGAGACAGATCCATTCGATTAGTGA
		ACGGATCCTTAGCACTTATCTGGGACGATCTGCGG
		AGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGA
		CTTACTCTTGATTGTAACGAGGATTGTGGAACTTCT
		GGGACGCAGGGGGTGGGAAGCCCTCAAATATTGG
		TGGAATCTCCTACAATATTGGAGTCAGGAGCTAAA
		GAATAGTCTAGA
89	Target sequence	ATGGCAGGAAGAAGCGGAG
90	shRNA sequence	ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGC
		TTCTTCCTGCCATTTTTT

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SEQ ID NO:	Description	Sequence
91	H1 promoter and shRT sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG
		CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC
		GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA
		CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC
		TATGTGTTCTGGGAAATCACCATAAACGTGAAATG
		TCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAG
		ACCACTTGGATCCGCGGAGACAGCGACGAAGAGC
		TTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT
92	H1 CCR5 sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG
		CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC
		GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA
		CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC
		TATGTGTTCTGGGAAATCACCATAAACGTGAAATG
		TCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAG
		ACCACTTGGATCCGTGTCAAGTCCAATCTATGTTCA
		AGAGACATAGATTGGACTTGACACTTTTT
93	Primer	AGGAATTGATGGCGAGAAGG
94	Primer	CCCCAAAGAAGGTCAAGGTAATCA
95	Primer	AGCGCGGCTACAGCTTCA
96	Primer	GGCGACGTAGCACAGCTTCP
97	AGT103 CCR5 miR30	TGTAAACTGAGCTTGCTCTA
98	AGT103-R5-1	TGTAAACTGAGCTTGCTCGC
99	AGT103-R5-2	CATAGATTGGACTTGACAC
100	CAG promoter	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG
		TTCATAGCCCATATATGGAGTTCCGCGTTACATAA
		CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA
		CGACCCCCGCCCATTGACGTCAATAATGACGTATG
		TTCCCATAGTAACGCCAATAGGGACTTTCCATTGA
		CGTCAATGGGTGGACTATTTACGGTAAACTGCCCA
		CTTGGCAGTACATCAAGTGTATCATATGCCAAGTA
		CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC
		GCCTGGCATTATGCCCAGTACATGACCTTATGGGA
		CTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT
		CGCTATTACCATGGGTCGAGGTGAGCCCCACGTTC
		TGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCC
		CCAATTTTGTATTTATTTATTTTTTAATTATTTTGT
		GCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGC
		CAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGG
		GGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAG
		AGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAG
		GCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGC
		GCGCGGCGGGCG
101	H1 element	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG
		CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC
		GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA
		CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC
		TATGTGTTCTGGGAAATCACCATAAACGTGAAATG
		TCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAG
		ACCACTT
102	3' LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT
		CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGAC
		CAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG
		GAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTT
		GAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG
		ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGT
		CAGTGTGGAAAATCTCTAGCAGTAGTAGTTCATGT
		CA

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SEQ ID NO:	Descrip-tion	Sequence
103	7SK promoter	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGG CATTCTGGATAGTGTCAAAACAGCCGGAAATCAAG TCCGTTTATCTCAAACTTTAGCATTTTGGGAATAAA TGATATTTGCTATGCTGGTTAAATTAGATTTTAGTT AAATTTCCTGCTGAAGCTCTAGTACGATAAGCAAC TTGACCTAAGTGTAAAGTTGAGATTTCCTTCAGGTT TATATAGCTTGTGCGCCGCCTGGCTACCTC
104	miR155 Tat	CTGGAGGCTTGCTGAAGGCTGTATGCTGTCCGCTT CTTCCTGCCATAGGGTTTTGGCCACTGACTGACCCT ATGGGGAAGAAGCGGACAGGACACAAGGCCTGTT ACTAGCACTCACATGGAACAAATGGCC

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 104

<210> SEQ ID NO 1
<211> LENGTH: 118
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 CCR5

<400> SEQUENCE: 1

aggtatattg ctgttgacag tgagcgactg taaactgagc ttgctctact gtgaagccac 60

agatgggtag agcaagcaca gtttaccgct gcctactgcc tcggacttca aggggctt 118

<210> SEQ ID NO 2
<211> LENGTH: 116
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 Vif

<400> SEQUENCE: 2

catctccatg gctgtaccac cttgtcgggg gatgtgtact tctgaacttg tgttgaatct 60

catggagttc agaagaacac atccgcactg acattttggt atctttcatc tgacca 116

<210> SEQ ID NO 3
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR185 Tat

<400> SEQUENCE: 3

gggcctggct cgagcagggg gcgagggatt ccgcttcttc ctgccatagc gtggtcccct 60

cccctatggc aggcagaagc ggcaccttcc ctcccaatga ccgcgtcttc gtcg 114

<210> SEQ ID NO 4
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

<400> SEQUENCE: 4

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc 60

gccttttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc 120

tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc 180

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ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg 240

cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct 300

tgcgcttaag gagccccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc 360

cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg ctgctttcga taagtctcta 420

gccatttaaa atttttgatg acctgctgcg acgcttttt tctggcaaga tagtcttgta 480

aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg 540

gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga 600

atcggacggg ggtagtctca agctggccgg cctgctctgg tgcctggcct cgcgccgccg 660

tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcggaa 720

agatggccgc ttcccggccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcggga 780

gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct ttccgtcctc agccgtcgct 840

tcatgtgact ccacggagta ccgggcgccg tccaggcacc tcgattagtt ctcgagcttt 900

tggagtacgt cgtctttagg ttgggggggag gggttttatg cgatggagtt tccccacact 960

gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt 1020

gccctttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt tcaaagtttt 1080

tttcttccat ttcaggtgtc gtga 1104

<210> SEQ ID NO 5
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence

<400> SEQUENCE: 5

gagcaagctc agtttaca 18

<210> SEQ ID NO 6
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Vif target sequence

<400> SEQUENCE: 6

gggatgtgta cttctgaact t 21

<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Tat target sequence

<400> SEQUENCE: 7

tccgcttctt cctgccatag 20

<210> SEQ ID NO 8
<211> LENGTH: 126
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAR decoy sequence

<400> SEQUENCE: 8

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cttgcaatga tgtcgtaatt tgcgtcttac ctcgttctcg acagcgacca gatctgagcc 60

tgggagctct ctggctgtca gtaagctggt acagaaggtt gacgaaaatt cttactgagc 120

aagaaa 126

<210> SEQ ID NO 9
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev/Tat target sequence

<400> SEQUENCE: 9

gcggagacag cgacgaagag c 21

<210> SEQ ID NO 10
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev/Tat shRNA sequence

<400> SEQUENCE: 10

gcggagacag cgacgaagag cttcaagaga gctcttcgtc gctgtctccg cttttt 56

<210> SEQ ID NO 11
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag target sequence

<400> SEQUENCE: 11

gaagaaatga tgacagcat 19

<210> SEQ ID NO 12
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag shRNA sequence

<400> SEQUENCE: 12

gaagaaatga tgacagcatt tcaagagaat gctgtcatca tttcttcttt tt 52

<210> SEQ ID NO 13
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pol target sequence

<400> SEQUENCE: 13

caggagcaga tgatacag 18

<210> SEQ ID NO 14
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pol shRNA sequence

<400> SEQUENCE: 14

caggagatga tacagttcaa gagactgtat catctgctcc tgttttt 47

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<210> SEQ ID NO 15
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #1

<400> SEQUENCE: 15

gtgtcaagtc caatctatg 19

<210> SEQ ID NO 16
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #1

<400> SEQUENCE: 16

gtgtcaagtc caatctatgt tcaagagaca tagattggac ttgacacttt tt 52

<210> SEQ ID NO 17
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #2

<400> SEQUENCE: 17

gagcatgact gacatctac 19

<210> SEQ ID NO 18
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #2

<400> SEQUENCE: 18

gagcatgact gacatctact tcaagagagt agatgtcagt catgctcttt tt 52

<210> SEQ ID NO 19
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #3

<400> SEQUENCE: 19

gtagctctaa caggttgga 19

<210> SEQ ID NO 20
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #3

<400> SEQUENCE: 20

gtagctctaa caggttggat tcaagagatc caacctgtta gagctacttt tt 52

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #4

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<400> SEQUENCE: 21

gttcagaaac tacctctta 19

<210> SEQ ID NO 22
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #4

<400> SEQUENCE: 22

gttcagaaac tacctcttat tcaagagata gaggtagtt tctgaacttt tt 52

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #5

<400> SEQUENCE: 23

gagcaagctc agtttacacc 20

<210> SEQ ID NO 24
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #5

<400> SEQUENCE: 24

gagcaagctc agtttacacc ttcaagagag gtgtaaactg agcttgctct tttt 54

<210> SEQ ID NO 25
<211> LENGTH: 141
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 1

<400> SEQUENCE: 25

atggattatc aagtgtcaag tccaatctat gacatcaatt attatacatc ggagccctgc 60

caaaaaatca atgtgaagca aatcgcagcc cgcctcctgc ctccgctcta ctcactggtg 120

ttcatctttg gttttgtggg c 141

<210> SEQ ID NO 26
<211> LENGTH: 633
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 2

<400> SEQUENCE: 26

aacatgctgg tcatcctcat cctgataaac tgcaaaaggc tgaagagcat gactgacatc 60

tacctgctca acctggccat ctctgacctg tttttccttc ttactgtccc cttctgggct 120

cactatgctg ccgcccagtg ggactttgga aatacaatgt gtcaactctt gacagggctc 180

tattttatag gcttcttctc tggaatcttc ttcatcatcc tcctgacaat cgataggtac 240

ctggctgtcg tccatgctgt gtttgcttta aaagccagga cggtcacctt tggggtggtg 300

acaagtgtga tcacttgggt ggtggctgtg tttgcgtctc tcccaggaat catctttacc 360

agatctcaaa aagaaggtct tcattacacc tgcagctctc attttccata cagtcagtat 420

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caattctgga agaatttcca gacattaaag atagtcatct tggggctggt cctgccgctg 480

cttgtcatgg tcatctgcta ctcgggaatc ctaaaaactc tgcttcggtg tcgaaatgag 540

aagaagaggc acagggctgt gaggcttatc ttcaccatca tgattgttta ttttctcttc 600

tgggctccct acaacattgt ccttctcctg aac 633

<210> SEQ ID NO 27
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 3

<400> SEQUENCE: 27

accttccagg aattctttgg cctgaataat tgcagtagct ctaacaggtt ggaccaagct 60

atgcaggtga 70

<210> SEQ ID NO 28
<211> LENGTH: 140
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 4

<400> SEQUENCE: 28

cagagactct tgggatgacg cactgctgca tcaaccccat catctatgcc tttgtcgggg 60

agaagttcag aaactacctc ttagtcttct tccaaaagca cattgccaaa cgcttctgca 120

aatgctgttc tattttccag 140

<210> SEQ ID NO 29
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 5

<400> SEQUENCE: 29

caagaggctc ccgagcgagc aagctcagtt tacacccgat ccactgggga gcaggaaata 60

tctgtgggct tgtga 75

<210> SEQ ID NO 30
<211> LENGTH: 541
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD4 promoter sequence

<400> SEQUENCE: 30

tgttggggtt caaatttgag ccccagctgt tagccctctg caaagaaaaa aaaaaaaaaa 60

aaagaacaaa gggcctagat ttcccttctg agccccaccc taagatgaag cctcttcttt 120

caagggagtg gggttggggt ggaggcggat cctgtcagct ttgctctctc tgtggctggc 180

agtttctcca aagggtaaca ggtgtcagct ggctgagcct aggctgaacc ctgagacatg 240

ctacctctgt cttctcatgg ctggaggcag cctttgtaag tcacagaaag tagctgaggg 300

gctctggaaa aaagacagcc agggtggagg tagattggtc tttgactcct gatttaagcc 360

tgattctgct taacttttttc ccttgacttt ggcattttca ctttgacatg ttccctgaga 420

gcctgggggg tggggaaccc agctccagct ggtgacgttt ggggccggcc caggcctagg 480

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gtgtggagga gccttgccat cgggcttcct gtctctcttc atttaagcac gactctgcag 540

a 541

<210> SEQ ID NO 31
<211> LENGTH: 359
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30-CCR5/miR21-Vif/miR185 Tat microRNA cluster sequence

<400> SEQUENCE: 31

aggtatattg ctgttgacag tgagcgactg taaactgagc ttgctctact gtgaagccac 60

agatgggtag agcaagcaca gtttaccgct gcctactgcc tcggacttca aggggcttcc 120

cgggcatctc catggctgta ccaccttgtc gggggatgtg tacttctgaa cttgtgttga 180

atctcatgga gttcagaaga acacatccgc actgacattt tggtatcttt catctgacca 240

gctagcgggc ctggctcgag caggggggcga gggattccgc ttcttcctgc catagcgtgg 300

tcccctcccc tatggcaggc agaagcggca ccttccctcc caatgaccgc gtcttcgtc 359

<210> SEQ ID NO 32
<211> LENGTH: 590
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Long WPRE sequence

<400> SEQUENCE: 32

aatcaacctc tgattacaaa atttgtgaaa gattgactgg tattcttaac tatgttgctc 60

cttttacgct atgtggatac gctgctttaa tgcctttgta tcatgctatt gcttcccgta 120

tggctttcat tttctcctcc ttgtataaat cctggttgct gtctctttat gaggagttgt 180

ggcccgttgt caggcaacgt ggcgtggtgt gcactgtgtt tgctgacgca acccccactg 240

gttggggcat tgccaccacc tgtcagctcc tttccgggac tttcgctttc cccctcccta 300

ttgccacggc ggaactcatc gccgcctgcc ttgcccgctg ctggacaggg gctcggctgt 360

tgggcactga caattccgtg gtgttgtcgg ggaaatcatc gtcctttcct tggctgctcg 420

cctgtgttgc cacctggatt ctgcgcggga cgtccttctg ctacgtccct tcggccctca 480

atccagcgga ccttccttcc cgcggcctgc tgccggctct gcggcctctt ccgcgtcttc 540

gccttcgccc tcagacgagt cggatctccc tttgggccgc ctccccgcct 590

<210> SEQ ID NO 33
<211> LENGTH: 1469
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter - miR30CCR5 - miR21Vif - miR185 Tat

<400> SEQUENCE: 33

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc 60

gccttttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc 120

tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc 180

ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg 240

cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct 300

tgcgcttaag gagccccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc 360

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cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg ctgctttcga taagtctcta 420

gccatttaaa atttttgatg acctgctgcg acgcttttt tctggcaaga tagtcttgta 480

aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg 540

gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga 600

atcggacggg ggtagtctca agctggccgg cctgctctgg tgcctggcct cgcgccgccg 660

tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcggaa 720

agatggccgc ttcccggccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcggga 780

gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct ttccgtcctc agccgtcgct 840

tcatgtgact ccacggagta ccgggcgccg tccaggcacc tcgattagtt ctcgagcttt 900

tggagtacgt cgtctttagg ttgggggggag gggttttatg cgatggagtt tccccacact 960

gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt 1020

gccctttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt tcaaagtttt 1080

tttcttccat ttcaggtgtc gtgatgtaca aggtatattg ctgttgacag tgagcgactg 1140

taaactgagc ttgctctact gtgaagccac agatgggtag agcaagcaca gtttaccgct 1200

gcctactgcc tcggacttca aggggcttcc cgggcatctc catggctgta ccaccttgtc 1260

gggggatgtg tacttctgaa cttgtgttga atctcatgga gttcagaaga acacatccgc 1320

actgacattt tggtatcttt catctgacca gctagcgggc ctggctcgag caggggggcga 1380

gggattccgc ttcttcctgc catagcgtgg tcccctcccc tatggcaggc agaagcggca 1440

ccttccctcc caatgaccgc gtcttcgtc 1469

<210> SEQ ID NO 34
<211> LENGTH: 228
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rous Sarcoma virus (RSV) promoter

<400> SEQUENCE: 34

gtagtcttat gcaatactct tgtagtcttg caacatggta acgatgagtt agcaacatgc 60

cttacaagga gagaaaaagc accgtgcatg ccgattggtg gaagtaaggt ggtacgatcg 120

tgccttatta ggaaggcaac agacgggtct gacatggatt ggacgaacca ctgaattgcc 180

gcattgcaga gatattgtat ttaagtgcct agctcgatac aataaacg 228

<210> SEQ ID NO 35
<211> LENGTH: 180
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5' Long terminal repeat (LTR)

<400> SEQUENCE: 35

ggtctctctg gttagaccag atctgagcct gggagctctc tggctaacta gggaacccac 60

tgcttaagcc tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt 120

gtgactctgg taactagaga tccctcagac cttttagtc agtgtggaaa atctctagca 180

<210> SEQ ID NO 36
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Psi Packaging signal

<400> SEQUENCE: 36

tacgccaaaa attttgacta gcggaggcta gaaggagaga g 41

<210> SEQ ID NO 37
<211> LENGTH: 233
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev response element (RRE)

<400> SEQUENCE: 37

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcctcaat 60

gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt 120

gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca 180

gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcc 233

<210> SEQ ID NO 38
<211> LENGTH: 118
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Central polypurine tract (cPPT)

<400> SEQUENCE: 38

ttttaaaaga aaaggggga ttgggggta cagtgcaggg gaaagaatag tagacataat 60

agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaattca aaatttta 118

<210> SEQ ID NO 39
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3' delta LTR

<400> SEQUENCE: 39

tggaagggct aattcactcc caacgaagat aagatctgct ttttgcttgt actgggtctc 60

tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta 120

agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact 180

ctggtaacta gagatccctc agacccttt agtcagtgtg gaaaatctct agcagtagta 240

gttcatgtca 250

<210> SEQ ID NO 40
<211> LENGTH: 352
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - CMV early (CAG) enhancer -
EnhanceTranscription

<400> SEQUENCE: 40

tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg 60

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 300

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catgaccttа tgggactttc ctacttggca gtacatctac gtattagtca tc 352

<210> SEQ ID NO 41
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - Chicken beta actin (CAG) promoter - Transcription

<400> SEQUENCE: 41

gctattacca tgggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc 60

ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120

gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180

ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt ccttttatgg 240

cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 290

<210> SEQ ID NO 42
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - Chicken beta actin intron - Enhance gene expression

<400> SEQUENCE: 42

ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60

cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180

cttaaagggc tccgggaggg ccctttgtgc ggggggggagc ggctcggggg gtgcgtgcgt 240

gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 300

gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg 360

gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt 420

gggggggtga gcaggggggtg tgggcgcggc ggtcgggctg taacccccccc ctgcaccccc 480

ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg gggcgtggcg 540

cggggctcgc cgtgccgggc gggggggtggc ggcaggtggg ggtgccgggc ggggcggggc 600

cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg ccccggagcg ccggcggctg 660

tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga gggcgcaggg 720

acttcctttg tcccaaatct ggcggagccg aaatctggga ggcgccgccg cacccccctct 780

agcgggcgcg ggcgaagcgg tgcggcgccg gcaggaagga aatgggcggg gagggccttc 840

gtgcgtcgcc gcgccgccgt cccccttctcc atctccagcc tcggggctgc cgcaggggga 900

cggctgcctt cgggggggac ggggcagggc ggggttcggc ttctggcgtg tgaccggcgg 960

<210> SEQ ID NO 43
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - HIV Gag - Viral capsid

<400> SEQUENCE: 43

atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60

ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag 120

-continued

ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata 180

ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240

acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct 300

ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct 360

gacacaggac acagcaatca ggtcagccaa aattacccta tagtgcagaa catccagggg 420

caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa 480

gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc 540

ccacaagatt taaacaccat gctaaacaca gtgggggggac atcaagcagc catgcaaatg 600

ttaaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcatcc agtgcatgca 660

gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact 720

agtacccttc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa 780

atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc 840

agcattctgg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc 900

tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc 960

ttgttggtcc aaaatgcgaa cccagattgt aagactattt taaaagcatt gggaccagga 1020

gcgacactag aagaaatgat gacagcatgt cagggagtgg ggggacccgg ccataaagca 1080

agagttttgg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa 1140

ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa agaagggcac 1200

atagccaaaa attgcagggc ccctaggaaa aagggctgtt ggaaatgtgg aaaggaagga 1260

caccaaatga aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc 1320

cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa 1380

gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac 1440

aaggaactgt atcctttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa 1500

taa 1503

<210> SEQ ID NO 44
<211> LENGTH: 1872
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - HIV Pol - Protease and reverse transcriptase

<400> SEQUENCE: 44

atgaatttgc caggaagatg gaaaccaaaa atgatagggg gaattggagg ttttatcaaa 60

gtaggacagt atgatcagat actcatagaa atctgcggac ataaagctat aggtacagta 120

ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc 180

actttaaatt ttcccattag tcctattgag actgtaccag taaaattaaa gccaggaatg 240

gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc attagtagaa 300

atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga aaatccatac 360

aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat 420

ttcagagaac ttaataagag aactcaagat ttctgggaag ttcaattagg aataccacat 480

cctgcagggt taaaacagaa aaaatcagta acagtactgg atgtgggcga tgcatatttt 540

tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac 600

aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg gaaaggatca 660

ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca 720

gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaatagggg 780

cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca 840

ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga actccatcct 900

gataaatgga cagtacagcc tatagtgctg ccagaaaagg acagctggac tgtcaatgac 960

atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta 1020

aggcaattat gtaaacttct taggggaacc aaagcactaa cagaagtagt accactaaca 1080

gaagaagcag agctagaact ggcagaaaac agggagattc taaaagaacc ggtacatgga 1140

gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa 1200

tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga 1260

atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc 1320

acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa 1380

acatgggaag catggtggac agagtattgg caagccacct ggattcctga gtgggagttt 1440

gtcaataccc ctcccttagt gaagttatgg taccagttag agaaagaacc cataatagga 1500

gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg aaaagcagga 1560

tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac aaatcagaag 1620

actgagttac aagcaattca tctagctttg caggattcgg gattagaagt aaacatagtg 1680

acagactcac aatatgcatt gggaatcatt caagcacaac cagataagag tgaatcagag 1740

ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggta 1800

ccagcacaca aaggaattgg aggaaatgaa caagtagatg ggttggtcag tgctggaatc 1860

aggaaagtac ta 1872

<210> SEQ ID NO 45
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper Rev - HIV Integrase - Integration of viral RNA

<400> SEQUENCE: 45

tttttagatg gaatagataa ggcccaagaa gaacatgaga aatatcacag taattggaga 60

gcaatggcta gtgattttaa cctaccacct gtagtagcaa aagaaatagt agccagctgt 120

gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag cccaggaata 180

tggcagctag attgtacaca tttagaagga aaagttatct tggtagcagt tcatgtagcc 240

agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc 300

ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat 360

ttcaccagta ctacagttaa ggccgcctgt tggtgggcgg ggatcaagca ggaatttggc 420

attccctaca atccccaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa 480

attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta 540

ttcatccaca attttaaaag aaaaggggggg attgggggtt acagtgcagg ggaaagaata 600

gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt 660

caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag 720

ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgacat aaaagtagtg 780

ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt 840

gtggcaagta gacaggatga ggattaa 867

<210> SEQ ID NO 46
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - HIV RRE- Binds Rev element

<400> SEQUENCE: 46

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat 60

gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt 120

gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca 180

gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct 234

<210> SEQ ID NO 47
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - HIV Rev - Nuclear export and stabilize viral mRNA

<400> SEQUENCE: 47

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag 60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat 120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt 180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga 240

cttactcttg attgtaacga ggattgtgga acttctggga cgcaggggt gggaagccct 300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g 351

<210> SEQ ID NO 48
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - Rabbit beta globin poly A - RNA stability

<400> SEQUENCE: 48

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180

ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg ctataaagag 240

gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga aaagccttga 300

cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 360

aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta ctcccagtca 420

tagctgtccc tcttctctta tgaagatc 448

<210> SEQ ID NO 49
<211> LENGTH: 352
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - CMV early (CAG) enhancer - Enhancetranscription

<400> SEQUENCE: 49

tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg 60

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 300

catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tc 352

<210> SEQ ID NO 50
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Chicken beta actin (CAG) promoter - Transcription

<400> SEQUENCE: 50

gctattacca tgggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc 60

ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120

gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180

ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt ccttttatgg 240

cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 290

<210> SEQ ID NO 51
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Chicken beta actin intron - Enhance gene expression

<400> SEQUENCE: 51

ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60

cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180

cttaaagggc tccgggaggg ccctttgtgc ggggggagc ggctcggggg gtgcgtgcgt 240

gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 300

gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg 360

gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt 420

gggggggtga gcagggggtg tgggcgcggc ggtcgggctg taacccccccc ctgcaccccc 480

ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg gggcgtggcg 540

cggggctcgc cgtgccgggc gggggggtggc ggcaggtggg ggtgccgggc ggggcggggc 600

cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg ccccggagcg ccggcggctg 660

tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga gggcgcaggg 720

acttcctttg tcccaaatct ggcggagccg aaatctggga ggcgccgccg cacccccctct 780

agcgggcgcg ggcgaagcgg tgcggcgccg gcaggaagga aatgggcggg gagggccttc 840

-continued

gtgcgtcgcc gcgccgccgt ccccttctcc atctccagcc tcggggctgc cgcaggggga 900

cggctgcctt cgggggggac ggggcagggc ggggttcggc ttctggcgtg tgaccggcgg 960

<210> SEQ ID NO 52
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV Gag - Viral capsid

<400> SEQUENCE: 52

atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60

ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag 120

ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata 180

ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240

acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct 300

ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct 360

gacacaggac acagcaatca ggtcagccaa aattacccta tagtgcagaa catccagggg 420

caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa 480

gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc 540

ccacaagatt taaacaccat gctaaacaca gtgggggggac atcaagcagc catgcaaatg 600

ttaaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcatcc agtgcatgca 660

gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact 720

agtacccttc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa 780

atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc 840

agcattctgg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc 900

tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc 960

ttgttggtcc aaaatgcgaa cccagattgt aagactattt taaaagcatt gggaccagga 1020

gcgacactag aagaaatgat gacagcatgt cagggagtgg ggggacccgg ccataaagca 1080

agagttttgg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa 1140

ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa agaagggcac 1200

atagccaaaa attgcagggc ccctaggaaa aagggctgtt ggaaatgtgg aaaggaagga 1260

caccaaatga aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc 1320

cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa 1380

gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac 1440

aaggaactgt atcctttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa 1500

taa 1503

<210> SEQ ID NO 53
<211> LENGTH: 1872
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV Pol - Protease and reverse
transcriptase

<400> SEQUENCE: 53

atgaatttgc caggaagatg gaaaccaaaa atgatagggg gaattggagg ttttatcaaa 60

-continued

gtaggacagt atgatcagat actcatagaa atctgcggac ataaagctat aggtacagta 120

ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc 180

actttaaatt ttcccattag tcctattgag actgtaccag taaaattaaa gccaggaatg 240

gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc attagtagaa 300

atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga aaatccatac 360

aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat 420

ttcagagaac ttaataagag aactcaagat ttctgggaag ttcaattagg aataccacat 480

cctgcagggt taaaacagaa aaaatcagta acagtactgg atgtgggcga tgcatatttt 540

tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac 600

aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg gaaaggatca 660

ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca 720

gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg 780

cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca 840

ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga actccatcct 900

gataaatgga cagtacagcc tatagtgctg ccagaaaagg acagctggac tgtcaatgac 960

atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta 1020

aggcaattat gtaaacttct taggggaacc aaagcactaa cagaagtagt accactaaca 1080

gaagaagcag agctagaact ggcagaaaac agggagattc taaaagaacc ggtacatgga 1140

gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa 1200

tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga 1260

atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc 1320

acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa 1380

acatgggaag catggtggac agagtattgg caagccacct ggattcctga gtgggagttt 1440

gtcaataccc ctcccttagt gaagttatgg taccagttag agaaagaacc cataatagga 1500

gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg aaaagcagga 1560

tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac aaatcagaag 1620

actgagttac aagcaattca tctagctttg caggattcgg gattagaagt aaacatagtg 1680

acagactcac aatatgcatt gggaatcatt caagcacaac cagataagag tgaatcagag 1740

ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggta 1800

ccagcacaca aaggaattgg aggaaatgaa caagtagatg ggttggtcag tgctggaatc 1860

aggaaagtac ta 1872

<210> SEQ ID NO 54
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV Integrase - Integration of viral RNA

<400> SEQUENCE: 54

tttttagatg gaatagataa ggcccaagaa gaacatgaga aatatcacag taattggaga 60

gcaatggcta gtgattttaa cctaccacct gtagtagcaa aagaaatagt agccagctgt 120

gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag cccaggaata 180

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tggcagctag attgtacaca tttagaagga aaagttatct tggtagcagt tcatgtagcc 240

agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc 300

ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat 360

ttcaccagta ctacagttaa ggccgcctgt tggtgggcgg ggatcaagca ggaatttggc 420

attccctaca atccccaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa 480

attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta 540

ttcatccaca attttaaaag aaaaggggg attgggggt acagtgcagg ggaaagaata 600

gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt 660

caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag 720

ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgacat aaaagtagtg 780

ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt 840

gtggcaagta gacaggatga ggattaa 867

<210> SEQ ID NO 55
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV RRE - Binds Rev element

<400> SEQUENCE: 55

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat 60

gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt 120

gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca 180

gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct 234

<210> SEQ ID NO 56
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Rabbit beta globin poly A - RNA stability

<400> SEQUENCE: 56

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180

ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg ctataaagag 240

gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga aaagccttga 300

cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 360

aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta ctcccagtca 420

tagctgtccc tcttctctta tgaagatc 448

<210> SEQ ID NO 57
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev - RSV promoter - Transcription

<400> SEQUENCE: 57

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atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag 60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat 120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt 180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga 240

cttactcttg attgtaacga ggattgtgga acttctggga cgcaggggt gggaagccct 300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g 351

<210> SEQ ID NO 58
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev - HIV Rev- Nuclear export and stabilize viral mRNA

<400> SEQUENCE: 58

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag 60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat 120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt 180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga 240

cttactcttg attgtaacga ggattgtgga acttctggga cgcaggggt gggaagccct 300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g 351

<210> SEQ ID NO 59
<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev- Rabbit beta globin poly A- RNA stability

<400> SEQUENCE: 59

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180

ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaaggtt ggctataaag 240

aggtcatcag tatatgaaac agccccctgc tgtccattcc ttattccata gaaaagcctt 300

gacttgaggt tagatttttt ttatattttg ttttgtgtta ttttttttctt taacatccct 360

aaaattttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt 420

catagctgtc cctcttctct tatggagatc 450

<210> SEQ ID NO 60
<211> LENGTH: 577
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- CMV promoter- Transcription

<400> SEQUENCE: 60

acattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcatagccc 60

atatatggag ttccgcgtta cataacttac ggtaaatggc ccgcctggct gaccgcccaa 120

cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac 180

tttccattga cgtcaatggg tggagtattt acggtaaact gcccacttgg cagtacatca 240

-continued

agtgtatcat atgccaagta cgccccctat tgacgtcaat gacggtaaat ggcccgcctg 300

gcattatgcc cagtacatga ccttatggga ctttcctact tggcagtaca tctacgtatt 360

agtcatcgct attaccatgg tgatgcggtt ttggcagtac atcaatgggc gtggatagcg 420

gtttgactca cggggatttc caagtctcca ccccattgac gtcaatggga gtttgttttg 480

gcaccaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat tgacgcaaat 540

gggcggtagg cgtgtacggt gggaggtcta tataagc 577

<210> SEQ ID NO 61
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Beta globin intron- Enhance gene expression

<400> SEQUENCE: 61

gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat 60

ggaggggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat 120

ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180

cttatttttct tttcattttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga 240

atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300

tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt 360

ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt 420

cttattggta gaaacaacta caccctggtc atcatcctgc ctttctcttt atggttacaa 480

tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540

aaccatgttc atgccttctt ctctttccta cag 573

<210> SEQ ID NO 62
<211> LENGTH: 1519
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- VSV-G- Glycoprotein envelope-cell entry

<400> SEQUENCE: 62

atgaagtgcc ttttgtactt agccttttta ttcattgggg tgaattgcaa gttcaccata 60

gtttttccac acaaccaaaa aggaaactgg aaaaatgttc cttctaatta ccattattgc 120

ccgtcaagct cagatttaaa ttggcataat gacttaatag gcacagcctt acaagtcaaa 180

atgcccaaga gtcacaaggc tattcaagca gacggttgga tgtgtcatgc ttccaaatgg 240

gtcactactt gtgatttccg ctggtatgga ccgaagtata taacacattc catccgatcc 300

ttcactccat ctgtagaaca atgcaaggaa agcattgaac aaacgaaaca aggaacttgg 360

ctgaatccag gcttccctcc tcaaagttgt ggatatgcaa ctgtgacgga tgccgaagca 420

gtgattgtcc aggtgactcc tcaccatgtg ctggttgatg aatacacagg agaatgggtt 480

gattcacagt tcatcaacgg aaaatgcagc aattacatat gccccactgt ccataactct 540

acaacctggc attctgacta taaggtcaaa gggctatgtg attctaacct catttccatg 600

gacatcacct tcttctcaga ggacggagag ctatcatccc tgggaaagga gggcacaggg 660

ttcagaagta actactttgc ttatgaaact ggaggcaagg cctgcaaaat gcaatactgc 720

aagcattggg gagtcagact cccatcaggt gtctggttcg agatggctga taaggatctc 780

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tttgctgcag ccagattccc tgaatgccca gaagggtcaa gtatctctgc tccatctcag 840

acctcagtgg atgtaagtct aattcaggac gttgagagga tcttggatta ttccctctgc 900

caagaaacct ggagcaaaat cagagcgggt cttccaatct ctccagtgga tctcagctat 960

cttgctccta aaaacccagg aaccggtcct gctttcacca taatcaatgg taccctaaaa 1020

tactttgaga ccagatacat cagagtcgat attgctgctc caatcctctc aagaatggtc 1080

ggaatgatca gtggaactac cacagaaagg gaactgtggg atgactgggc accatatgaa 1140

gacgtggaaa ttggacccaa tggagttctg aggaccagtt caggatataa gtttccttta 1200

tacatgattg gacatggtat gttggactcc gatcttcatc ttagctcaaa ggctcaggtg 1260

ttcgaacatc ctcacattca agacgctgct tcgcaacttc ctgatgatga gagtttattt 1320

tttggtgata ctgggctatc caaaaatcca atcgagcttg tagaaggttg gttcagtagt 1380

tggaaaagct ctattgcctc ttttttcttt atcatagggt taatcattgg actattcttg 1440

gttctccgag ttggtatcca tctttgcatt aaattaaagc acaccaagaa aagacagatt 1500

tatacagaca tagagatga 1519

<210> SEQ ID NO 63
<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Rabbit beta globin poly A- RNA stability

<400> SEQUENCE: 63

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180

ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaaggtt ggctataaag 240

aggtcatcag tatatgaaac agccccctgc tgtccattcc ttattccata gaaaagcctt 300

gacttgaggt tagatttttt ttatattttg ttttgtgtta tttttttctt taacatccct 360

aaaatttttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt 420

catagctgtc cctcttctct tatggagatc 450

<210> SEQ ID NO 64
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter- EF-1

<400> SEQUENCE: 64

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc 60

gccttttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc 120

tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc 180

ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg 240

cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct 300

tgcgcttaag gagcccccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc 360

cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg ctgctttcga taagtctcta 420

gccatttaaa atttttgatg acctgctgcg acgcttttttt tctggcaaga tagtcttgta 480

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aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg 540

gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga 600

atcggacggg ggtagtctca agctggccgg cctgctctgg tgcctggcct cgcgccgccg 660

tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcggaa 720

agatggccgc ttcccggccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcggga 780

gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct ttccgtcctc agccgtcgct 840

tcatgtgact ccacggagta ccgggcgccg tccaggcacc tcgattagtt ctcgagcttt 900

tggagtacgt cgtctttagg ttgggggggag gggttttatg cgatggagtt tccccacact 960

gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt 1020

gccctttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt tcaaagtttt 1080

tttcttccat ttcaggtgtc gtga 1104

<210> SEQ ID NO 65
<211> LENGTH: 511
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter- PGK

<400> SEQUENCE: 65

ggggttgggg ttgcgccttt tccaaggcag ccctgggttt gcgcagggac gcggctgctc 60

tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgcacat tcttcacgtc 120

cgttcgcagc gtcacccgga tcttcgccgc tacccttgtg ggcccccggg cgacgcttcc 180

tgctccgccc ctaagtcggg aaggttcctt gcggttcgcg gcgtgccgga cgtgacaaac 240

ggaagccgca cgtctcacta gtaccctcgc agacggacag cgccagggag caatggcagc 300

gcgccgaccg cgatgggctg tggccaatag cggctgctca gcagggcgcg ccgagagcag 360

cggccgggaa ggggcggtgc gggaggcggg gtgtggggcg gtagtgtggg ccctgttcct 420

gcccgcgcgg tgttccgcat tctgcaagcc tccggagcgc acgtcggcag tcggctccct 480

cgttgaccga atcaccgacc tctctcccca g 511

<210> SEQ ID NO 66
<211> LENGTH: 1162
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter- UbC

<400> SEQUENCE: 66

gcgccgggtt ttggcgcctc ccgcgggcgc ccccctcctc acggcgagcg ctgccacgtc 60

agacgaaggg cgcaggagcg ttcctgatcc ttccgcccgg acgctcagga cagcggcccg 120

ctgctcataa gactcggcct tagaacccca gtatcagcag aaggacattt taggacggga 180

cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg aggaaaagta 240

gtcccttctc ggcgattctg cggagggatc tccgtggggc ggtgaacgcc gatgattata 300

taaggacgcg ccgggtgtgg cacagctagt tccgtcgcag ccgggatttg ggtcgcggtt 360

cttgtttgtg gatcgctgtg atcgtcactt ggtgagttgc gggctgctgg gctggccggg 420

gctttcgtgg ccgccgggcc gctcggtggg acggaagcgt gtggagagac cgccaagggc 480

tgtagtctgg gtccgcgagc aaggttgccc tgaactgggg gttgggggga gcgcacaaaa 540

-continued

tggcggctgt tcccgagtct tgaatggaag acgcttgtaa ggcgggctgt gaggtcgttg 600

aaacaaggtg gggggcatgg tgggcggcaa gaacccaagg tcttgaggcc ttcgctaatg 660

cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc ctgacgtgaa 720

gtttgtcact gactggagaa ctcgggtttg tcgtctggtt gcgggggcgg cagttatgcg 780

gtgccgttgg gcagtgcacc cgtacctttg ggagcgcgcg cctcgtcgtg tcgtgacgtc 840

acccgttctg ttggcttata atgcagggtg gggccacctg ccggtaggtg tgcggtaggc 900

ttttctccgt cgcaggacgc agggttcggg cctagggtag gctctcctga atcgacaggc 960

gccggacctc tggtgagggg agggataagt gaggcgtcag tttctttggt cggttttatg 1020

tacctatctt cttaagtagc tgaagctccg gttttgaact atgcgctcgg ggttggcgag 1080

tgtgttttgt gaagtttttt aggcaccttt tgaaatgtaa tcatttgggt caatatgtaa 1140

ttttcagtgt tagactagta aa 1162

<210> SEQ ID NO 67
<211> LENGTH: 120
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- SV40

<400> SEQUENCE: 67

gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa 60

agcatttttt tcactgcatt ctagttgtgg tttgtccaaa ctcatcaatg tatcttatca 120

<210> SEQ ID NO 68
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- bGH

<400> SEQUENCE: 68

gactgtgcct tctagttgcc agccatctgt tgtttgcccc tcccccgtgc cttccttgac 60

cctggaaggt gccactccca ctgtcctttc ctaataaaat gaggaaattg catcgcattg 120

tctgagtagg tgtcattcta ttctgggggg tggggtgggg caggacagca agggggagga 180

ttgggaagac aatagcaggc atgctgggga tgcggtgggc tctatgg 227

<210> SEQ ID NO 69
<211> LENGTH: 1512
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Gag- Bal

<400> SEQUENCE: 69

atgggtgcga gagcgtcagt attaagcggg ggagaattag ataggtggga aaaaattcgg 60

ttaaggccag ggggaaagaa aaaatataga ttaaaacata tagtatgggc aagcagggaa 120

ctagaaagat tcgcagtcaa tcctggcctg ttagaaacat cagaaggctg cagacaaata 180

ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240

acagtagcaa ccctctattg tgtacatcaa aagatagagg taaaagacac caaggaagct 300

ttagacaaaa tagaggaaga gcaaaacaaa tgtaagaaaa aggcacagca agcagcagct 360

gacacaggaa acagcggtca ggtcagccaa aatttcccta tagtgcagaa cctccagggg 420

caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtaatagaa 480

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gagaaagctt tcagcccaga agtaataccc atgttttcag cattatcaga aggagccacc 540

ccacaagatt taaacaccat gctaaacaca gtgggggggac atcaagcagc catgcaaatg 600

ttaaaagaac ccatcaatga ggaagctgca agatgggata gattgcatcc cgtgcaggca 660

gggcctgttg caccaggcca gataagagat ccaaggggaa gtgacatagc aggaactacc 720

agtacccttc aggaacaaat aggatggatg acaagtaatc cacctatccc agtaggagaa 780

atctataaaa gatggataat cctgggatta aataaaatag taaggatgta tagccctacc 840

agcattttgg acataagaca aggaccaaag gaacccttta gagactatgt agaccggttc 900

tataaaactc taagagccga gcaagcttca caggaggtaa aaaattggat gacagaaacc 960

ttgttggtcc aaaatgcgaa cccagattgt aagactattt taaaagcatt gggaccagca 1020

gctacactag aagaaatgat gacagcatgt cagggagtgg gaggacccag ccataaagca 1080

agaattttgg cagaagcaat gagccaagta acaaattcag ctaccataat gatgcagaaa 1140

ggcaatttta ggaaccaaag aaagattgtt aaatgtttca attgtggcaa agaagggcac 1200

atagccagaa actgcagggc ccctaggaaa aggggctgtt ggaaatgtgg aaaggaagga 1260

caccaaatga aagactgtac tgagagacag gctaattttt tagggaaaat ctggccttcc 1320

cacaaaggaa ggccagggaa tttccttcag agcagaccag agccaacagc cccaccagcc 1380

ccaccagaag agagcttcag gtttggggaa gagacaacaa ctccctctca gaagcaggag 1440

ctgatagaca aggaactgta tcctttagct tccctcagat cactctttgg caacgacccc 1500

tcgtcacaat aa 1512

<210> SEQ ID NO 70
<211> LENGTH: 1872
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Pol- Bal

<400> SEQUENCE: 70

atgaatttgc caggaagatg gaaaccaaaa atgatagggg gaattggagg ttttatcaaa 60

gtaagacagt atgatcagat actcatagaa atctgtggac ataaagctat aggtacagta 120

ttaataggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggttgc 180

actttaaatt ttcccattag tcctattgaa actgtaccag taaaattaaa accaggaatg 240

gatggcccaa aagttaaaca atggccactg acagaagaaa aaataaaagc attaatggaa 300

atctgtacag aaatggaaaa ggaagggaaa atttcaaaaa ttgggcctga aaatccatac 360

aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat 420

ttcagagaac ttaataagaa aactcaagac ttctgggaag tacaattagg aatacacatc 480

ccgcaggggt taaaaaagaa aaaatcagta acagtactgg atgtgggtga tgcatatttt 540

tcagttccct tagataaaga attcaggaag tatactgcat ttaccatacc tagtataaac 600

aatgaaacac cagggatcag atatcagtac aatgtacttc cacagggatg gaaaggatca 660

ccagcaatat ttcaaagtag catgacaaga atcttagagc cttttagaaa acaaaatcca 720

gaaatagtga tctatcaata catggatgat ttgtatgtag gatctgactt agaaatagggg 780

cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca 840

ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga actccatcct 900

gataaatgga cagtacagcc tatagtgctg ccagaaaaag acagctggac tgtcaatgac 960

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atacagaagt tagtgggaaa attgaattgg gcaagtcaga tttacccagg aattaaagta 1020

aagcaattat gtaggctcct taggggaacc aaggcattaa cagaagtaat accactaaca 1080

aaagaaacag agctagaact ggcagagaac agggaaattc taaaagaacc agtacatggg 1140

gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa 1200

tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga 1260

atgaggggtg cccacactaa tgatgtaaaa caattaacag aggcagtgca aaaaataacc 1320

acagaaagca tagtaatatg gggaaagact cctaaattta aactacccat acaaaaagaa 1380

acatgggaaa catggtggac agagtattgg caagccacct ggattcctga gtgggagttt 1440

gtcaataccc ctcccttagt gaaattatgg taccagttag agaaagaacc cataatagga 1500

gcagaaacat tctatgtaga tggagcagct aaccgggaga ctaaattagg aaaagcagga 1560

tatgttacta acagaggaag acaaaaagtt gtctccctaa ctgacacaac aaatcagaag 1620

actgagttac aagcaattca tctagcttta caagattcag gattagaagt aaacatagta 1680

acagactcac aatatgcatt aggaatcatt caagcacaac cagataaaag tgaatcagag 1740

ttagtcagtc aaataataga acagttaata aaaaaggaaa aggtctacct ggcatgggta 1800

ccagcgcaca aaggaattgg aggaaatgaa caagtagata aattagtcag tactggaatc 1860

aggaaagtac ta 1872

<210> SEQ ID NO 71
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Integrase- Bal

<400> SEQUENCE: 71

tttttagatg gaatagatat agcccaagaa gaacatgaga aatatcacag taattggaga 60

gcaatggcta gtgattttaa cctgccacct gtggtagcaa aagaaatagt agccagctgt 120

gataaatgtc agctaaaagg agaagccatg catggacaag tagactgtag tccaggaata 180

tggcaactag attgtacaca tttagaagga aaaattatcc tggtagcagt tcatgtagcc 240

agtggatata tagaagcaga agttattcca gcagagacag ggcaggaaac agcatacttt 300

ctcttaaaat tagcaggaag atggccagta aaaacaatac atacagacaa tggcagcaat 360

ttcactagta ctacagtcaa ggccgcctgt tggtgggcgg ggatcaagca ggaatttggc 420

attccctaca atccccaaag tcagggagta gtagaatcta taaataaaga attaaagaaa 480

attataggac aggtaagaga tcaggctgaa catcttaaaa cagcagtaca aatggcagta 540

ttcatccaca attttaaaag aaaaggggg attgggggt atagtgcagg ggaaagaata 600

gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt 660

caaaattttc gggtttatta cagggacagc agagatccac tttggaaagg accagcaaag 720

cttctctgga aaggtgaagg ggcagtagta atacaagata atagtgacat aaaagtagta 780

ccaagaagaa aagcaaagat cattagggat tatggaaaac agatggcagg tgatgattgt 840

gtggcaagta gacaggatga ggattag 867

<210> SEQ ID NO 72
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- RD114

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<400> SEQUENCE: 72

atgaaactcc caacaggaat ggtcatttta tgtagcctaa taatagttcg ggcagggttt 60

gacgaccccc gcaaggctat cgcattagta caaaaacaac atggtaaacc atgcgaatgc 120

agcggagggc aggtatccga ggccccaccg aactccatcc aacaggtaac ttgcccaggc 180

aagacggcct acttaatgac caaccaaaaa tggaaatgca gagtcactcc aaaaaatctc 240

acccctagcg ggggagaact ccagaactgc ccctgtaaca ctttccagga ctcgatgcac 300

agttcttgtt atactgaata ccggcaatgc agggcgaata ataagacata ctacacggcc 360

accttgctta aaatacggtc tgggagcctc aacgaggtac agatattaca aaaccccaat 420

cagctcctac agtccccttg taggggctct ataaatcagc ccgtttgctg gagtgccaca 480

gcccccatcc atatctccga tggtggagga cccctcgata ctaagagagt gtggacagtc 540

caaaaaaggc tagaacaaat tcataaggct atgcatcctg aacttcaata ccacccctta 600

gccctgccca aagtcagaga tgaccttagc cttgatgcac ggacttttga tatcctgaat 660

accactttta ggttactcca gatgtccaat tttagccttg cccaagattg ttggctctgt 720

ttaaaactag gtacccctac ccctcttgcg atacccactc cctctttaac ctactcccta 780

gcagactccc tagcgaatgc ctcctgtcag attatacctc ccctcttggt tcaaccgatg 840

cagttctcca actcgtcctg tttatcttcc cctttcatta acgatacgga acaaatagac 900

ttaggtgcag tcacctttac taactgcacc tctgtagcca atgtcagtag tcctttatgt 960

gccctaaacg ggtcagtctt cctctgtgga aataacatgg catacaccta tttaccccaa 1020

aactggacag gactttgcgt ccaagcctcc ctcctccccg acattgacat catcccgggg 1080

gatgagccag tccccattcc tgccattgat cattatatac atagacctaa acgagctgta 1140

cagttcatcc ctttactagc tggactggga atcaccgcag cattcaccac cggagctaca 1200

ggcctaggtg tctccgtcac ccagtataca aaattatccc atcagttaat atctgatgtc 1260

caagtcttat ccggtaccat acaagattta caagaccagg tagactcgtt agctgaagta 1320

gttctccaaa ataggagggg actggaccta ctaacggcag aacaaggagg aatttgttta 1380

gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag aaacaaaata 1440

agaaccctac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa ccctctctgg 1500

accgggctgc agggctttct tccgtacctc ctacctctcc tgggacccct actcaccctc 1560

ctactcatac taaccattgg gccatgcgtt ttcaatcgat tggtccaatt tgttaaagac 1620

aggatctcag tggtccaggc tctggttttg actcagcaat atcaccagct aaaacccata 1680

gagtacgagc catga 1695

<210> SEQ ID NO 73
<211> LENGTH: 2013
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- GALV

<400> SEQUENCE: 73

atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtcctgg gagctggaaa 60

agactgatca tcctcttaag ctgcgtattc ggagacggca aaacgagtct gcagaataag 120

aacccccacc agcctgtgac cctcacctgg caggtactgt cccaaactgg ggacgttgtc 180

tgggacaaaa aggcagtcca gccccttttgg acttggtggc cctctcttac acctgatgta 240

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tgtgccctgg cggccggtct tgagtcctgg gatatcccgg gatccgatgt atcgtcctct 300

aaaagagtta gacctcctga ttcagactat actgccgctt ataagcaaat cacctgggga 360

gccatagggt gcagctaccc tcgggctagg accaggatgg caaattcccc cttctacgtg 420

tgtccccgag ctggccgaac ccattcagaa gctaggaggt gtgggggggct agaatcccta 480

tactgtaaag aatggagttg tgagaccacg ggtaccgttt attggcaacc caagtcctca 540

tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa atttcaaaag 600

tgtgaacaaa ccggctggtg taacccccctc aagatagact tcacagaaaa aggaaaactc 660

tccagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata tggacaccca 720

ggcatacagt tgactatccg cttagaggtc actaacatgc cggttgtggc agtgggccca 780

gaccctgtcc ttgcggaaca gggacctcct agcaagcccc tcactctccc tctctcccca 840

cggaaagcgc cgcccacccc tctacccccg gcggctagtg agcaaacccc tgcggtgcat 900

ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact ctttggcctt 960

gtgcaggggg ccttcctaac cttgaatgct accaacccag gggccactaa gtcttgctgg 1020

ctctgtttgg gcatgagccc cccttattat gaagggatag cctcttcagg agaggtcgct 1080

tatacctcca accatacccg atgccactgg ggggcccaag gaaagcttac cctcactgag 1140

gtctccggac tcgggtcatg catagggaag gtgcctctta cccatcaaca tctttgcaac 1200

cagaccttac ccatcaattc ctctaaaaac catcagtatc tgctcccctc aaaccatagc 1260

tggtgggcct gcagcactgg cctcaccccc tgcctctcca cctcagtttt taatcagtct 1320

aaagacttct gtgtccaggt ccagctgatc ccccgcatct attaccattc tgaagaaacc 1380

ttgttacaag cctatgacaa atcacccccc aggtttaaaa gagagcctgc ctcacttacc 1440

ctagctgtct tcctggggtt agggattgcg gcaggtatag gtactggctc aaccgcccta 1500

attaaagggc ccatagacct ccagcaaggc ctaaccagcc tccaaatcgc cattgacgct 1560

gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac ttccctatct 1620

gaggtagtac tccaaaatag gagaggcctt gacttactat tccttaaaga aggaggcctc 1680

tgcgcggccc taaaagaaga gtgctgtttt tatgtagacc actcaggtgc agtacgagac 1740

tccatgaaaa aacttaaaga aagactagat aaaagacagt tagagcgcca gaaaaaccaa 1800

aactggtatg aagggtggtt caataactcc ccttggttta ctaccctact atcaaccatc 1860

gctgggcccc tattgctcct ccttttgtta ctcactcttg ggccctgcat catcaataaa 1920

ttaatccaat tcatcaatga taggataagt gcagtcaaaa ttttagtcct tagacagaaa 1980

tatcagaccc tagataacga ggaaaaccttaa 2013

<210> SEQ ID NO 74
<211> LENGTH: 1530
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- FUG

<400> SEQUENCE: 74

atggttccgc aggttctttt gtttgtactc cttctgggtt tttcgttgtg tttcgggaag 60

ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga catacaccat 120

ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct gtccgagttc 180

tcctacatgg aactcaaagt gggatacatc tcagccatca aagtgaacgg gttcacttgc 240

acaggtgttg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca 300

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ttcaagagaa agcatttccg ccccacccca gacgcatgta gagccgcgta taactggaag 360

atggccggtg accccagata tgaagagtcc ctacacaatc catacccga ctaccactgg 420

cttcgaactg taagaaccac caaagagtcc ctcattatca tatccccaag tgtgacagat 480

ttggacccat atgacaaatc ccttcactca agggtcttcc ctggcggaaa gtgctcagga 540

ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg gatgcccgag 600

aatccgagac caaggacacc ttgtgacatt tttaccaata gcagagggaa gagagcatcc 660

aacgggaaca agacttgcgg ctttgtggat gaaagaggcc tgtataagtc tctaaaagga 720

gcatgcaggc tcaagttatg tggagttctt ggacttagac ttatggatgg aacatgggtc 780

gcgatgcaaa catcagatga gaccaaatgg tgccctccag atcagttggt gaatttgcac 840

gactttcgct cagacgagat cgagcatctc gttgtggagg agttagttaa gaaaagagag 900

gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt cagacgtctc 960

agtcacctga gaaaacttgt cccagggttt ggaaaagcat ataccatatt caacaaaacc 1020

ttgatggagg ctgatgctca ctacaagtca gtccggacct ggaatgagat catcccctca 1080

aaagggtgtt tgaaagttgg aggaaggtgc catcctcatg tgaacggggt gtttttcaat 1140

ggtataatat tagggcctga cgaccatgtc ctaatcccag agatgcaatc atccctcctc 1200

cagcaacata tggagttgtt ggaatcttca gttatccccc tgatgcaccc cctggcagac 1260

ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt tcacctcccc 1320

gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg aaagtatgta 1380

ttgatgactg caggggccat gattggcctg gtgttgatat tttccctaat gacatggtgc 1440

agagttggta tccatctttg cattaaatta aagcacacca agaaaagaca gatttataca 1500

gacatagaga tgaaccgact tggaaagtaa 1530

<210> SEQ ID NO 75
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- LCMV

<400> SEQUENCE: 75

atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatcgatga ggtgatcaac 60

attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa ttttgccacc 120

tgtgggatat tcgcattgat cagtttccta cttctggctg gcaggtcctg tggcatgtac 180

ggtcttaagg gacccgacat ttacaaagga gtttaccaat ttaagtcagt ggagtttgat 240

atgtcacatc tgaacctgac catgcccaac gcatgttcag ccaacaactc ccaccattac 300

atcagtatgg ggacttctgg actagaattg accttcacca atgattccat catcagtcac 360

aacttttgca atctgacctc tgccttcaac aaaaagacct ttgaccacac actcatgagt 420

atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc agtatcctgc 480

gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg acaaagtgct 540

cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac tgccttcggg 600

gggaaatata tgaggagtgg ctggggctgg acaggctcag atggcaagac cacctggtgt 660

agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa ccactgcaca 720

tatgcaggtc cttttgggat gtccaggatt ctcctttccc aagagaagac taagttcttc 780

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actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg ggtggagaat 840

ccaggtggtt attgcctgac caaatggatg attcttgctg cagagcttaa gtgtttcggg 900

aacacagcag ttgcgaaatg caatgtaaat catgatgccg aattctgtga catgctgcga 960

ctaattgact acaacaaggc tgctttgagt aagttcaaag aggacgtaga atctgccttg 1020

cacttattca aaacaacagt gaattctttg atttcagatc aactactgat gaggaaccac 1080

ttgagagatc tgatgggggt gccatattgc aattactcaa agttttggta cctagaacat 1140

gcaaagaccg gcgaaactag tgtccccaag tgctggcttg tcaccaatgg ttcttactta 1200

aatgagaccc acttcagtga tcaaatcgaa caggaagccg ataacatgat tacagagatg 1260

ttgaggaagg attacataaa gaggcagggg agtacccccc tagcattgat ggaccttctg 1320

atgttttcca catctgcata tctagtcagc atcttcctgc accttgtcaa aataccaaca 1380

cacaggcaca taaaaggtgg ctcatgtcca aagccacacc gattaaccaa caaaggaatt 1440

tgtagttgtg gtgcatttaa ggtgcctggt gtaaaaaccg tctggaaaag acgctga 1497

<210> SEQ ID NO 76
<211> LENGTH: 1692
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- FPV

<400> SEQUENCE: 76

atgaacactc aaatcctggt tttcgccctt gtggcagtca tccccacaaa tgcagacaaa 60

atttgtcttg gacatcatgc tgtatcaaat ggcaccaaag taaacacact cactgagaga 120

ggagtagaag ttgtcaatgc aacggaaaca gtggagcgga caaacatccc caaaatttgc 180

tcaaaaggga aaagaaccac tgatcttggc caatgcggac tgttagggac cattaccgga 240

ccacctcaat gcgaccaatt tctagaattt tcagctgatc taataatcga gagacgagaa 300

ggaaatgatg tttgttaccc ggggaagttt gttaatgaag aggcattgcg acaaatcctc 360

agaggatcag gtgggattga caaagaaaca atgggattca catatagtgg aataaggacc 420

aacggaacaa ctagtgcatg tagaagatca gggtcttcat tctatgcaga aatggagtgg 480

ctcctgtcaa atacagacaa tgctgctttc ccacaaatga caaaatcata caaaaacaca 540

aggagagaat cagctctgat agtctgggga atccaccatt caggatcaac caccgaacag 600

accaaactat atgggagtgg aaataaactg ataacagtcg ggagttccaa atatcatcaa 660

tcttttgtgc cgagtccagg aacacgaccg cagataaatg gccagtccgg acggattgat 720

tttcattggt tgatcttgga tcccaatgat acagttactt ttagtttcaa tggggctttc 780

atagctccaa atcgtgccag cttcttgagg ggaaagtcca tggggatcca gagcgatgtg 840

caggttgatg ccaattgcga aggggaatgc taccacagtg gagggactat aacaagcaga 900

ttgccttttc aaaacatcaa tagcagagca gttggcaaat gcccaagata tgtaaaacag 960

gaaagtttat tattggcaac tgggatgaag aacgttcccg aaccttccaa aaaaaggaaa 1020

aaaagaggcc tgtttggcgc tatagcaggg tttattgaaa atggttggga aggtctggtc 1080

gacgggtggt acggtttcag gcatcagaat gcacaaggag aaggaactgc agcagactac 1140

aaaagcaccc aatcggcaat tgatcagata accggaaagt taaatagact cattgagaaa 1200

accaaccagc aatttgagct aatagataat gaattcactg aggtggaaaa gcagattggc 1260

aatttaatta actggaccaa agactccatc acagaagtat ggtcttacaa tgctgaactt 1320

cttgtggcaa tggaaaacca gcacactatt gatttggctg attcagagat gaacaagctg 1380

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tatgagcgag tgaggaaaca attaagggaa aatgctgaag aggatggcac tggttgcttt 1440

gaaatttttc ataaatgtga cgatgattgt atggctagta taaggaacaa tacttatgat 1500

cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgaccc agtcaaattg 1560

agtagtggct acaaagatgt gatactttgg tttagcttcg gggcatcatg ctttttgctt 1620

cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaaacat gcggtgcact 1680

atttgtatat aa 1692

<210> SEQ ID NO 77
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- RRV

<400> SEQUENCE: 77

agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc acattgcgcc 60

gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag 120

gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc 180

acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga atctaagaga 240

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc 300

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg 360

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag 420

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg 480

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg 540

ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaac 600

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc 660

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca 720

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg 780

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggtaagaag 840

gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag gagtttagga 900

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg 960

acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct gtgggcgcaa 1020

ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta ctattatgga 1080

ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc cctcctaact 1140

ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac accgtacgcc 1200

ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc accgagggcg 1260

aatgca 1266

<210> SEQ ID NO 78
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- MLV 10A1

<400> SEQUENCE: 78

agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc acattgcgcc 60

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gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag 120

gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc 180

acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga atctaagaga 240

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc 300

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg 360

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag 420

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg 480

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg 540

ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaac 600

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc 660

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca 720

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg 780

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggtaagaag 840

gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag gagtttagga 900

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg 960

acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct gtgggcgcaa 1020

ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta ctattatgga 1080

ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc cctcctaact 1140

ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac accgtacgcc 1200

ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc accgagggcg 1260

aatgca 1266

<210> SEQ ID NO 79
<211> LENGTH: 2030
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Ebola

<400> SEQUENCE: 79

atgggtgtta caggaatatt gcagttacct cgtgatcgat tcaagaggac atcattcttt 60

ctttgggtaa ttatcctttt ccaaagaaca ttttccatcc cacttggagt catccacaat 120

agcacattac aggttagtga tgtcgacaaa ctggtttgcc gtgacaaact gtcatccaca 180

aatcaattga gatcagttgg actgaatctc gaagggaatg gagtggcaac tgacgtgcca 240

tctgcaacta aaagatgggg cttcaggtcc ggtgtcccac caaaggtggt caattatgaa 300

gctggtgaat gggctgaaaa ctgctacaat cttgaaatca aaaaacctga cgggagtgag 360

tgtctaccag cagcgccaga cgggattcgg ggcttccccc ggtgccggta tgtgcacaaa 420

gtatcaggaa cgggaccgtg tgccggagac tttgccttcc acaaagaggg tgctttcttc 480

ctgtatgacc gacttgcttc cacagttatc taccgaggaa cgactttcgc tgaaggtgtc 540

gttgcatttc tgatactgcc ccaagctaag aaggacttct tcagctcaca ccccttgaga 600

gagccggtca atgcaacgga ggacccgtct agtggctact attctaccac aattagatat 660

caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga caatttgacc 720

tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa tgagacaata 780

tatacaagtg ggaaaaggag caataccacg ggaaaactaa tttggaaggt caaccccgaa 840

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attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaaacctc actagaaaaa 900

ttcgcagtga agagttgtct ttcacagctg tatcaaacag agccaaaaac atcagtggtc 960

agagtccggc gcgaacttct tccgacccag ggaccaacac aacaactgaa gaccacaaaa 1020

tcatggcttc agaaaattcc tctgcaatgg ttcaagtgca cagtcaagga agggaagctg 1080

cagtgtcgca tctgacaacc cttgccacaa tctccacgag tcctcaaccc cccacaacca 1140

aaccaggtcc ggacaacagc acccacaata cacccgtgta taaacttgac atctctgagg 1200

caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc tccgacactc 1260

cccccgccac gaccgcagcc ggacccctaa aagcagagaa caccaacacg agcaagggta 1320

ccgacctcct ggaccccgcc accacaacaa gtccccaaaa ccacagcgag accgctggca 1380

acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg aagctaggct 1440

taattaccaa tactattgct ggagtcgcag gactgatcac aggcgggagg agagctcgaa 1500

gagaagcaat tgtcaatgct caacccaaat gcaaccctaa tttacattac tggactactc 1560

aggatgaagg tgctgcaatc ggactggcct ggataccata tttcgggcca gcagccgagg 1620

gaatttacat agaggggctg atgcacaatc aagatggttt aatctgtggg ttgagacagc 1680

tggccaacga gacgactcaa gctcttcaac tgttcctgag agccacaacc gagctacgca 1740

ccttttcaat cctcaaccgt aaggcaattg atttcttgct gcagcgatgg ggcggcacat 1800

gccacatttt gggaccggac tgctgtatcg aaccacatga ttggaccaag aacataacag 1860

acaaaattga tcagattatt catgattttg ttgataaaac ccttccggac caggggggaca 1920

atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga gttacaggcg 1980

ttataattgc agttatcgct ttattctgta tatgcaaatt tgtcttttag 2030

<210> SEQ ID NO 80
<211> LENGTH: 389
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Short WPRE sequence

<400> SEQUENCE: 80

aatcaacctc tggattacaa aatttgtgaa agattgactg atattcttaa ctatgttgct 60

ccttttacgc tgtgtggata tgctgcttta atgcctctgt atcatgctat tgcttcccgt 120

acggctttcg ttttctcctc cttgtataaa tcctggttgc tgtctcttta tgaggagttg 180

tggcccgttg tccgtcaacg tggcgtggtg tgctctgtgt ttgctgacgc aacccccact 240

ggctggggca ttgccaccac ctgtcaactc ctttctggga ctttcgcttt ccccctcccg 300

atcgccacgg cagaactcat cgccgcctgc cttgcccgct gctggacagg ggctaggttg 360

ctgggcactg ataattccgt ggtgttgtc 389

<210> SEQ ID NO 81
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 81

taagcagaat tcatgaattt gccaggaaga t 31

<210> SEQ ID NO 82

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<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 82

ccatacaatg aatggacact aggcggccgc acgaat 36

<210> SEQ ID NO 83
<211> LENGTH: 2745
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag, Pol, Integrase fragment

<400> SEQUENCE: 83

gaattcatga atttgccagg aagatggaaa ccaaaaatga tagggggaat tggaggtttt 60

atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa agctataggt 120

acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt gactcagatt 180

ggctgcactt taaattttcc cattagtcct attgagactg taccagtaaa attaaagcca 240

ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat aaaagcatta 300

gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg gcctgaaaat 360

ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg gagaaaatta 420

gtagatttca gagaacttaa taagagaact caagatttct gggaagttca attaggaata 480

ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt gggcgatgca 540

tatttttcag ttcccttaga taaagacttc aggaagtata ctgcatttac catacctagt 600

ataaacaatg agacaccagg gattagatat cagtacaatg tgcttccaca gggatggaaa 660

ggatcaccag caatattcca gtgtagcatg acaaaaatct tagagccttt tagaaaacaa 720

aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggatc tgacttagaa 780

atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag gtggggattt 840

accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg ttatgaactc 900

catcctgata aatggacagt acagcctata gtgctgccag aaaaggacag ctggactgtc 960

aatgacatac agaaattagt gggaaaattg aattgggcaa gtcagattta tgcagggatt 1020

aaagtaaggc aattatgtaa acttcttagg ggaaccaaag cactaacaga agtagtacca 1080

ctaacagaag aagcagagct agaactggca gaaaacaggg agattctaaa agaaccggta 1140

catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa gcaggggcaa 1200

ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac aggaaagtat 1260

gcaagaatga agggtgccca cactaatgat gtgaaacaat taacagaggc agtacaaaaa 1320

atagccacag aaagcatagt aatatgggga aagactccta aatttaaatt acccatacaa 1380

aaggaaacat gggaagcatg gtggacagag tattggcaag ccacctggat tcctgagtgg 1440

gagtttgtca ataccctcc cttagtgaag ttatggtacc agttagagaa agaacccata 1500

ataggagcag aaactttcta tgtagatggg gcagccaata gggaaactaa attaggaaaa 1560

gcaggatatg taactgacag aggaagacaa aaagttgtcc ccctaacgga cacaacaaat 1620

cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt agaagtaaac 1680

atagtgacag actcacaata tgcattggga atcattcaag cacaaccaga taagagtgaa 1740

tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt ctacctggca 1800

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tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt ggtcagtgct 1860

ggaatcagga aagtactatt tttagatgga atagataagg cccaagaaga acatgagaaa 1920

tatcacagta attggagagc aatggctagt gattttaacc taccacctgt agtagcaaaa 1980

gaaatagtag ccagctgtga taaatgtcag ctaaaagggg aagccatgca tggacaagta 2040

gactgtagcc caggaatatg gcagctagat tgtacacatt tagaaggaaa agttatcttg 2100

gtagcagttc atgtagccag tggatatata gaagcagaag taattccagc agagacaggg 2160

caagaaacag catacttcct cttaaaatta gcaggaagat ggccagtaaa aacagtacat 2220

acagacaatg gcagcaattt caccagtact acagttaagg ccgcctgttg gtgggcgggg 2280

atcaagcagg aatttggcat tccctacaat ccccaaagtc aaggagtaat agaatctatg 2340

aataaagaat taaagaaaat tataggacag gtaagagatc aggctgaaca tcttaagaca 2400

gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat tgggggtac 2460

agtgcagggg aaagaatagt agacataata gcaacagaca tacaaactaa agaattacaa 2520

aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag agatccagtt 2580

tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat acaagataat 2640

agtgacataa aagtagtgcc aagaagaaaa gcaaagatca tcagggatta tggaaaacag 2700

atggcaggtg atgattgtgt ggcaagtaga caggatgagg attaa 2745

<210> SEQ ID NO 84
<211> LENGTH: 1586
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA Fragment containing Rev, RRE and rabbit beta globin poly A

<400> SEQUENCE: 84

tctagaatgg caggaagaag cggagacagc gacgaagagc tcatcagaac agtcagactc 60

atcaagcttc tctatcaaag caacccacct cccaatcccg aggggacccg acaggcccga 120

aggaatagaa gaagaaggtg gagagagaga cagagacaga tccattcgat tagtgaacgg 180

atccttggca cttatctggg acgatctgcg gagcctgtgc ctcttcagct accaccgctt 240

gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca gggggtggga 300

agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa agaatagagg 360

agctttgttc cttgggttct tgggagcagc aggaagcact atgggcgcag cgtcaatgac 420

gctgacggta caggccagac aattattgtc tggtatagtg cagcagcaga acaatttgct 480

gagggctatt gaggcgcaac agcatctgtt gcaactcaca gtctggggca tcaagcagct 540

ccaggcaaga atcctggctg tggaaagata cctaaaggat caacagctcc tagatctttt 600

tccctctgcc aaaaattatg gggacatcat gaagcccctt gagcatctga cttctggcta 660

ataaaggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgtc tctcactcgg 720

aaggacatat gggagggcaa atcatttaaa acatcagaat gagtatttgg tttagagttt 780

ggcaacatat gccatatgct ggctgccatg aacaaaggtg gctataaaga ggtcatcagt 840

atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg acttgaggtt 900

agatttttt tatattttgt tttgtgttat ttttttcttt aacatcccta aaattttcct 960

tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc 1020

ctctttctctt atgaagatcc ctcgacctgc agcccaagct tggcgtaatc atggtcatag 1080

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ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc 1140

ataaagtgta aagcctgggg tgcctaatga gtgagctaac tcacattaat tgcgttgcgc 1200

tcactgcccg ctttccagtc gggaaacctg tcgtgccagc ggatccgcat ctcaattagt 1260

cagcaaccat agtcccgccc ctaactccgc ccatcccgcc cctaactccg cccagttccg 1320

cccattctcc gccccatggc tgactaattt tttttattta tgcagaggcc gaggccgcct 1380

cggcctctga gctattccag aagtagtgag gaggcttttt tggaggccta ggcttttgca 1440

aaaagctaac ttgtttattg cagcttataa tggttacaaa taaagcaata gcatcacaaa 1500

tttcacaaat aaagcatttt tttcactgca ttctagttgt ggtttgtcca aactcatcaa 1560

tgtatcttat cagcggccgc cccggg 1586

<210> SEQ ID NO 85
<211> LENGTH: 1614
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA fragment containing the CAG
enhancer/promoter/intron sequence

<400> SEQUENCE: 85

acgcgttagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga 60

gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca acgacccccg 120

cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg 180

acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca 240

tatgccaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgcct ggcattatgc 300

ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat tagtcatcgc 360

tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc tccccccccc 420

ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg atgggggcgg 480

gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg cggggcgagg 540

cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc ttttatggcg 600

aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg agtcgctgcg 660

ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg gctctgactg 720

accgcgttac tcccacaggt gagcgggcgg gacggcccct ctcctccggg ctgtaattag 780

cgcttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct taaagggctc 840

cgggagggcc ctttgtgcgg gggggagcgg ctcgggggggt gcgtgcgtgt gtgtgtgcgt 900

ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgctgcgg gcgcggcgcg 960

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agggggtgtg ggcgcggcgg tcgggctgta acccccccct gcacccccct ccccgagttg 1140

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tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg cctcgggccg 1260

gggagggctc gggggagggg cgcggcggcc ccggagcgcc ggcggctgtc gaggcgcggc 1320

gagccgcagc cattgccttt tatggtaatc gtgcgagagg gcgcagggac ttcctttgtc 1380

ccaaatctgg cggagccgaa atctgggagg cgccgccgca ccccctctag cgggcgcggg 1440

cgaagcggtg cggcgccggc aggaaggaaa tgggcgggga gggccttcgt gcgtcgccgc 1500

-continued

gccgccgtcc ccttctccat ctccagcctc ggggctgccg caggggacg gctgccttcg 1560

gggggacgg ggcagggcgg ggttcggctt ctggcgtgtg accggcggga attc 1614

<210> SEQ ID NO 86
<211> LENGTH: 1531
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA fragment containing VSV-G

<400> SEQUENCE: 86

gaattcatga agtgcctttt gtacttagcc tttttattca ttggggtgaa ttgcaagttc 60

accatagttt ttccacacaa ccaaaaagga aactggaaaa atgttccttc taattaccat 120

tattgcccgt caagctcaga tttaaattgg cataatgact taataggcac agccttacaa 180

gtcaaaatgc ccaagagtca caaggctatt caagcagacg gttggatgtg tcatgcttcc 240

aaatgggtca ctacttgtga tttccgctgg tatggaccga agtatataac acattccatc 300

cgatccttca ctccatctgt agaacaatgc aaggaaagca ttgaacaaac gaaacaagga 360

acttggctga atccaggctt ccctcctcaa agttgtggat atgcaactgt gacggatgcc 420

gaagcagtga ttgtccaggt gactcctcac catgtgctgg ttgatgaata cacaggagaa 480

tgggttgatt cacagttcat caacggaaaa tgcagcaatt acatatgccc cactgtccat 540

aactctacaa cctggcattc tgactataag gtcaaagggc tatgtgattc taacctcatt 600

tccatggaca tcaccttctt ctcagaggac ggagagctat catccctggg aaaggagggc 660

acagggttca gaagtaacta ctttgcttat gaaactggag gcaaggcctg caaaatgcaa 720

tactgcaagc attggggagt cagactccca tcaggtgtct ggttcgagat ggctgataag 780

gatctctttg ctgcagccag attccctgaa tgcccagaag ggtcaagtat ctctgctcca 840

tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt ggattattcc 900

ctctgccaag aaacctggag caaaatcaga gcgggtcttc caatctctcc agtggatctc 960

agctatcttg ctcctaaaaa cccaggaacc ggtcctgctt tcaccataat caatggtacc 1020

ctaaaatact ttgagaccag atacatcaga gtcgatattg ctgctccaat cctctcaaga 1080

atggtcggaa tgatcagtgg aactaccaca gaaagggaac tgtgggatga ctgggcacca 1140

tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg atataagttt 1200

cctttataca tgattggaca tggtatgttg gactccgatc ttcatcttag ctcaaaggct 1260

caggtgttcg aacatcctca cattcaagac gctgcttcgc aacttcctga tgatgagagt 1320

ttatttttttg gtgatactgg gctatccaaa aatccaatcg agcttgtaga aggttggttc 1380

agtagttgga aaagctctat tgcctctttt ttctttatca tagggttaat cattggacta 1440

ttcttggttc tccgagttgg tatccatctt tgcattaaat taaagcacac caagaaaaga 1500

cagatttata cagacataga gatgagaatt c 1531

<210> SEQ ID NO 87
<211> LENGTH: 1227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper plasmid containing RRE and rabbit beta
globin poly A

<400> SEQUENCE: 87

tctagaagga gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc 60

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gtcaatgacg ctgacggtac aggccagaca attattgtct ggtatagtgc agcagcagaa 120

caatttgctg agggctattg aggcgcaaca gcatctgttg caactcacag tctggggcat 180

caagcagctc caggcaagaa tcctggctgt ggaaagatac ctaaaggatc aacagctcct 240

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 300

ttctggctaa taaaggaaat ttatttttcat tgcaatagtg tgttggaatt ttttgtgtct 360

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 420

ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg ctataaagag 480

gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga aaagccttga 540

cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 600

aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta ctcccagtca 660

tagctgtccc tcttctctta tgaagatccc tcgacctgca gcccaagctt ggcgtaatca 720

tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga 780

gccggaagca taaagtgtaa agcctggggt gcctaatgag tgagctaact cacattaatt 840

gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagcg gatccgcatc 900

tcaattagtc agcaaccata gtcccgcccc taactccgcc catcccgccc ctaactccgc 960

ccagttccgc ccattctccg ccccatggct gactaatttt ttttatttat gcagaggccg 1020

aggccgcctc ggcctctgag ctattccaga agtagtgagg aggcttttt ggaggcctag 1080

gcttttgcaa aaagctaact tgtttattgc agcttataat ggttacaaat aaagcaatag 1140

catcacaaat ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa 1200

actcatcaat gtatcttatc acccggg 1227

<210> SEQ ID NO 88
<211> LENGTH: 884
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: RSV promoter and HIV Rev

<400> SEQUENCE: 88

caattgcgat gtacgggcca gatatacgcg tatctgaggg gactagggtg tgtttaggcg 60

aaaagcgggg cttcggttgt acgcggttag gagtcccctc aggatatagt agtttcgctt 120

ttgcataggg agggggaaat gtagtcttat gcaatacact tgtagtcttg caacatggta 180

acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg ccgattggtg 240

gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct gacatggatt 300

ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct agctcgatac 360

aataaacgcc atttgaccat tcaccacatt ggtgtgcacc tccaagctcg agctcgttta 420

gtgaaccgtc agatcgcctg gagacgccat ccacgctgtt ttgacctcca tagaagacac 480

cgggaccgat ccagcctccc ctcgaagcta gcgattaggc atctcctatg gcaggaagaa 540

gcggagacag cgacgaagaa ctcctcaagg cagtcagact catcaagttt ctctatcaaa 600

gcaacccacc tcccaatccc gaggggaccc gacaggcccg aaggaataga agaagaaggt 660

ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg 720

gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt 780

gtaacgagga ttgtggaact tctgggacgc agggggtggg aagccctcaa atattggtgg 840

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aatctcctac aatattggag tcaggagcta aagaatagtc taga 884

<210> SEQ ID NO 89
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Target sequence

<400> SEQUENCE: 89

atggcaggaa gaagcggag 19

<210> SEQ ID NO 90
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 90

atggcaggaa gaagcggagt tcaagagact ccgcttcttc ctgccatttt tt 52

<210> SEQ ID NO 91
<211> LENGTH: 279
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H1 promoter and shRT sequence

<400> SEQUENCE: 91

gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa 60

cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accacttgga tccgcggaga cagcgacgaa 240

gagcttcaag agagctcttc gtcgctgtct ccgcttttt 279

<210> SEQ ID NO 92
<211> LENGTH: 275
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H1 CCR5 sequence

<400> SEQUENCE: 92

gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa 60

cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accacttgga tccgtgtcaa gtccaatcta 240

tgttcaagag acatagattg gacttgacac ttttt 275

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 93

aggaattgat ggcgagaagg 20

-continued

<210> SEQ ID NO 94
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 94

ccccaaagaa ggtcaaggta atca 24

<210> SEQ ID NO 95
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 95

agcgcggcta cagcttca 18

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: n = p

<400> SEQUENCE: 96

ggcgacgtag cacagcttcn 20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AGT103 CCR5 miR30

<400> SEQUENCE: 97

tgtaaactga gcttgctcta 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AGT103-R5-1

<400> SEQUENCE: 98

tgtaaactga gcttgctcgc 20

<210> SEQ ID NO 99
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AGT103-R5-2

<400> SEQUENCE: 99

catagattgg acttgacac 19

<210> SEQ ID NO 100
<211> LENGTH: 642
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CAG promoter

<400> SEQUENCE: 100

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cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 300

catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac 360

catgggtcga ggtgagcccc acgttctgct tcactctccc catctccccc ccctccccac 420

ccccaatttt gtatttattt atttttaat tattttgtgc agcgatgggg gcgggggggg 480

gggggcgcg cgccaggcgg ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga 540

ggtgcggcgg cagccaatca gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg 600

cggcggcggc ggccctataa aaagcgaagc gcgcggcggg cg 642

<210> SEQ ID NO 101
<211> LENGTH: 217
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H1 element

<400> SEQUENCE: 101

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cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accactt 217

<210> SEQ ID NO 102
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3' LTR

<400> SEQUENCE: 102

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tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta 120

agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact 180

ctggtaacta gagatccctc agacccttttt agtcagtgtg gaaaatctct agcagtagta 240

gttcatgtca 250

<210> SEQ ID NO 103
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 7SK promoter

<400> SEQUENCE: 103

ctgcagtatt tagcatgccc cacccatctg caaggcattc tggatagtgt caaaacagcc 60

ggaaatcaag tccgtttatc tcaaacttta gcattttggg aataaatgat atttgctatg 120

-continued

ctggttaaat tagattttag ttaaatttcc tgctgaagct ctagtacgat aagcaacttg 180

acctaagtgt aaagttgaga tttccttcag gtttatatag cttgtgcgcc gcctggctac 240

ctc 243

<210> SEQ ID NO 104
<211> LENGTH: 132
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 Tat

<400> SEQUENCE: 104

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tgactgaccc tatggggaag aagcggacag gacacaaggc ctgttactag cactcacatg 120

gaacaaatgg cc 132

What is claimed is:

1. A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO: 31.

2. The lentiviral vector of claim **1**, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO: 31.

3. A lentiviral particle produced by a packaging cell and capable of infecting a target cell, the lentiviral particle comprising:

a. an envelope protein capable of infecting the target cell; and

b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO: 31.

4. The lentiviral particle of claim **3**, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO: 31.

5. The lentiviral particle of claim **3**, wherein the target cell is a CD4+ T cell.

6. A modified cell comprising a primary T cell infected with a lentiviral particle, wherein the lentiviral particle comprises:

a. an envelope protein capable of infecting the target cell; and

b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO: 31.

7. The modified cell of claim **6**, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO: 31.

8. The modified cell of claim **6**, wherein the primary T cell is a primary CD4+ T cell.

9. A method of treating cells infected with HIV, the method comprising:

a. contacting or having contacted peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of an ex vivo stimulatory agent, wherein the contacting is conducted ex vivo;

b. transducing or having transduced the PBMC ex vivo with a lentiviral particle, wherein the lentiviral particle comprises:

i. an envelope protein capable of infecting the PBMC; and

ii. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO: 31; and

c. culturing or having cultured the transduced PBMC for at least about 1 day.

10. The method of claim **9**, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO: 31.

11. The method of claim **9**, further comprising infusing or having infused the transduced PBMC into a subject.

12. The method of claim **9**, further comprising positively selecting or having positively selected HIV-specific CD4+ T cells from the PBMC.

13. The method of claim **9**, further comprising immunizing or having immunized the subject with an effective amount of an in vivo stimulatory agent, wherein the immunization occurs prior to contacting the peripheral blood mononuclear cells (PMBC) with the ex vivo stimulatory agent.

14. The method of claim **13**, wherein each of the in vivo stimulatory agent and ex vivo stimulatory agent is independently selected from a peptide and a vaccine.

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