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(54) **VECTOR SYSTEM**

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(73) Assignee: **Oxford Biomedica (UK) Limited**

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(30) **Foreign Application Priority Data**

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(52) **U.S. Cl.** **424/93.2**; 435/456; 977/802

(57) **ABSTRACT**

The present invention relates to retroviral vector genomes and to vector systems comprising such genomes. In particular the present invention relates to a retroviral vector genome comprising two or more NOIs operably linked by one or more Internal Ribosome Entry Site(s); a lentiviral vector genome comprising two or more NOIs suitable for treating a neurodegenerative disorder; and a lentiviral vector genome which encodes tyrosine hydroxylase, GTP-cyclohydrolase I and optionally Aromatic Amino Acid Dopa Decarboxylase.

Figure 1

5'hTH2

5'-GC GGATCC *GCC ACC* **ATG GAA AAA CTC ATC TCA GAA GAG GAT**
BamH I

CTG CCC ACCCCC GAC GCC ACC ACG –3'

3'hTH2

5'- GAA CCG CGG GGA CTG CCC TCT TAC C- 3'

5'hTH3

5'-GGT AAA GAG GGC AGT CCC CGC GGT TC- 3'

3'-hTH1

5'- CG AAGCTT CTA GCC AATGGC ACT CAG CGC ATG GGC-3'
HindIII

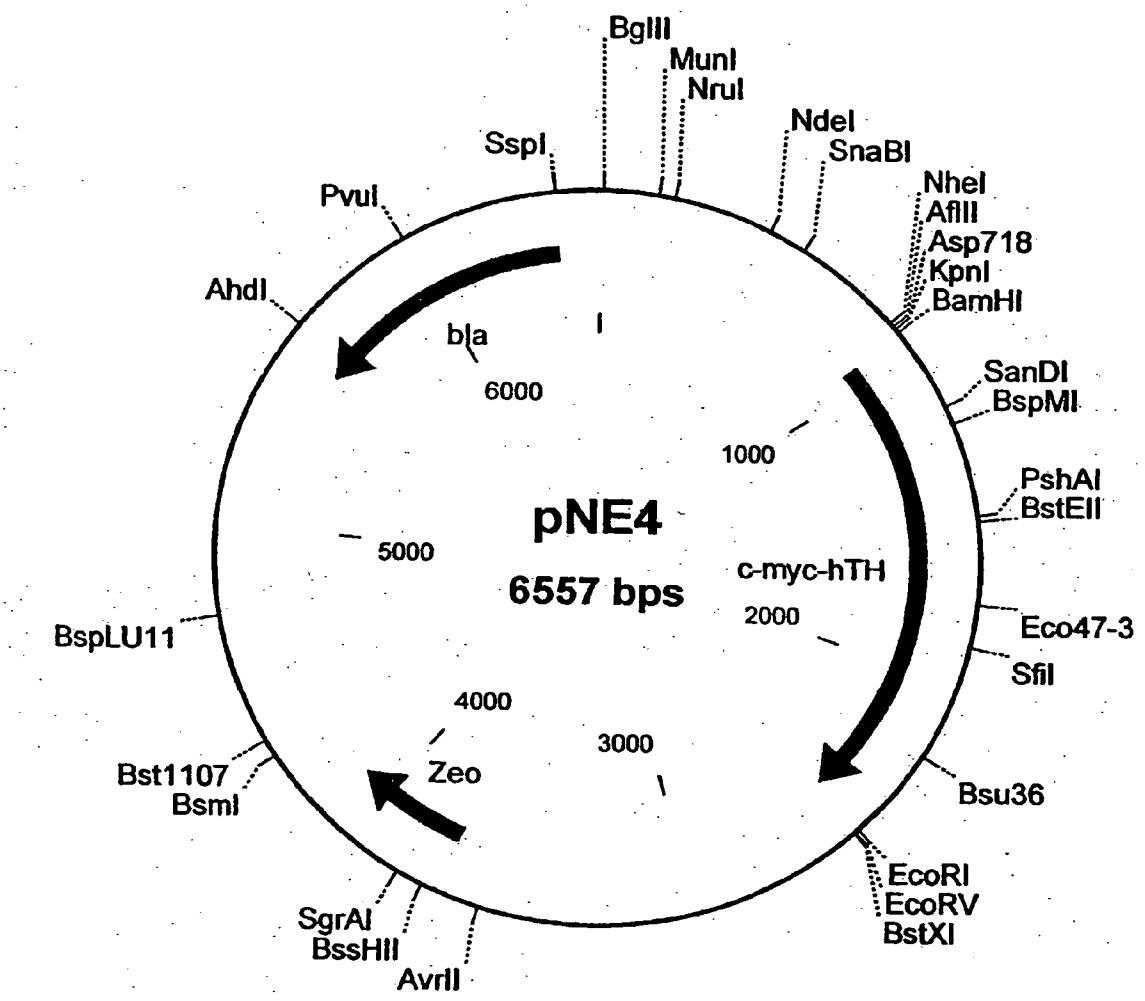

BglII
MunI
NruI
NdeI
SnaBI
NheI
AflII
Asp718
KpnI
BamHI
SanDI
BspMI
PshAI
BstEII
Eco47-3
SfiI
Bsu36
EcoRI
EcoRV
BstXI
AvrII
SgrAI
BssHII
Bst1107
BsmI
BspLU11
AhdI
PvuI
SspI
bla
Zeo
c-myc-hTH
pNE4
6557 bps
1000
2000
3000
4000
5000
6000

Figure 2

Figure 3

5'hAADC

5'- CG AGA TCT *GCC ACC* **ATG TAC CCC TAC GAC GTG CCC GAC TAC**
Bgl II

GCC AAC GCA AGT GAA TTC CGA AGG-3'

3'hAADC

5'- CG AAG CTT CTA CTC CCT CTC TGC TCG C-3'
HindIII

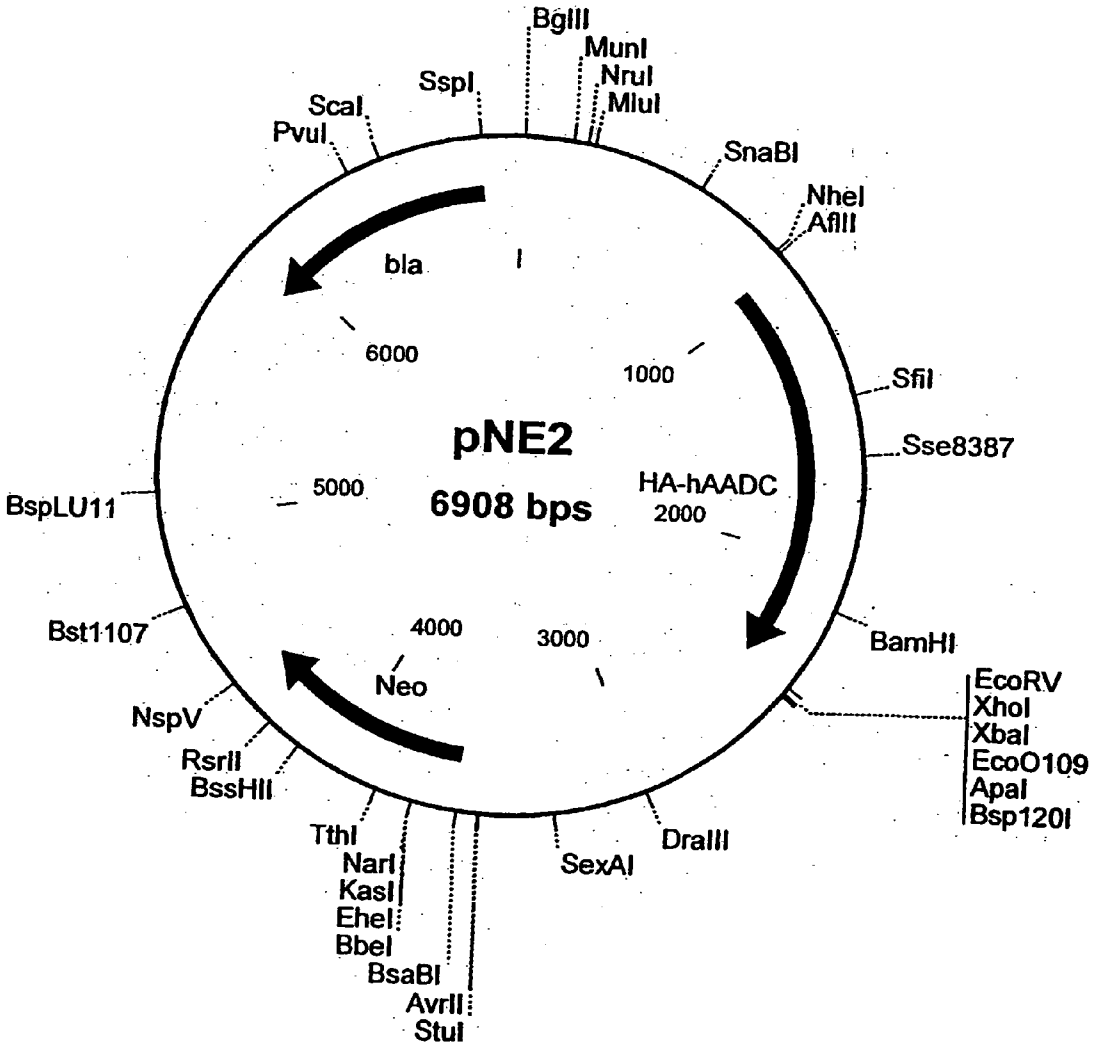

pNE2
6908 bps
bla
Neo
HA-hAADC
1000
2000
3000
4000
5000
6000
BglII
MunI
NruI
MluI
SnaBI
NheI
AflII
SfiI
Sse8387
BamHI
EcoRV
XhoI
XbaI
EcoO109
ApaI
Bsp120I
DraIII
SexAI
TthI
NarI
KasI
EheI
BbeI
BsaBI
AvrII
StuI
NspV
RsrII
BssHII
Bst1107
BspLU11
PvuI
ScaI
SspI

Figure 4

Figure 5

5'hGTP

5'- CG AGA TCT *GCC ACC* **ATG GAC TAC AAG GAC GAC GAT GAC** GAG
Bgl II

AAG GGC CCT GTG CGG CG-3'

3'hGTP

5'- CG AAG CTT TCA GCT CCT AAT GAG AGT CAG GAA-3'
HindIII

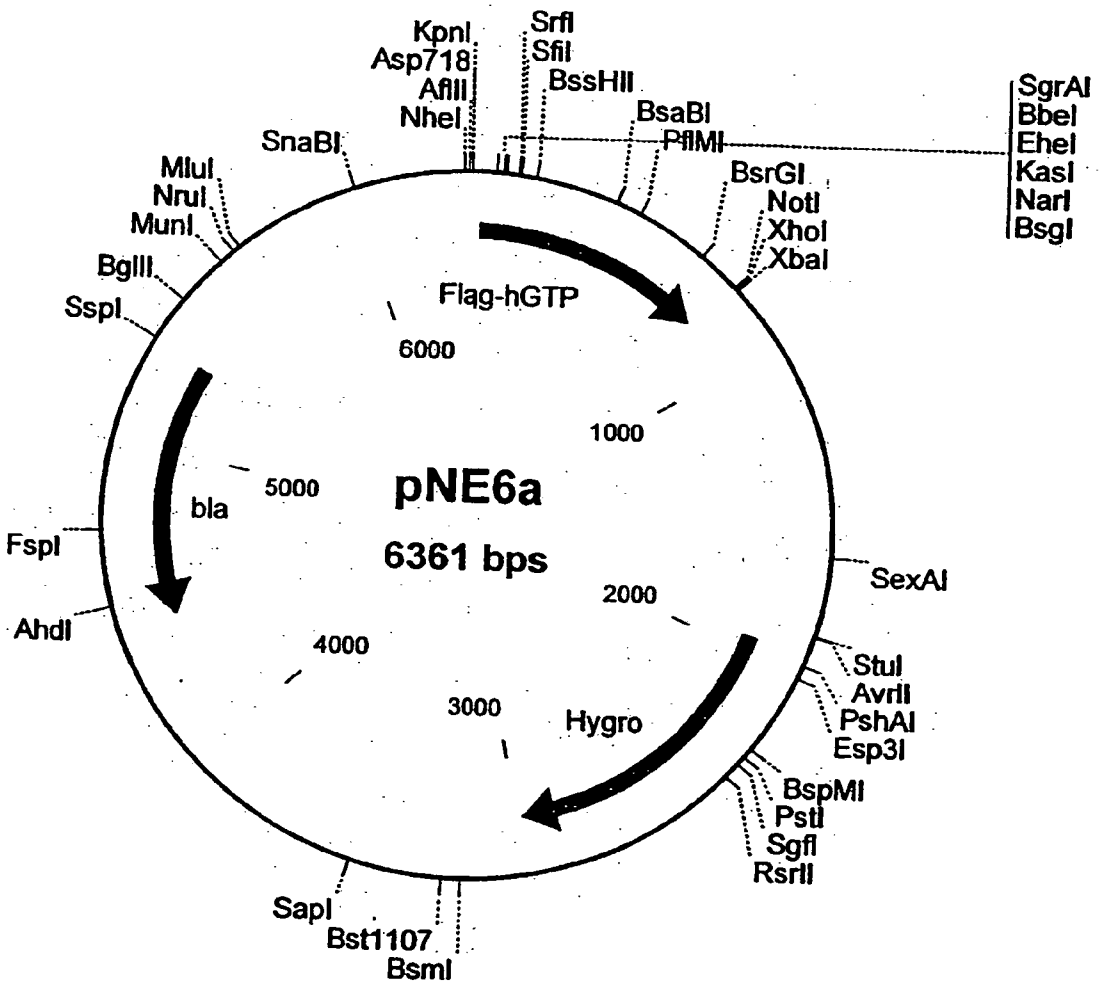

pNE6a
6361 bps
Flag-hGTP
Hygro
bla
1000
2000
3000
4000
5000
6000
KpnI
Asp718
AflII
NheI
SrfI
SfiI
BssHII
BsaBI
PflMI
SgrAI
BbeI
EheI
KasI
NarI
BsgI
BsrGI
NotI
XhoI
XbaI
SexAI
StuI
AvrII
PshAI
Esp3I
BspMI
PstI
SgfI
RsrII
SapI
Bst1107
BsmI
AhdI
FspI
SspI
BglII
MunI
NruI
MluI
SnaBI

Figure 6

Figure 7

5'hTHt

5'-CG AAG CTT GGA TCC *GCC ACC* **ATG GAA CAA AAA CTC ATC TCA**
HindIII BamHI

GAA GAG GAT CTG AAG GTC CCC TGG TTC CCA AGA AAA-3'

3'hTHt

5'- CG GAA TTC CTA GCC AAT GGC ACT CAG CGC ATG GGC-3'
EcoRI

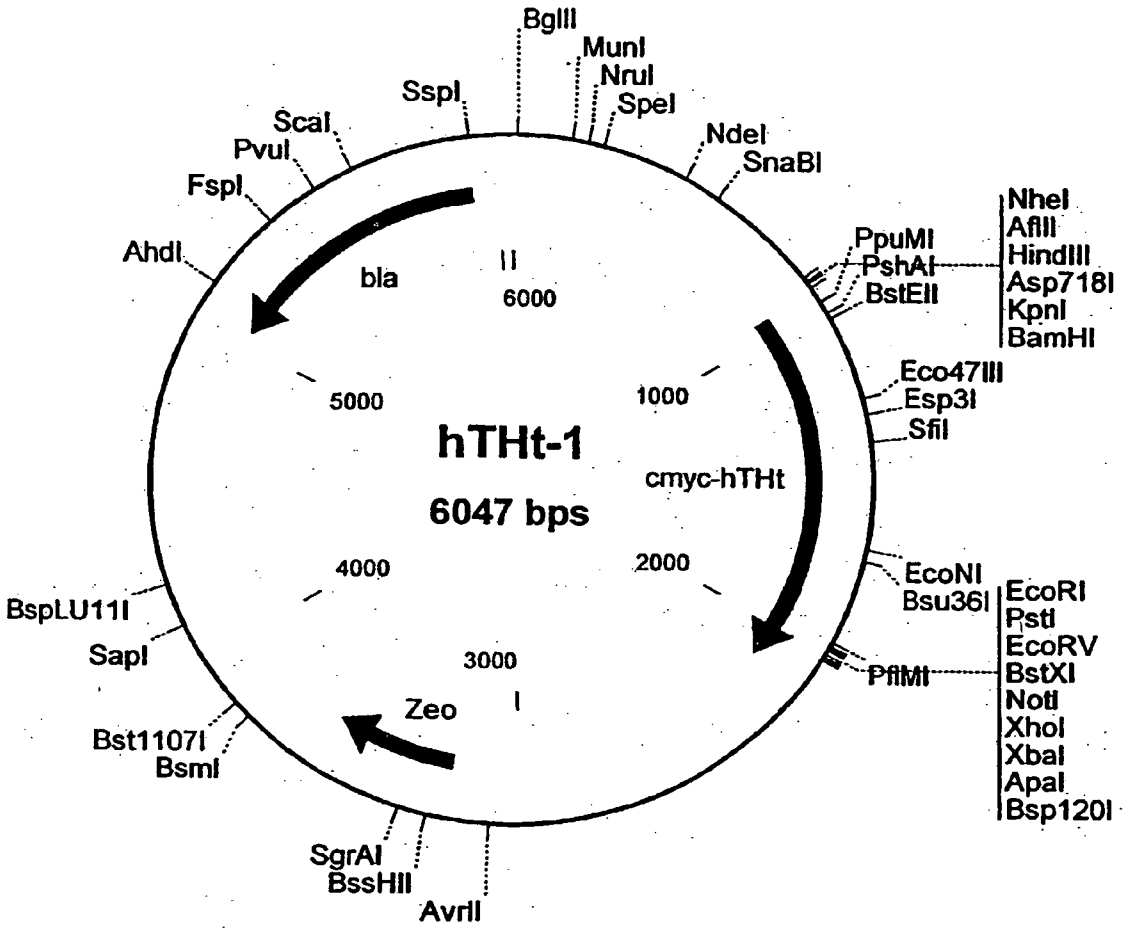

BglII
MunI
NruI
SpeI
NdeI
SnaBI
SspI
ScaI
PvuI
FspI
AhdI
bla
6000
5000
1000
hTHt-1
6047 bps
cmyc-hTHt
4000
2000
3000
Zeo
NheI
AflII
HindIII
Asp718I
KpnI
BamHI
PpuMI
PshAI
BstEII
Eco47III
Esp3I
SfiI
EcoNI
Bsu36I
EcoRI
PstI
EcoRV
BstXI
NotI
XhoI
XbaI
ApaI
Bsp120I
PflMI
BspLU11I
SapI
Bst1107I
BsmI
SgrAI
BssHII
AvrII

Figure 8

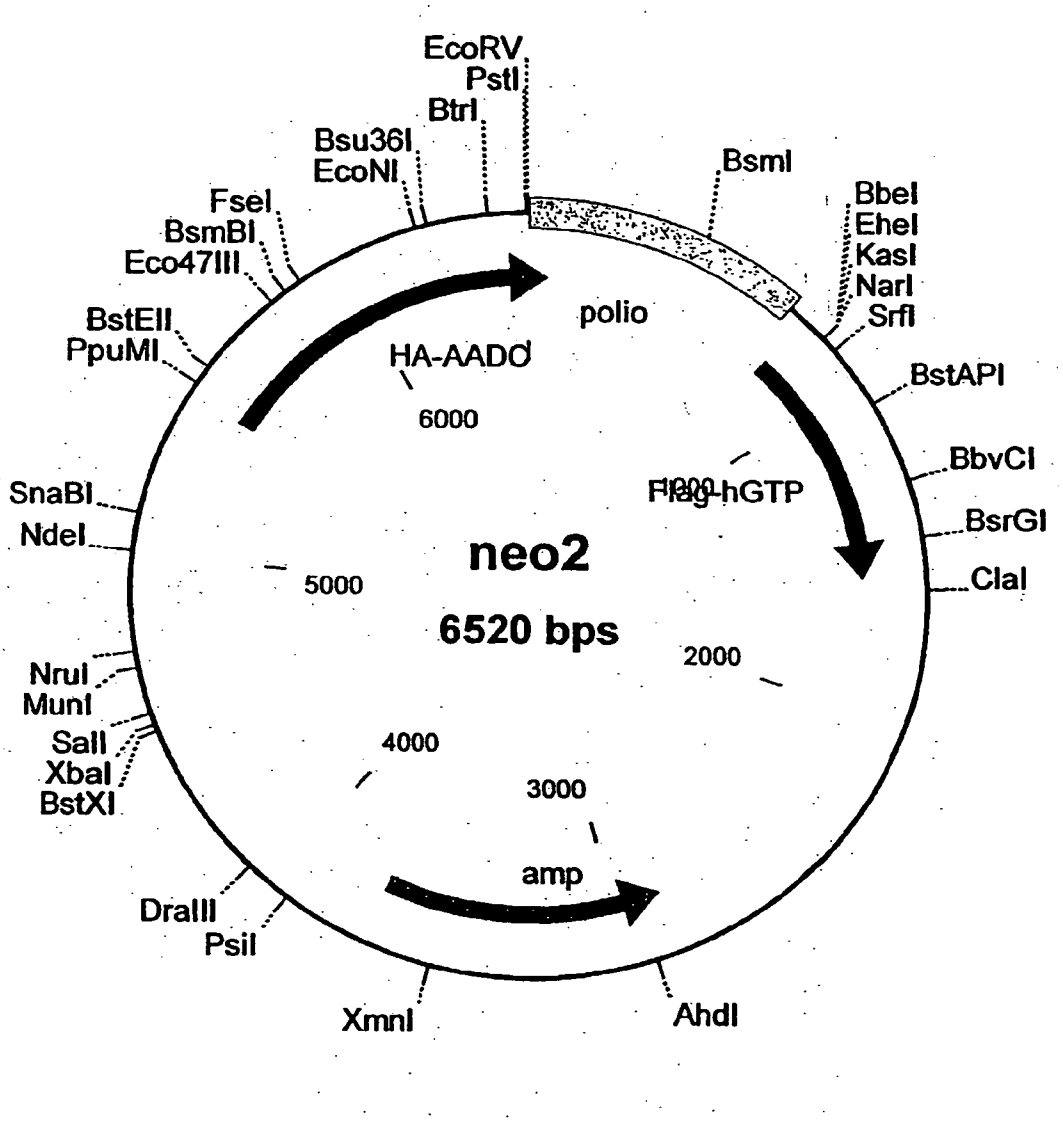

EcoRV
PstI
BtrI
Bsu36I
EcoNI
FseI
BsmBI
Eco47III
BstEII
PpuMI
SnaBI
NdeI
NruI
MunI
SalI
XbaI
BstXI
DraIII
PsiI
XmnI
AhdI
BsmI
BbeI
EheI
KasI
NarI
SrfI
BstAPI
BbvCI
BsrGI
ClaI
polio
HA-AADO
6000
Flag-hGTP
neo2
6520 bps
5000
2000
4000
3000
amp

Figure 9

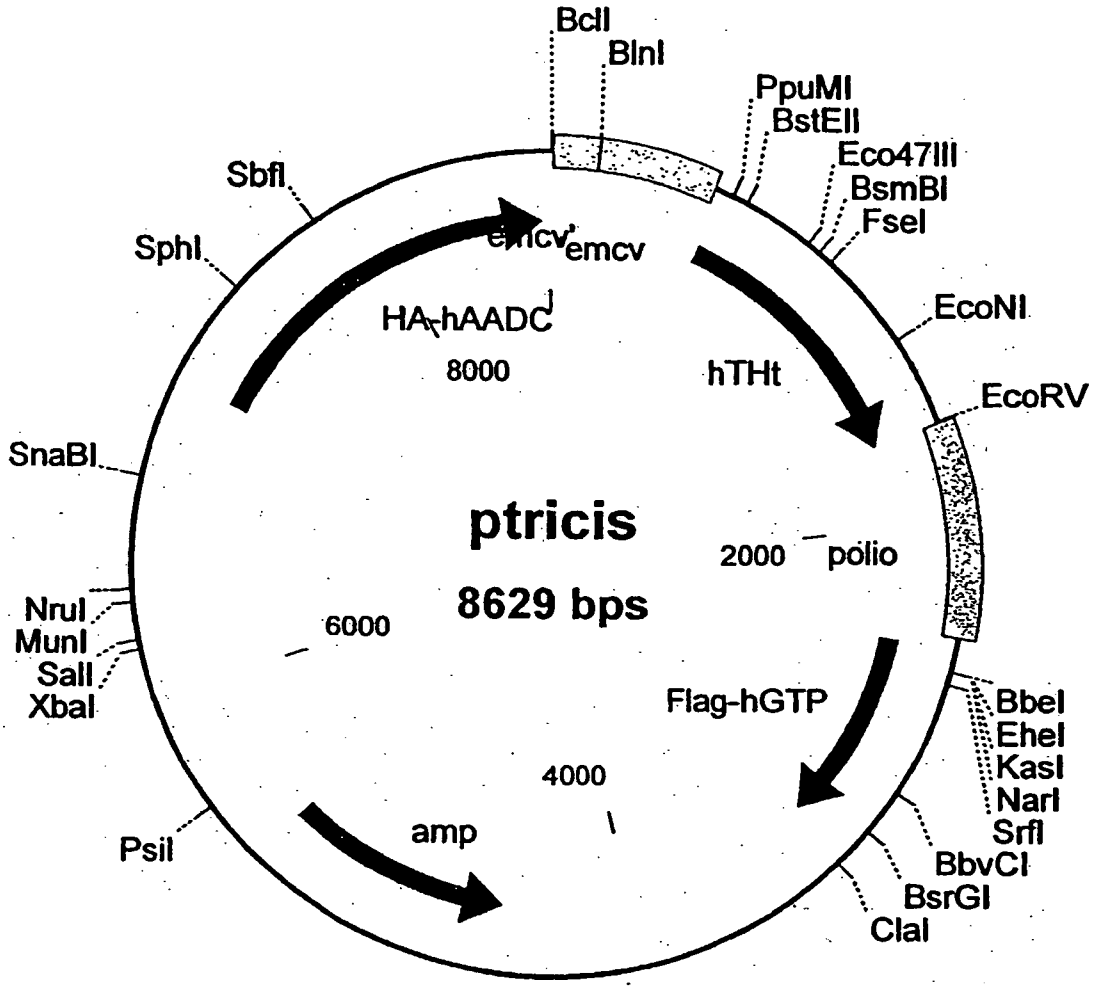

BclI
BlnI
PpuMI
BstEII
Eco47III
BsmBI
FseI
EcoNI
EcoRV
BbeI
EheI
KasI
NarI
SrfI
BbvCI
BsrGI
ClaI
PsiI
SnaBI
NruI
MunI
SalI
XbaI
SbfI
SphI
emcv
HA-hAADC
8000
hTHt
ptricis
8629 bps
2000
polio
6000
Flag-hGTP
4000
amp

Figure 10

Figure 11

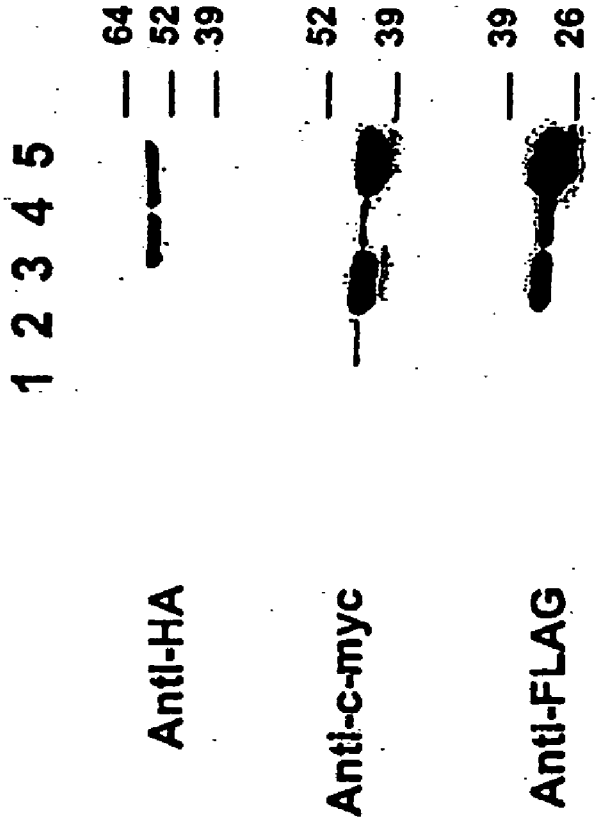


Figure 12

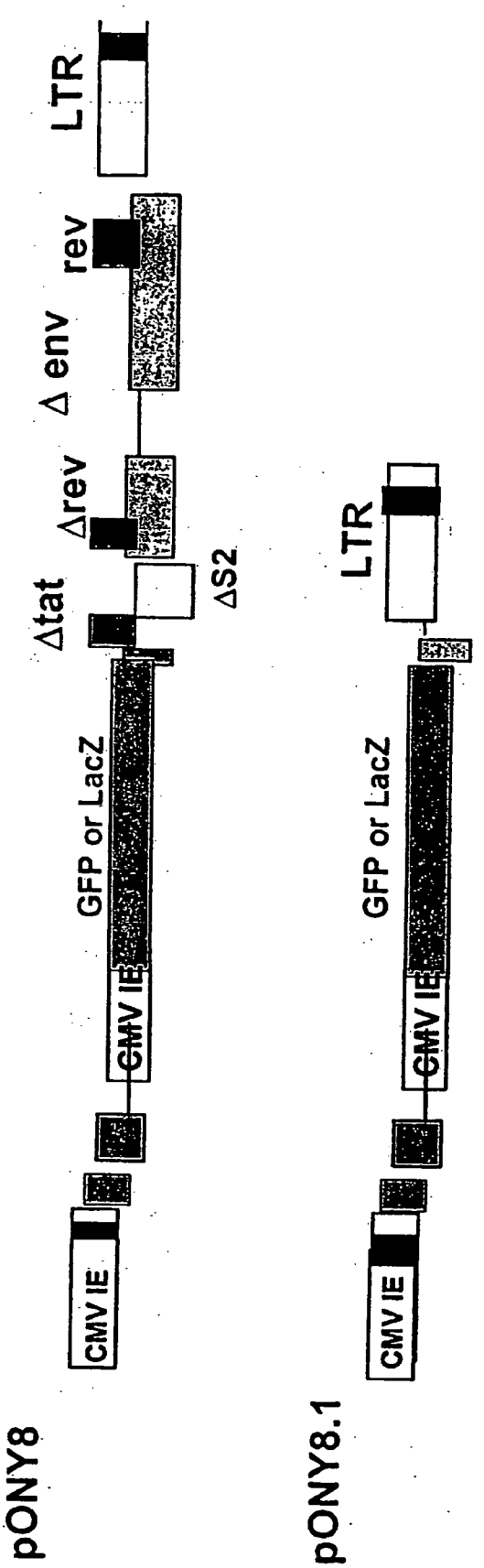

LTR
rev
Δ env
Δrev
Δtat
ΔS2
GFP or LacZ
CMV IE
CMV IE
pONY8
LTR
GFP or LacZ
CMV IE
CMV IE
pONY8.1

Figure 13

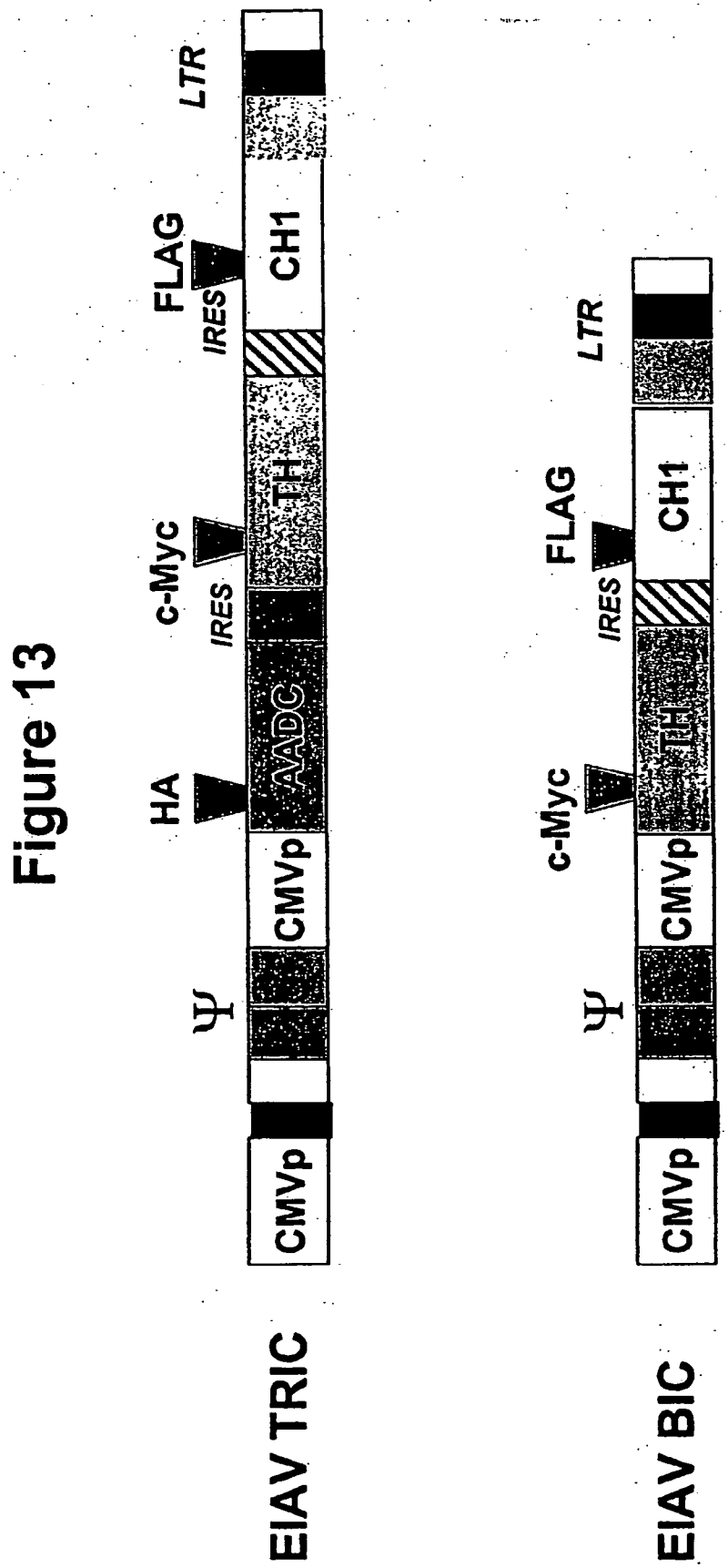

CMVp
Ψ
CMVp
HA
AADC
c-Myc
IRES
TH
FLAG
IRES
CH1
LTR
EIAV TRIC
CMVp
Ψ
CMVp
c-Myc
TH
FLAG
IRES
CH1
LTR
EIAV BIC

Figure 14

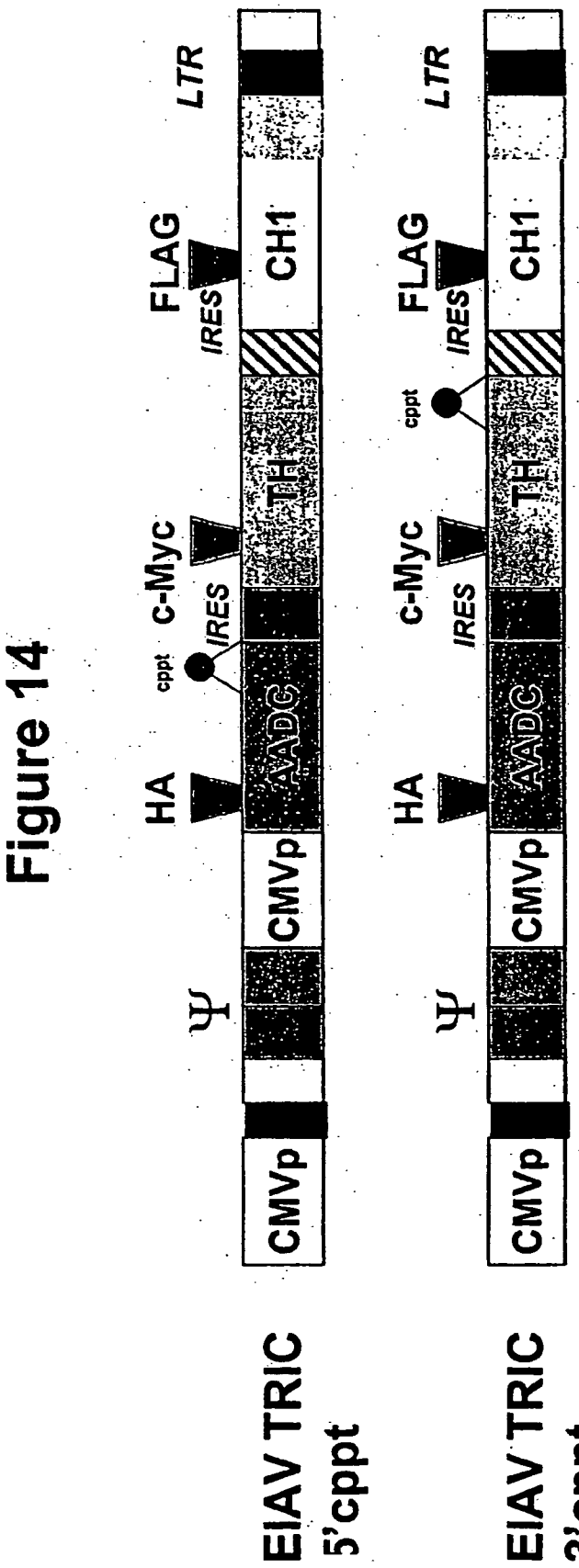

EIAV TRIC 5'cppt
CMVp
Ψ
HA
AADC
cppt
c-Myc
IRES
TH
FLAG
IRES
CH1
LTR
EIAV TRIC 3'cppt
CMVp
Ψ
HA
AADC
c-Myc
IRES
cppt
TH
FLAG
IRES
CH1
LTR

Figure 15

Comparison of Lentiviral preps by PERT/RNA ratio

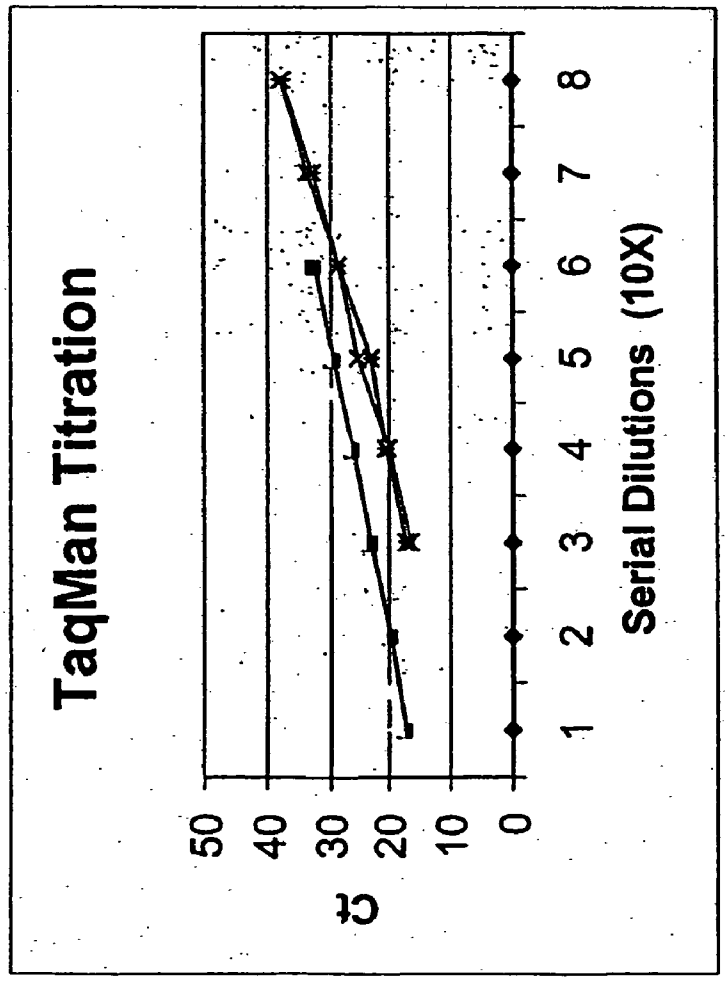


Figure 16

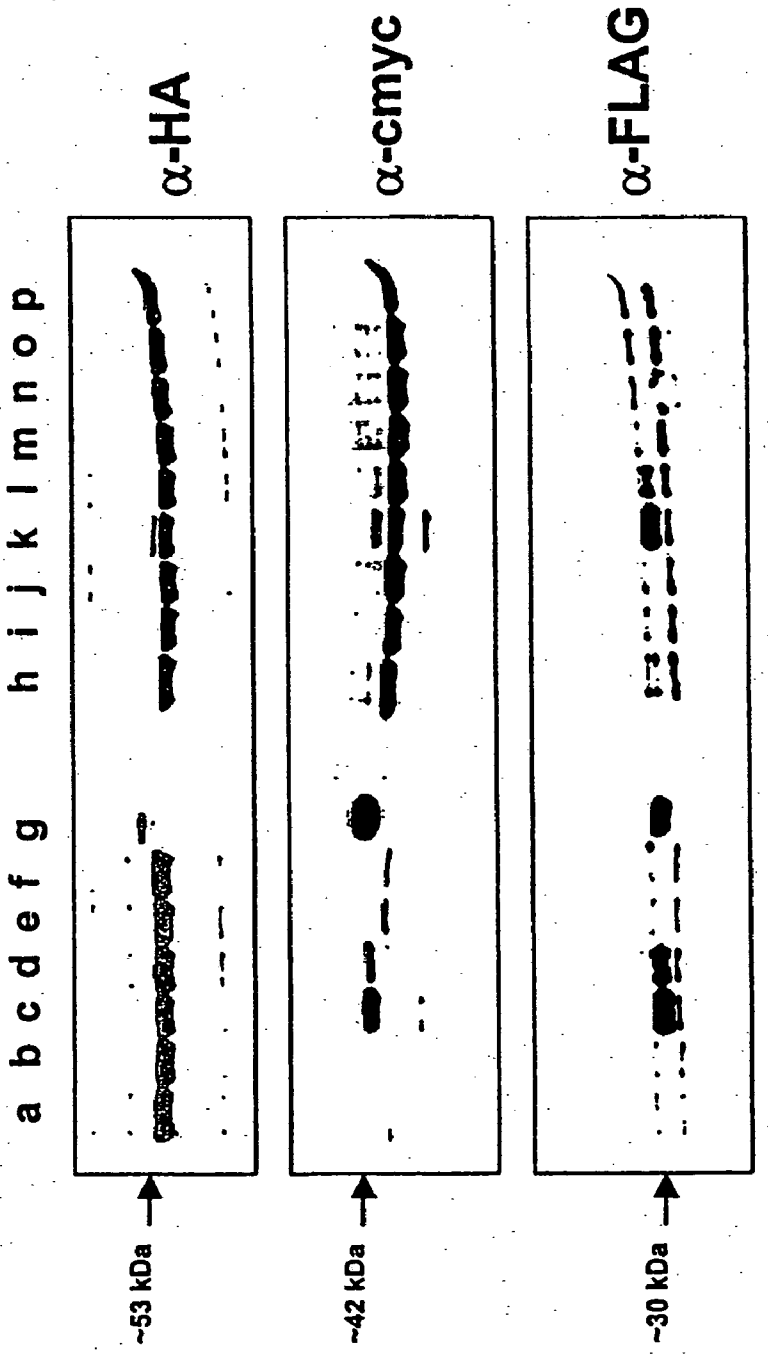


Figure 17

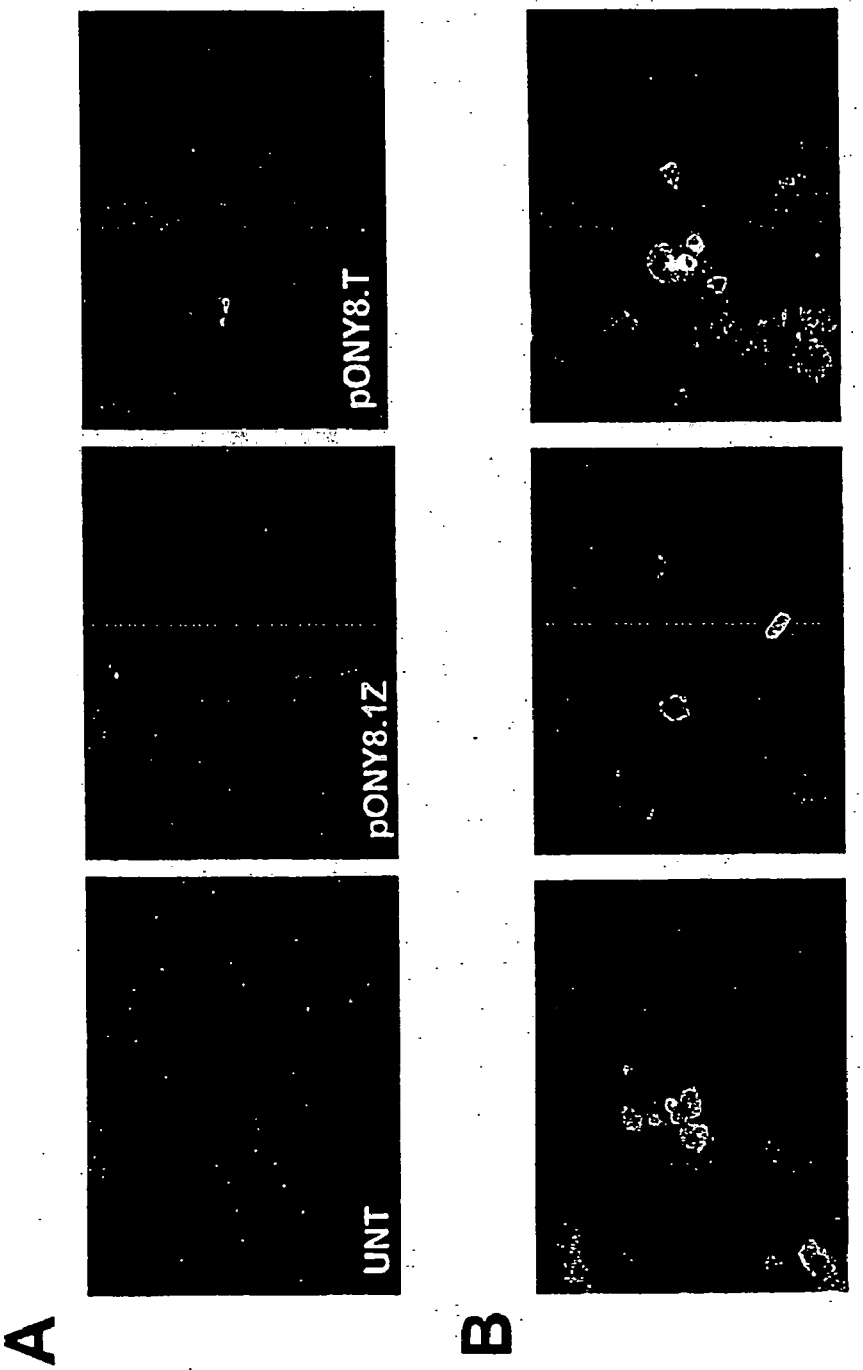


Figure 18

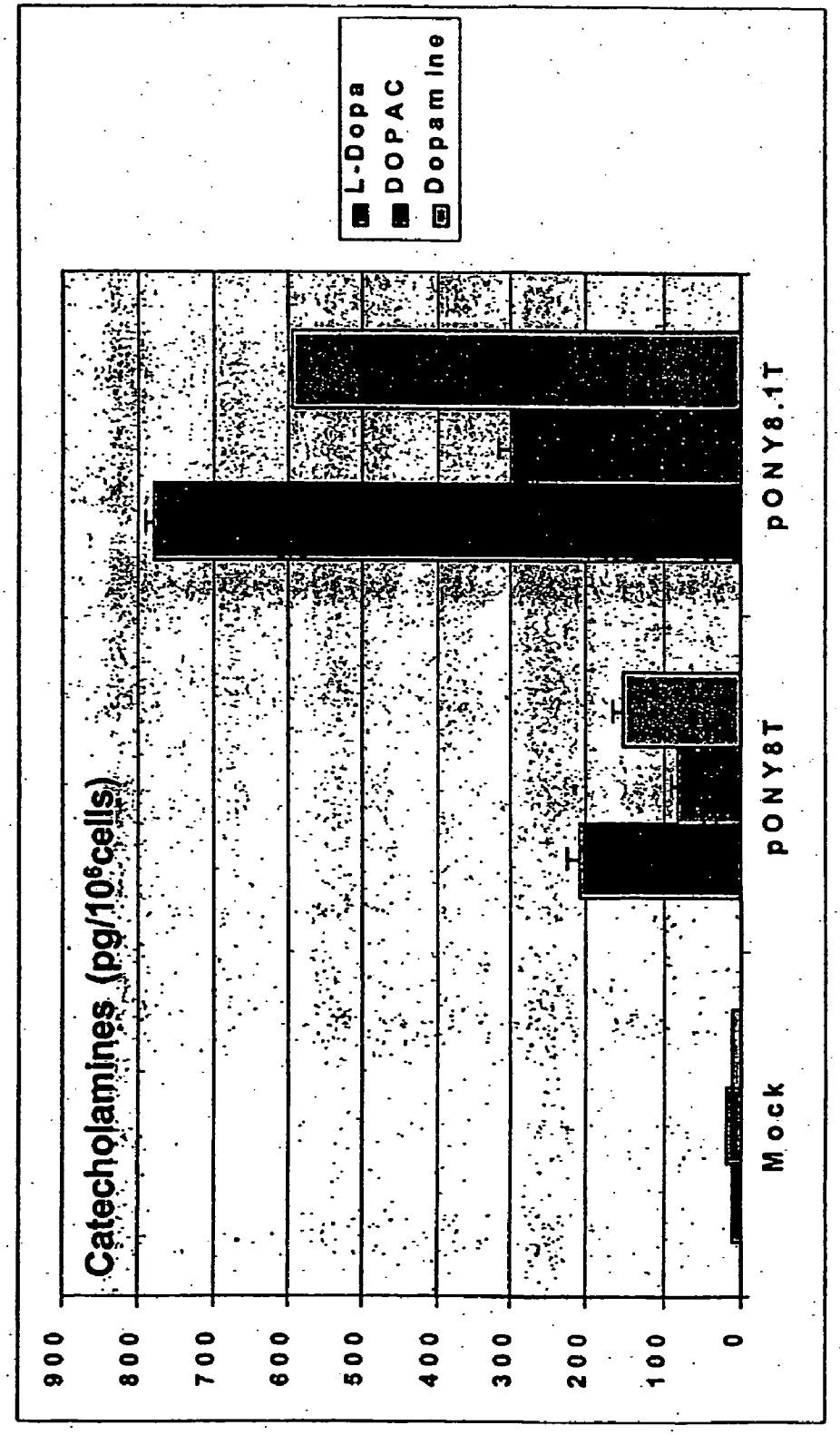

L-Dopa
DOPAC
Dopamine
Catecholamines (pg/10⁶cells)
900
800
700
600
500
400
300
200
100
0
Mock
pONY8T
pONY8.1T

Figure 19

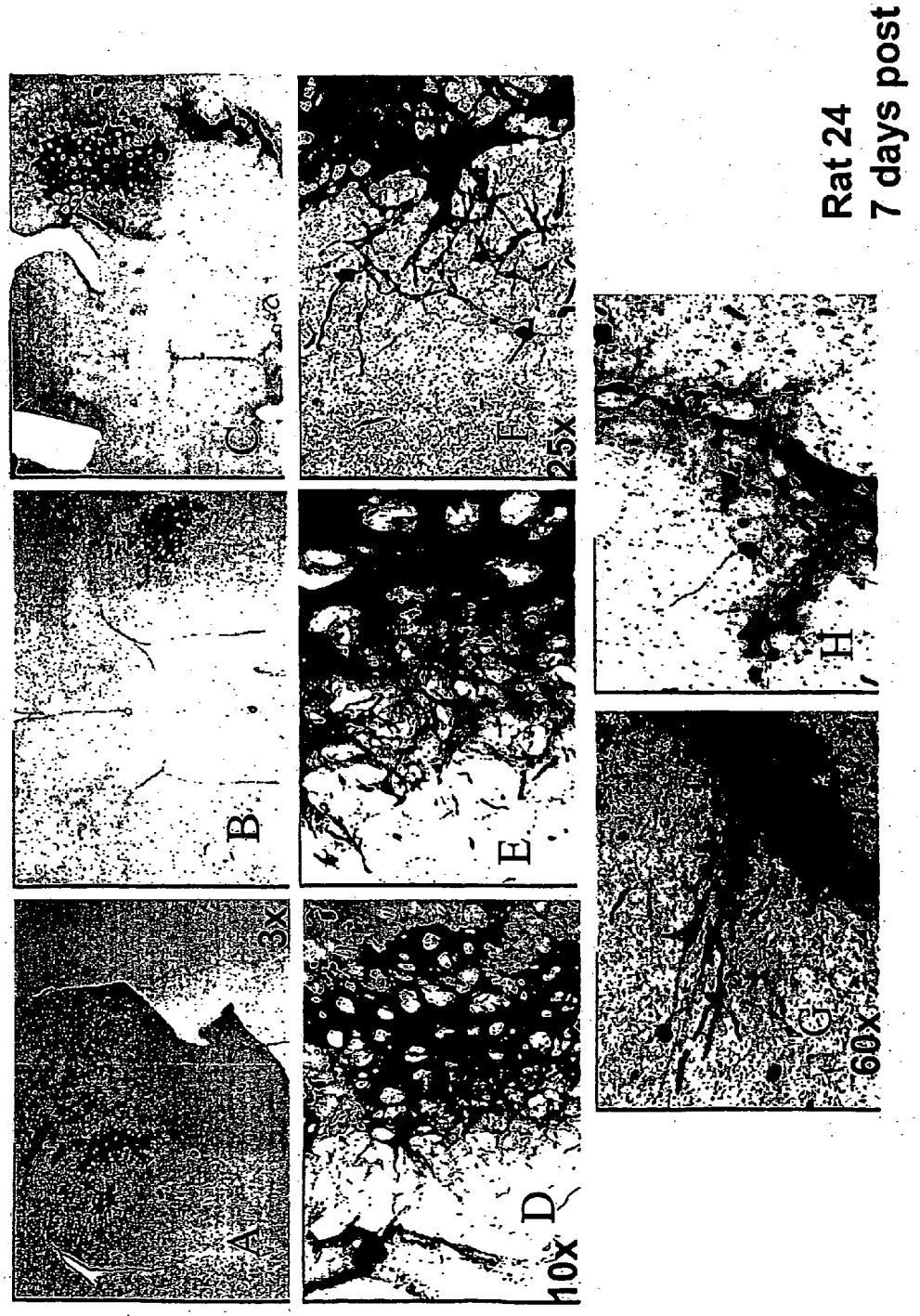
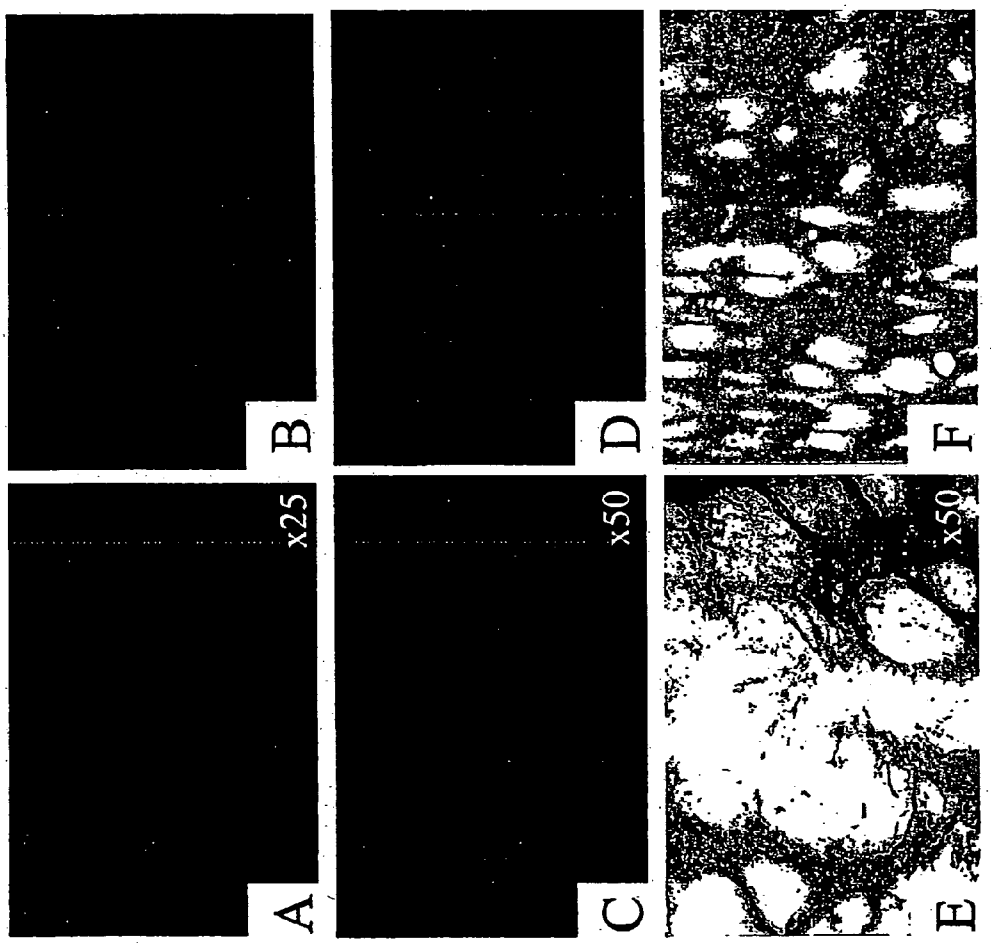


FIGURE 20



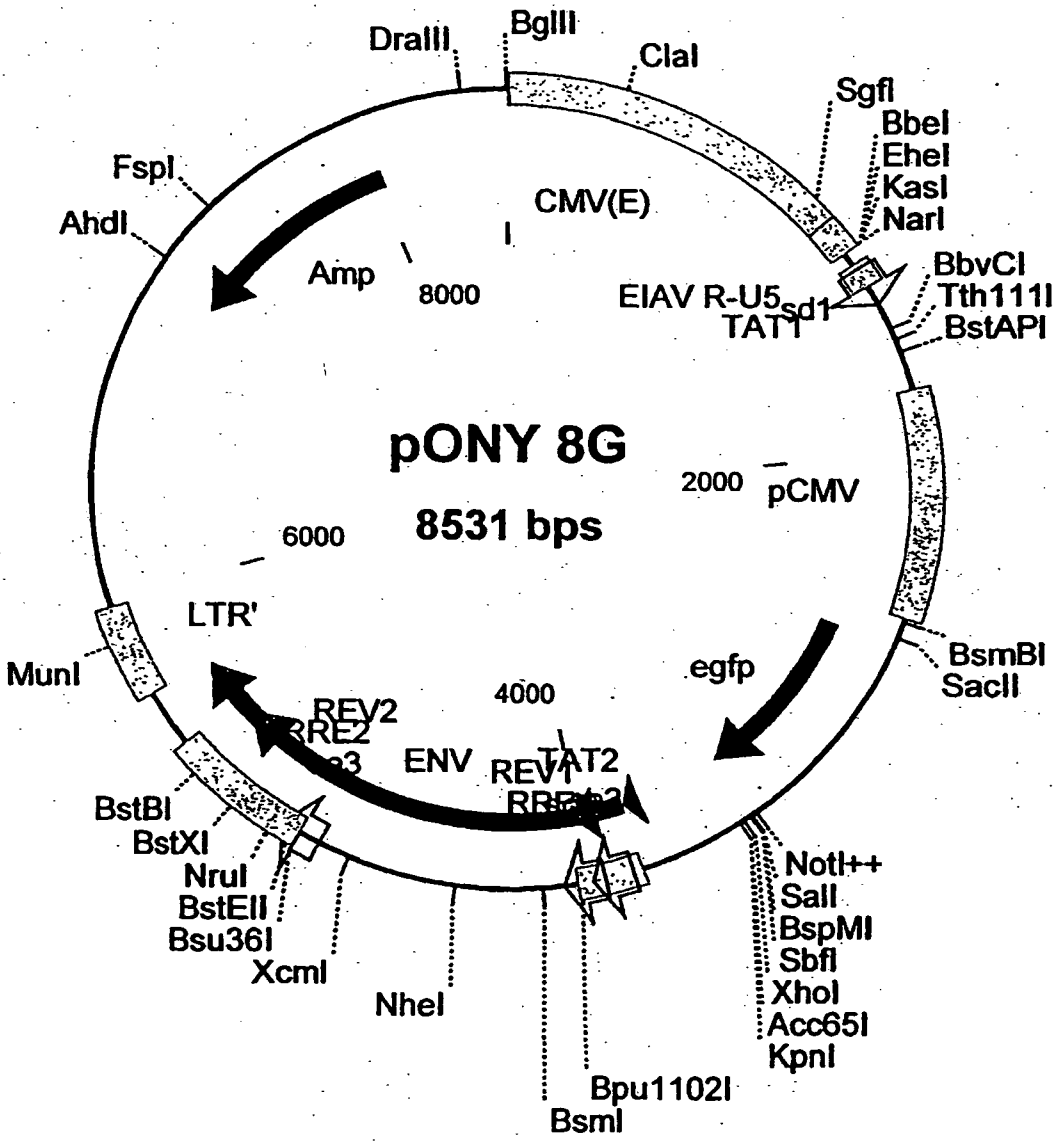

DraIII
BglII
ClaI
SgfI
BbeI
EheI
KasI
NarI
FspI
AhdI
CMV(E)
Amp
8000
EIAV R-U5
sd1
TAT1
BbvCI
Tth111I
BstAPI
pONY 8G
8531 bps
2000
pCMV
6000
LTR'
MunI
egfp
BsmBI
SacII
4000
REV2
RRE2
ENV
REV1
TAT2
BstBI
BstXI
NruI
BstEII
Bsu36I
XcmI
NheI
BsmI
Bpu1102I
NotI++
SalI
BspMI
SbfI
XhoI
Acc65I
KpnI

Figure 21

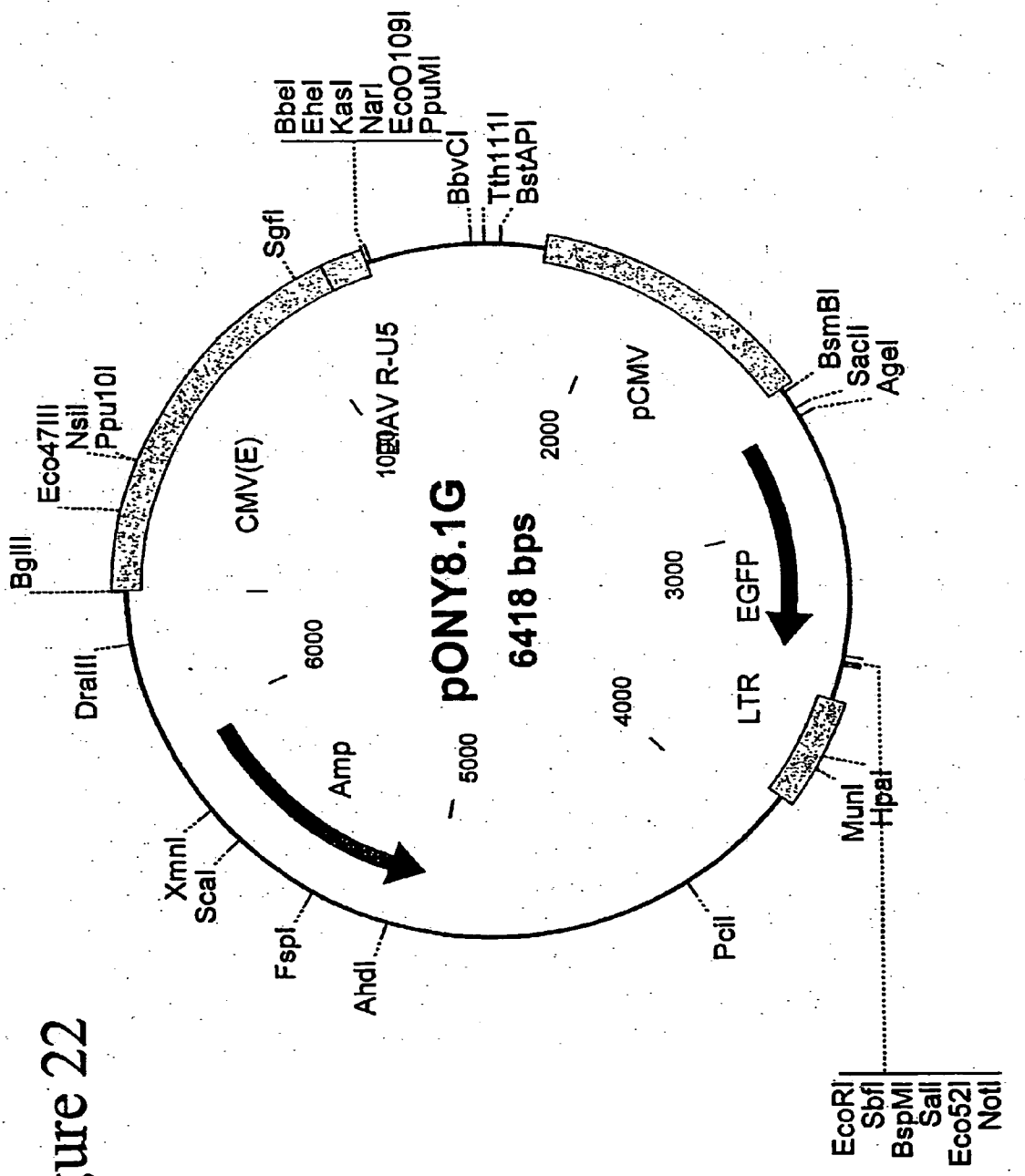

pONY8.1G
6418 bps
CMV(E)
MAV R-U5
pCMV
EGFP
LTR
Amp
1000
2000
3000
4000
5000
6000
BglII
Eco47III
NsiI
Ppu10I
DraIII
XmnI
ScaI
FspI
AhdI
PciI
MunI
HpaI
EcoRI
SbfI
BspMI
SalI
Eco52I
NotI
BsmBI
SacII
AgeI
BbeI
EheI
KasI
NarI
EcoO109I
PpuMI
BbvCI
Tth111I
BstAPI
SgfI

Figure 22

Figure 23

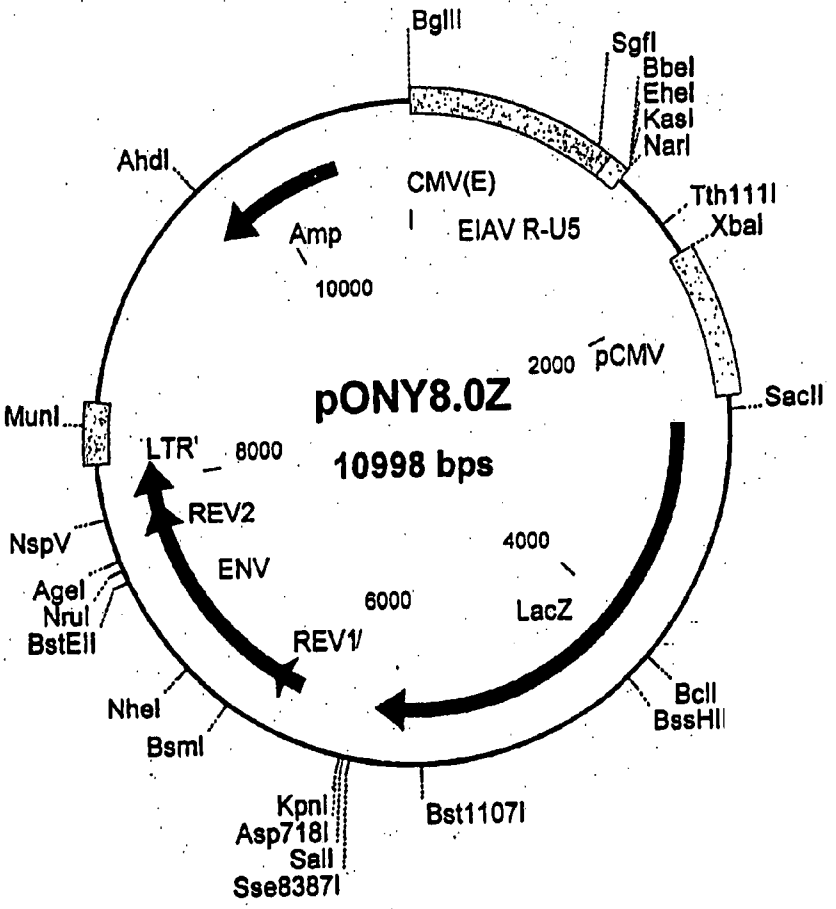

BglII
SgfI
BbeI
EheI
KasI
NarI
Tth111I
XbaI
SacII
pCMV
2000
CMV(E)
EIAV R-U5
Amp
10000
AhdI
pONY8.0Z
10998 bps
MunI
LTR'
8000
REV2
NspV
ENV
4000
AgeI
NruI
BstEII
6000
LacZ
REV1
NheI
BsmI
BclI
BssHII
KpnI
Asp718I
SalI
Sse8387I
Bst1107I

Figure 24.A

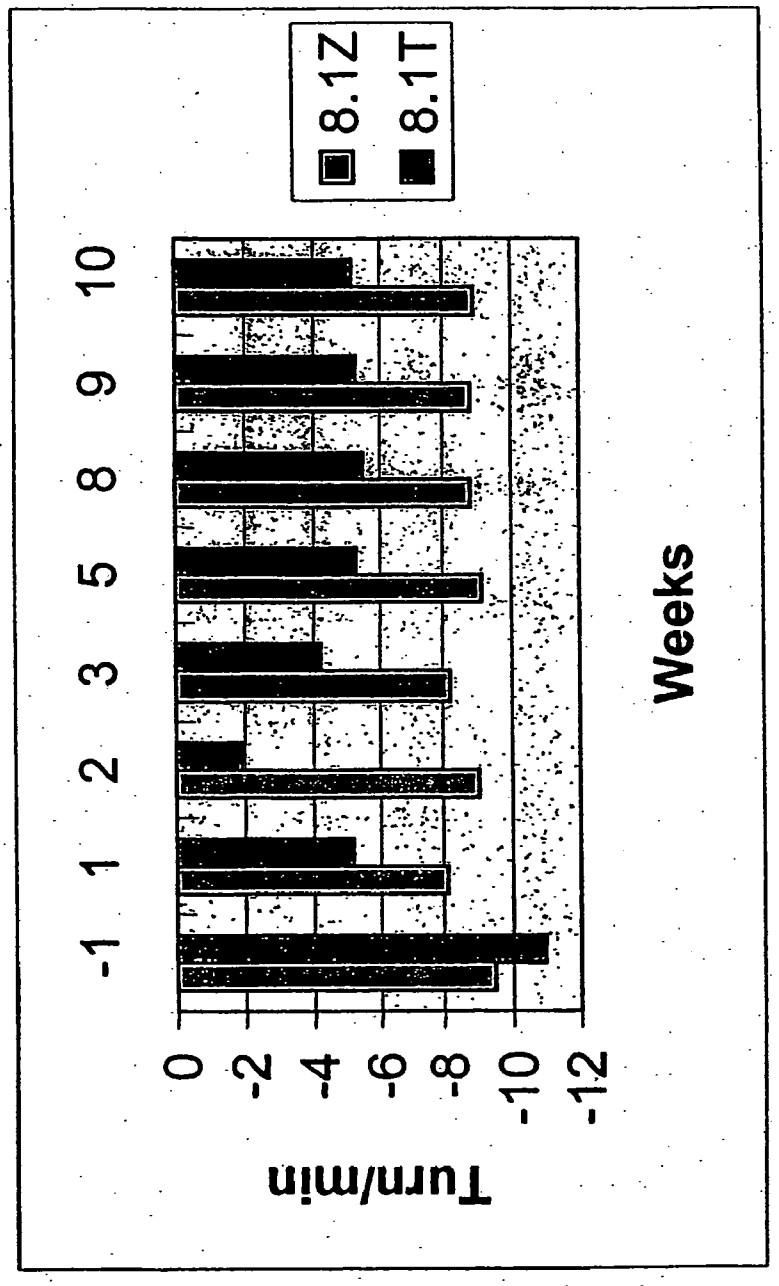


Figure 24.B

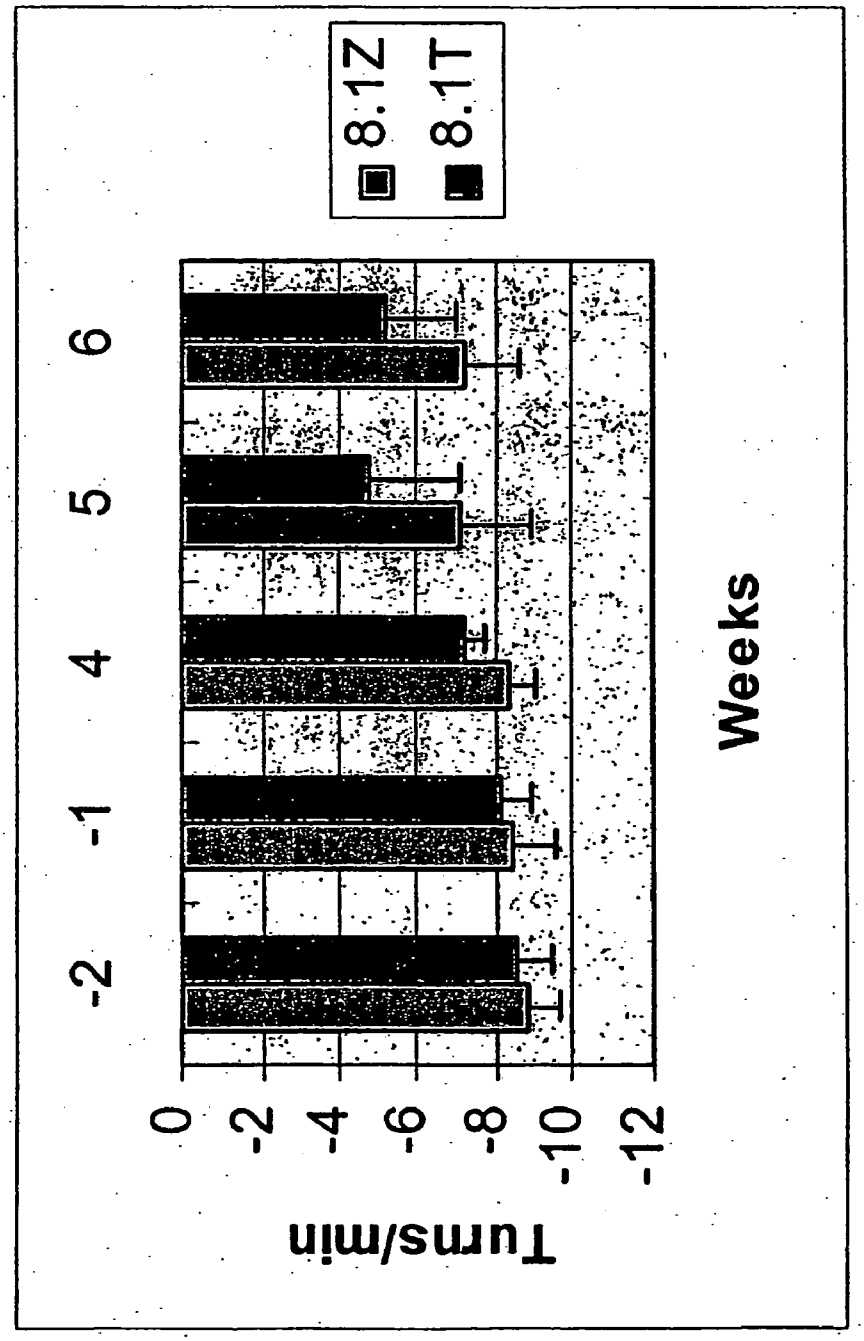


Figure 25.A

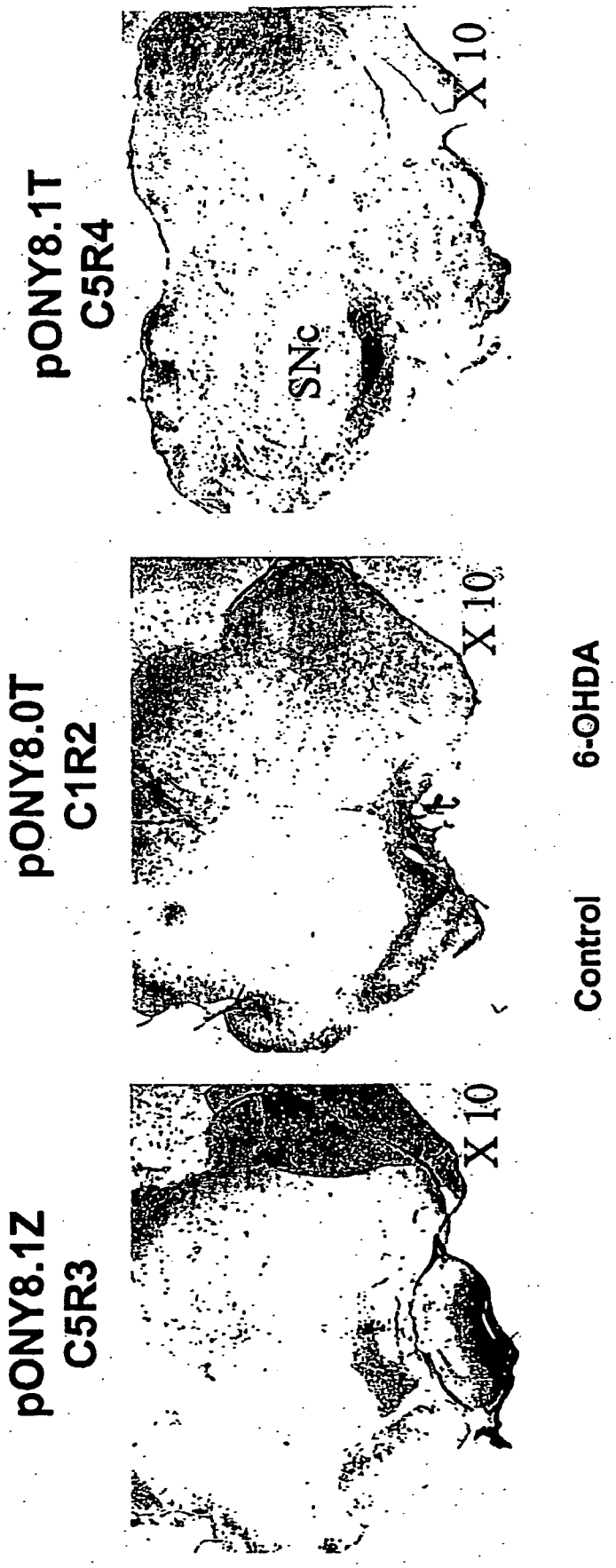

pONY8.1T
C5R4
SNc
X 10
pONY8.0T
C1R2
X 10
pONY8.1Z
C5R3
X 10
Control
6-OHDA

Figure 25.B

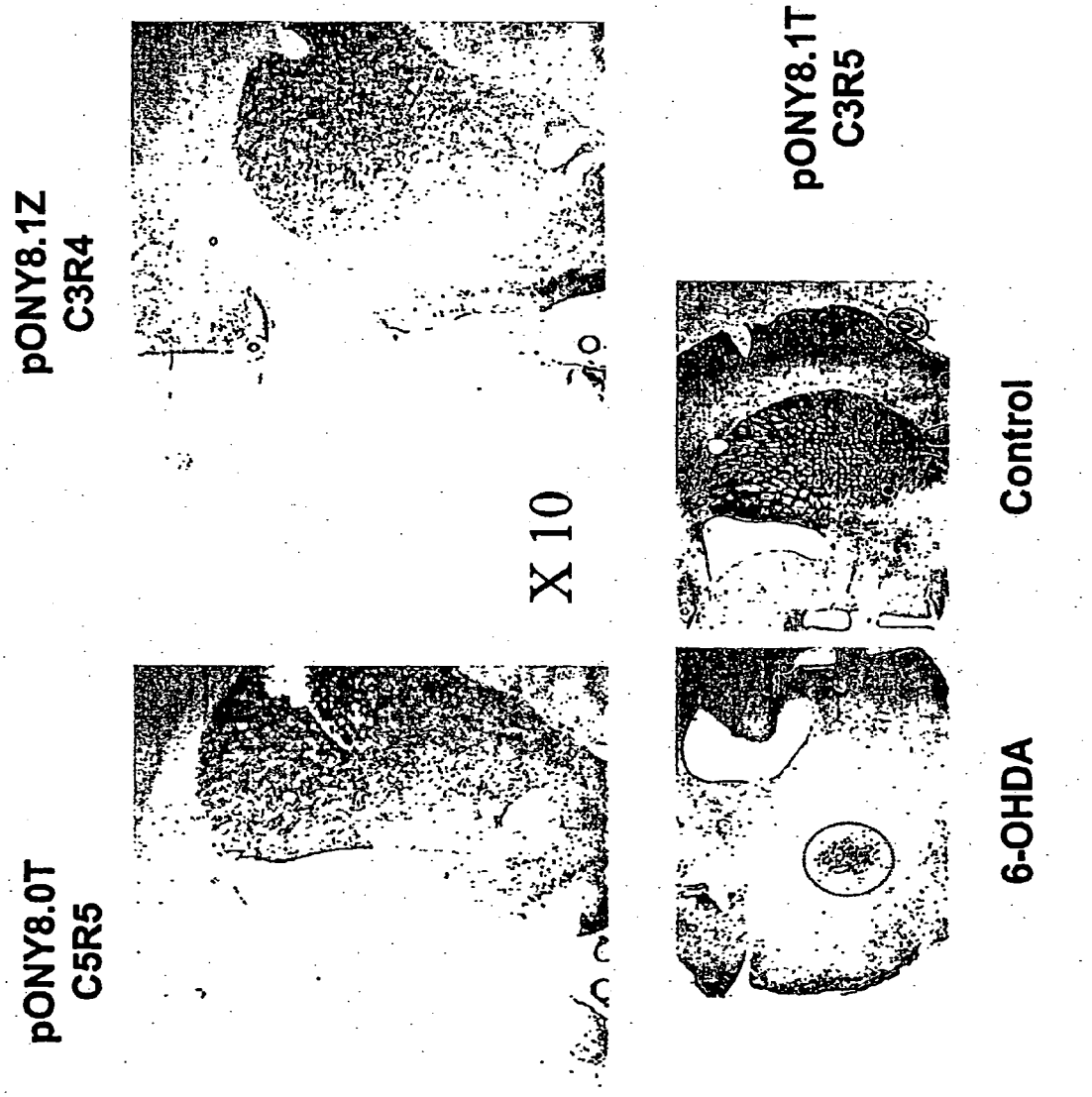
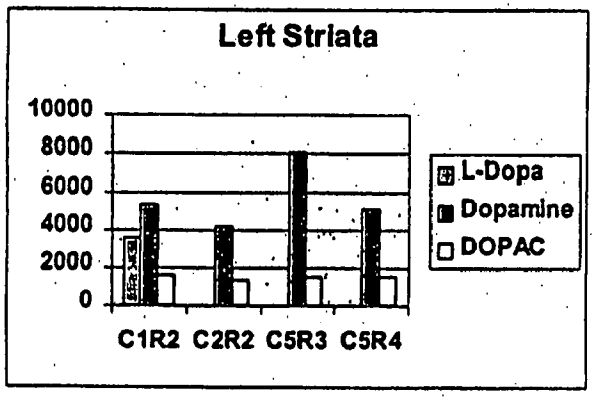
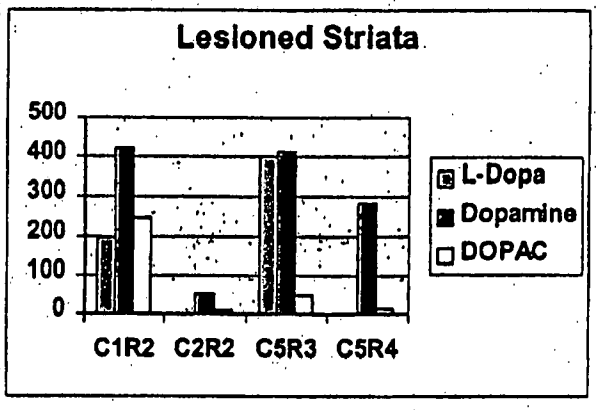


Figure 26: Catecholamines (pg/mg wet tissue)

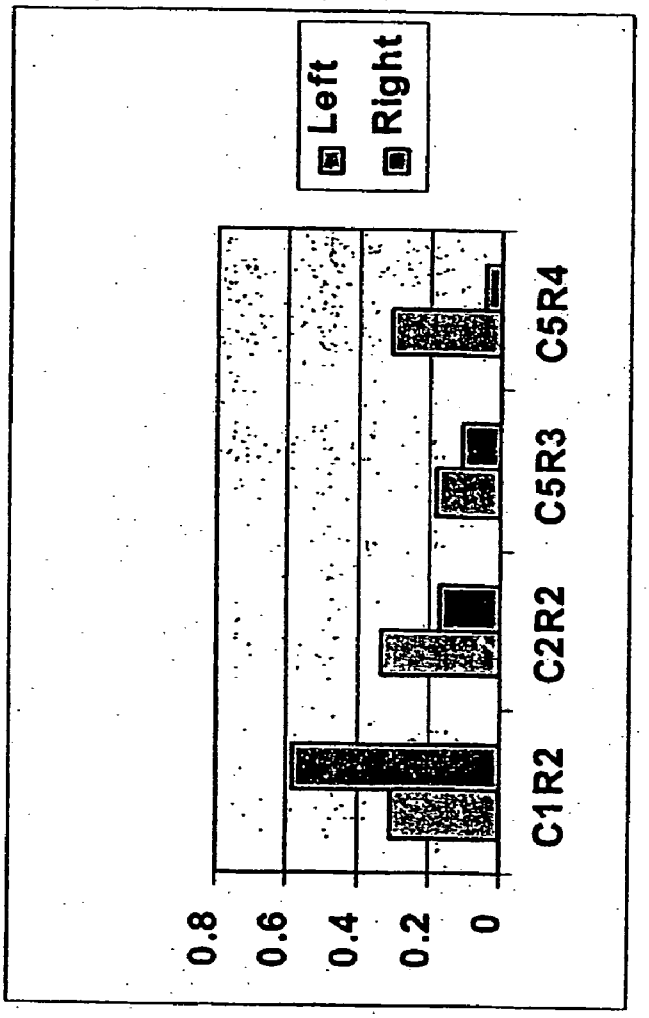




	Striata	Virus	Volume	L-Dopa	opamine	DOPAC	DOPAC/DA
C1R2	Right	pony8.T	8 ul	194	423	247.6	0.58
C2R2	Right	pony8.1T	8 ul	-	50.9	8.7	0.17
C5R3	Right	pony8.1Z	8 ul	402.4	415.6	46	0.11
C5R4	Right	pony8.1T	6 ul	-	284.4	14	0.05

	Striata	Virus	Volume	L-Dopa	Dopamine	DOPAC	Dopac/DA
C1R2	Left	-	-	3600	5274	1612	0.31
C2R2	Left	-	-	-	4216	1424	0.34
C5R3	Left	-	-	-	8112	1532	0.18
C5R4	Left	-	-	-	5112	1586	0.31

Figure 27: DOPAC/Dopamine ratio



	Left	Right
C1R2	0.31	0.58
C2R2	0.34	0.17
C5R3	0.18	0.11
C5R4	0.31	0.05

Right/Left Striata Dopamine DOPAC	
C1R2	8% 15.40%
C2R2	1.20% 0.60%
C5R3	5.10% 3%
C5R4	5.60% 0.90%

Figure 28

A. Amino acid sequence of codon-optimized GTP-cyclohydrolase I (SEQ ID NO:18)

```
  1 MEKGPVRAPA EKPRGARCSN GFPERDPPRP GPSRPAEKPP
    RPEAKSAQPA
 51 DGWKGERPRS EEDNELNLPN LAAAYSSILS SLGENPQRQG
    LLKTPWRAAS
101 AMQFFTKGYQ ETISDVLNDA IFDEDHDEMV IVKDIDMFSM
    CEHHLVPFVG
151 KVHIGYLPNK QVLGLSKLAR IVEIYSRRLQ VQERLTKQIA
    VAITEALRPA
201 GVGVVVEATH MCMVMRGVQK MNSKTVTSTM LGVFREDPKT
    REEFLTLIRS
```

B. Nucleotide sequence of codon-optimized GTP-cyclohydrolase I (SEQ ID NO:19)

```
atggagaagggccctgtgcgcgccccggccgagaagccgcgcggcgcccgctgcagcaatgggttccccgagc
gcgacccgccgcgccccgggcccagcaggccggccgagaagcccccgcgccccgaggccaagagcgcgcag
cccgcggacggctggaagggcgagcgcccccgcagcgaggaggacaacgagctgaacctccctaacctggcc
gccgcctactcctccatcctgagctcgctgggcgagaacccccagcggcaggggctgctcaagacccccctggagg
gcggcctcggccatgcagttcttcaccaagggctaccaggagaccatctcagacgtcctgaacgacgctatcttcga
cgaagatcacgatgagatggtgatcgtgaaggacatagacatgttctccatgtgcgagcaccacctggtgccatttgt
gggaaaggtccatatcggctacctgcctaacaagcaggtcctgggcctcagcaagctggcgaggattgtggaaatct
atagtagaagactacaggttcaggagcgccttaccaaacaaattgctgtggcaatcacggaagccttgcggcctgct
ggagtcggggtcgtggtggaagcaacacacatgtgtatggtgatgcgaggtgtacagaaaatgaacagcaaaacc
gtgaccagcacaatgctgggtgtgttccgggaggatccaaagactcgggaagagttcctgactctcatcaggagctg
a
```

C. Amino acid sequence of wild type GTP-cyclohydrolase I (SEQ ID NO:20)

```
1   MEKGPVRAPA EKPRGARCSN GFPERDPPRP GPSRPAEKPP
    RPEAKSAQPA
 51 DGWKGERPRS EEDNELNLPN LAAAYSSILS SLGENPQRQG
    LLKTPWRAAS
101 AMQFFTKGYQ ETISDVLNDA IFDEDHDEMV IVKDIDMFSM
    CEHHLVPFVG
151 KVHIGYLPNK QVLGLSKLAR IVEIYSRRLQ VQERLTKQIA
    VAITEALRPA
201 GVGVVVEATH MCMVMRGVQK MNSKTVTSTM LGVFREDPKT
    REEFLTLIRS
```

Figure 28 (cont.)

D. Nucleotide sequence of wild type GTP-cyclohydrolase I (SEQ ID NO:21)

atggagaagggccctgtgcgggcaccggcggagaagccgcggggcgccaggtgcagcaatgggttccccgagc
gggatccgccgcggcccgggcccagcaggccggcggagaagcccccgcggcccgaggccaagagcgcgcag
cccgcggacggctggaagggcgagcggccccgcagcgaggaggataacgagctgaacctccctaacctggcag
ccgcctactcgtccatcctgagctcgctgggcgagaacccccagcggcaagggctgctcaagacgccctggaggg
cggcctcggccatgcagttcttcaccaagggctaccaggagaccatctcagatgtcctaaacgatgctatatttgatga
agatcatgatgagatggtgattgtgaaggacatagacatgttttccatgtgtgagcatcacttggttccatttgttggaaag
gtccatattggttatcttcctaacaagcaagtccttggcctcagcaaacttgcgaggattgtagaaatctatagtagaag
actacaagttcaggagcgccttacaaaacaaattgctgtagcaatcacggaagccttgcggcctgctggagtcgggg
tagtggttgaagcaacacacatgtgtatggtaatgcgaggtgtacagaaaatgaacagcaaaactgtgaccagcac
aatgttgggtgtgttccgggaggatccaaagactcgggaagagttcctgactctcattaggagctga

Figure 29

A. Amino acid sequence of codon-optimized truncated tyrosine hydroxylase, type 2, from 8.9.4 MV opti Y (SEQ ID NO:22)

```
  1 MVKVPWFPRK VSELDKCHHL VTKFDPDLDL DHPGFSDQVY
    RQRRKLIAEI
 51 AFQYRHGDPI PRVEYTAEEI ATWKEVYTTL KGLYATHACG
    EHLEAFALLE
101 RFSGYREDNI PQLEDVSRFL KERTGFQLRP VAGLLSARDF
    LASLAFRVFQ
151 CTQYIRHASS PMHSPEPDCC HELLGHVPML ADRTFAQFSQ
    DIGLASLGAS
201 DEEIEKLSTL YWFTVEFGLC KQNGEVKAYG AGLLSSYGEL
    LHCLSEEPEI
251 RAFDPEAAAV QPYQDQTYQS VYFVSESFSD AKDKLRSYAS
    RIQRPFSVKF
301 DPYTLAIDVL DSPQAVRRSL EGVQDELDTL AHALSAIG
```

B. Nucleotide sequence of codon-optimized truncated tyrosine hydroxylase, type 2, from 8.9.4 MV opti Y (SEQ ID NO:23)

```
atggtgaaggtaccctggttcccaagaaaagtgtcagagctggacaagtgtcatcacctggtcaccaagttcgaccc
cgacctggacttggaccaccccggcttctcggaccaggtgtaccgccagcgcaggaagctgatcgctgagatcgcc
ttccagtacaggcacggcgacccgatcccccgtgtggagtacaccgccgaggagatcgccacctggaaggaggtc
tacaccaccctgaagggcctctacgccacccacgcctgcggggagcacctggaggcctttgctttgctggagcgcttc
agcggctaccgggaagacaacatcccccagctggaggacgtctcccgcttcctgaaggagcgcacaggcttccag
ctgcggcccgtggccggcctgctgtccgcccgggacttcctggccagcctggccttccgcgtgttccagtgcacccag
tatatccgccacgcgtcctcgcccatgcactcccctgagccggactgctgccacgagctgctggggcacgtgcccat
gctggccgaccgcaccttcgcgcagttcagccaggacatcggcctggcgtccctgggggccagcgatgaggaaat
cgagaagctgtccactctgtactggttcacggtggagttcgggctgtgtaagcagaacggggaggtgaaggcctatg
gtgccgggctgctgtcctcctacggggagctcctgcactgcctgtctgaggagcctgagatccgggccttcgaccctg
aggctgcggccgtgcagccctaccaagaccagacgtaccagtcagtctacttcgtgtctgagagcttcagcgacgcc
aaggacaagctcaggagctatgccagccgcatccagcgccccttctccgtgaagttcgacccgtacaccctggccat
cgacgtgctggacagcccccaggccgtgcggcgctccctggagggtgtccaggatgagctggacacccttgcccat
gcgctgagcgccatcggc
```

Figure 29 (cont.)

C. Amino acid sequence of codon-optimized truncated tyrosine hydroxylase, type 2 with serine at position 211 (SEQ ID NO:24)

```
  1 MVKVPWFPRK VSELDKCHHL VTKFDPDLDL DHPGFSDQVY
    RQRRKLIAEI
 51 AFQYRHGDPI PRVEYTAEEI ATWKEVYTTL KGLYATHACG
    EHLEAFALLE
101 RFSGYREDNI PQLEDVSRFL KERTGFQLRP VAGLLSARDF
    LASLAFRVFQ
151 CTQYIRHASS PMHSPEPDCC HELLGHVPML ADRTFAQFSQ
    DIGLASLGAS
201 DEEIEKLSTL SWFTVEFGLC KQNGEVKAYG AGLLSSYGEL
    LHCLSEEPEI
251 RAFDPEAAAV QPYQDQTYQS VYFVSESFSD AKDKLRSYAS
    RIQRPFSVKF
301 DPYTLAIDVL DSPQAVRRSL EGVQDELDTL AHALSAIG*
```

D. Nucleotide sequence of codon-optimized tyrosine hydroxylase, type 2 with serine encoded by nucleotides 631-633 (SEQ ID NO:25)

```
atggtgaaggtaccctggttcccaagaaaagtgtcagagctggacaagtgtcatcacctggtcaccaagttcgaccc
cgacctggacttggaccaccccggcttctcggaccaggtgtaccgccagcgcaggaagctgatcgctgagatcgcc
ttccagtacaggcacggcgacccgatcccccgtgtggagtacaccgccgaggagatcgccacctggaaggaggtc
tacaccaccctgaagggcctctacgccacccacgcctgcggggagcacctggaggcctttgctttgctggagcgcttc
agcggctaccgggaagacaacatcccccagctggaggacgtctcccgcttcctgaaggagcgcacaggcttccag
ctgcggcccgtggccggcctgctgtccgcccgggacttcctggccagcctggccttccgcgtgttccagtgcacccag
tatatccgccacgcgtcctcgcccatgcactcccctgagccggactgctgccacgagctgctggggcacgtgcccat
gctggccgaccgcaccttcgcgcagttcagccaggacatcggcctggcgtccctgggggccagcgatgaggaaat
cgagaagctgtccactctgtcatggttcacggtggagttcgggctgtgtaagcagaacggggaggtgaaggcctatg
gtgccgggctgctgtcctcctacggggagctcctgcactgcctgtctgaggagcctgagatccgggccttcgaccctg
aggctgcggccgtgcagccctaccaagaccagacgtaccagtcagtctacttcgtgtctgagagcttcagcgacgcc
aaggacaagctcaggagctatgccagccgcatccagcgccccttctccgtgaagttcgacccgtacaccctggccat
cgacgtgctggacagcccccaggccgtgcggcgctccctggagggtgtccaggatgagctggacacccttgcccat
gcgctgagcgccatcggctga
```

Figure 29 (cont.)

E. Amino acid sequence of wild type truncated tyrosine hydroxylase, type 2 with tyrosine at position 211 (SEQ ID NO:26)

```
  1 MVKVPWFPRK VSELDKCHHL VTKFDPDLDL DHPGFSDQVY
    RQRRKLIAEI
 51 AFQYRHGDPI PRVEYTAEEI ATWKEVYTTL KGLYATHACG
    EHLEAFALLE
101 RFSGYREDNI PQLEDVSRFL KERTGFQLRP VAGLLSARDF
    LASLAFRVFQ
151 CTQYIRHASS PMHSPEPDCC HELLGHVPML ADRTFAQFSQ
    DIGLASLGAS
201 DEEIEKLSTL YWFTVEFGLC KQNGEVKAYG AGLLSSYGEL
    LHCLSEEPEI
251 RAFDPEAAAV QPYQDQTYQS VYFVSESFSD AKDKLRSYAS
    RIQRPFSVKF
301 DPYTLAIDVL DSPQAVRRSL EGVQDELDTL AHALSAIG
```

F. Nucleotide sequence of truncated wild type tyrosine hydroxylase, type 2,with tyrosine encoded by nucleotides 631-633 (SEQ ID NO:27)

```
atggtgaaggtaccctggttcccaagaaaagtgtcagagctggacaagtgtcatcacctggtcaccaagttcgaccct
gacctggacttggaccacccgggcttctcggaccaggtgtaccgccagcgcaggaagctgattgctgagatcgcctt
ccagtacaggcacggcgacccgattccccgtgtggagtacaccgccgaggagattgccacctggaaggaggtcta
caccacgctgaagggcctctacgccacgcacgcctgcggggagcacctggaggcctttgctttgctggagcgcttca
gcggctaccgggaagacaatatcccccagctggaggacgtctcccgcttcctgaaggagcgcacgggcttccagct
gcggcctgtggccggcctgctgtccgcccgggacttcctggccagcctggccttccgcgtgttccagtgcacccagtat
atccgccacgcgtcctcgcccatgcactcccctgagccggactgctgccacgagctgctggggcacgtgcccatgct
ggccgaccgcaccttcgcgcagttctcgcaggacattggcctggcgtccctgggggcctcggatgaggaaattgag
aagctgtccacgctgtactggttcacggtggagttcgggctgtgtaagcagaacggggaggtgaaggcctatggtgc
cgggctgctgtcctcctacggggagctcctgcactgcctgtctgaggagcctgagattcgggccttcgaccctgaggct
gcggccgtgcagccctaccaagaccagacgtaccagtcagtctacttcgtgtctgagagcttcagtgacgccaagga
caagctcaggagctatgcctcacgcatccagcgccccttctccgtgaagttcgacccgtacacgctggccatcgacgt
gctggacagcccccaggccgtgcggcgctccctggagggtgtccaggatgagctggacacccttgcccatgcgctg
agtgccattggctag
```

Figure 29 (cont.)

G. Amino acid sequence of full-length tyrosine hydroxylase, type 2, with tyrosine at position 374 (SEQ ID NO:28)

```
  1  MPTPDATTPQ AKGFRRAVSE LDAKQAEAIM VRGQSPRFIG
     RRQSLIEDAR
 51  KEREAAVAAA AAAVPSEPGD PLEAVAFEEK EGKAVLNLLF
     SPRATKPSAL
101  SRAVKVFETF EAKIHHLETR PAQRPRAGGP HLEYFVRLEV
     RRGDLAALLS
151  GVRQVSEDVR SPAGPKVPWF PRKVSELDKC HHLVTKFDPD
     LDLDHPGFSD
201  QVYRQRRKLI AEIAFQYRHG DPIPRVEYTA EEIATWKEVY
     TTLKGLYATH
251  ACGEHLEAFA LLERFSGYRE DNIPQLEDVS RFLKERTGFQ
     LRPVAGLLSA
301  RDFLASLAFR VFQCTQYIRH ASSPMHSPEP DCCHELLGHV
     PMLADRTFAQ
351  FSQDIGLASL GASDEEIEKL STLYWFTVEF GLCKQNGEVK
     AYGAGLLSSY
401  GELLHCLSEE PEIRAFDPEA AAVQPYQDQT YQSVYFVSES
     FSDAKDKLRS
451  YASRIQRPFS VKFDPYTLAI DVLDSPQAVR RSLEGVQDEL
     DTLAHALSAI
501  G*
```

H. Nucleotide sequence of full-length tyrosine hydroxylase, type 2, which encodes tyrosine with nucleotides 1120-1122 (SEQ ID NO:29)

```
atgcccacccccgacgccaccacgccacaggccaagggcttccgcagggccgtgtctgagctggacgccaagcaggcaga
ggccatcatggtaagagggcagtccccgcggttcattgggcgcaggcagagcctcatcgaggacgcccgcaaggagcggga
ggcggcggtggcagcagcggccgctgcagtcccctcggagcccggggaccccctggaggctgtggcctttgaggagaagga
ggggaaggccgtgctaaacctgctcttctccccgagggccaccaagccctcggcgctgtcccgagctgtgaaggtgtttgagac
gtttgaagccaaaatccaccatctagagacccggcccgcccagaggccgcgagctgggggcccccacctggagtacttcgtg
cgcctcgaggtgcgccgaggggacctggccgccctgctcagtggtgtgcgccaggtgtcagaggacgtgcgcagccccgcgg
ggcccaaggtcccctggttcccaagaaaagtgtcagagctggacaagtgtcatcacctggtcaccaagttcgaccctgacctgg
acttggaccacccgggcttctcggaccaggtgtaccgccagcgcaggaagctgattgctgagatcgccttccagtacaggcacg
gcgacccgattccccgtgtggagtacaccgccgaggagattgccacctggaaggaggtctacaccacgctgaagggcctctac
gccacgcacgcctgcggggagcacctggaggcctttgctttgctggagcgcttcagcggctaccgggaagacaatatccccca
gctggaggacgtctcccgcttcctgaaggagcgcacgggcttccagctgcggcctgtggccggcctgctgtccgcccgggactt
cctggccagcctggccttccgcgtgttccagtgcacccagtatatccgccacgcgtcctcgcccatgcactcccctgagccggact
gctgccacgagctgctggggcacgtgcccatgctggccgaccgcaccttcgcgcagttctcgcaggacattggcctggcgtccct
gggggcctcggatgaggaaattgagaagctgtccacgctgtactggttcacggtggagttcgggctgtgtaagcagaacgggg
aggtgaaggcctatggtgccgggctgctgtcctcctacggggagctcctgcactgcctgtctgaggagcctgagattcgggccttc
gaccctgaggctgcggccgtgcagccctaccaagaccagacgtaccagtcagtctacttcgtgtctgagagcttcagtgacgcc
aaggacaagctcaggagctatgcctcacgcatccagcgcccccttctccgtgaagttcgacccgtacacgctggccatcgacgtg
ctggacagcccccaggccgtgcggcgctccctggagggtgtccaggatgagctggacacccttgcccatgcgctgagtgccatt
ggctag
```

Figure 30

A. Amino acid sequence of codon-optimized aromatic amino acid decarboxylase in pONY8.9.4 MV opti Y (SEQ ID NO:30)

```
  1 MDASEFRRRG KEMVDYVANY MEGIEGRQVY PDVEPGYLRP
    LIPAAAPQEP
 51 DTFEDIINDV EKIIMPGVTH WHSPYFFAYF PTASSYPAML
    ADMLCGAIGC
101 IGFSWAASPA CTELETVMMD WLGKMLELPK AFLNEKAGEG
    GGVIQGSASE
151 ATLVALLAAR TKVIHRLQAA SPELTQAAIM EKLVAYSSDQ
    AHSSVERAGL
201 IGGVKLKAIP SDGNFAMRAS ALQEALERDK AAGLIPFFMV
    ATLGTTTCCS
251 FDNLLEVGPI CNKEDIWLHV DAAYAGSAFI CPEFRHLLNG
    VEFADSFNFN
301 PHKWLLVNFD CSAMWVKKRT DLTGAFRLDP TYLKHSHQDS
    GLITDYRHWQ
351 IPLGRRFRSL KMWFVFRMYG VKGLQAYIRK HVQLSHEFES
    LVRQDPRFEI
401 CVEVILGLVC FRLKGSNKVN EALLQRINSA KKIHLVPCHL
    RDKFVLRFAI
451 CSRTVESAHV QRAWEHIKEL AADVLRAERE *
```

B. Nucleotide sequence of codon-optimized aromatic amino acid decarboxylase in pONY8.9.4 MV opti Y (SEQ ID NO:31)

```
atggacgccagtgagttccgaaggcgcggcaaggagatggtggactacgtggccaactacatggaaggcatcga
gggccgccaagtctaccccgacgtggagcccggctacctgcgcccgctgatccccgccgctgcccctcaggagcc
cgacaccttcgaggacatcatcaacgacgtggagaagatcatcatgcctggcgtgacgcactggcacagcccctac
ttcttcgcctacttccccaccgccagctcgtacccggccatgctggcggacatgctgtgcggggccattggctgcatcg
gcttctcctgggcggcgagcccagcgtgcaccgagctggagaccgtgatgatggactggctcgggaagatgctgga
gctcccaaaggcgttcttgaacgagaaggctggcgagggggcggcgtgatccagggcagcgccagcgaggcc
accctggtggccctgctggccgctcggaccaaagtgatccaccggctgcaggcagcgtccccagagctcacccag
gccgctatcatggagaagctggtggcttactcctccgatcaggcacactcctccgtggaacgcgctgggctcattggtg
gagtgaagctcaaggccatccccagcgatggcaacttcgccatgcgtgcgagcgccctgcaggaagccctggaga
gagacaaggcggctggcctgattcctttcttcatggtggccaccctggggaccacaacatgctgctccttcgacaacct
cctcgaagtcggtcctatctgcaacaaggaagacatctggctgcacgttgatgcagcctacgcaggcagcgcattca
tctgccctgagttccggcaccttctgaacggagtggagttcgcagatagcttcaacttcaatccccacaagtggctattg
gtgaatttcgactgcagcgccatgtgggtgaagaagcgcaccgacctcacgggagccttccgcctggaccccactta
cctgaagcacagccaccaggattcagggcttatcactgactaccggcactggcagatcccactgggccgcagattc
cgcagcttgaagatgtggttcgtattcaggatgtatggagtcaagggactgcaggcttatatccgcaagcatgtccagc
tgtcccatgagtttgagtcactggtgcgccaggatccccgctttgaaatctgtgtggaagtcattctggggcttgtctgcttt
cggctaaagggttccaacaaagtgaatgaagctcttctgcaaaggatcaacagtgccaaaaaaatccacttggttcc
atgtcacctcagggacaagtttgtcctgcgctttgccatctgttctcgcaccgtggaatctgcccatgtgcagcgggcctg
ggaacacatcaaagagctggcggccgacgtgctgcgagcagagagggagtag
```

Figure 30 (cont.)

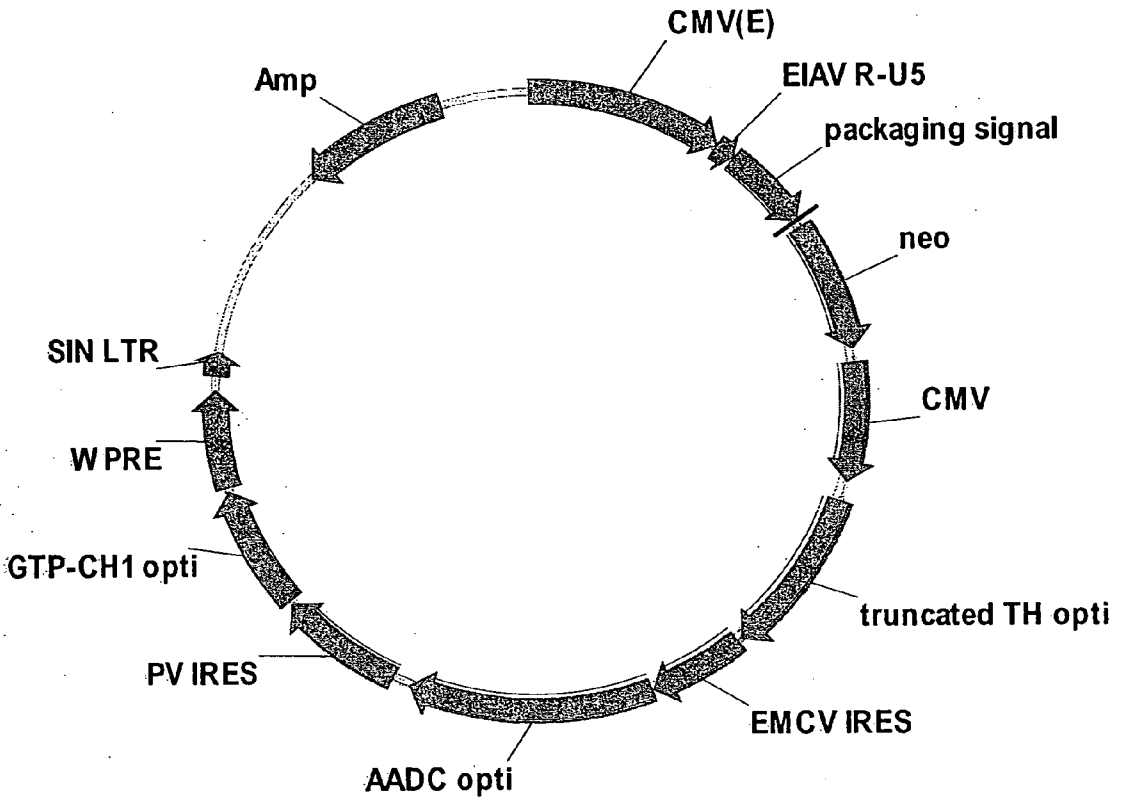
C. Amino acid sequence of wild type aromatic amino acid decarboxylase (SEQ ID NO:32)

```
  1 MNASEFRRRG KEMVDYVANY MEGIEGRQVY PDVEPGYLRP
    LIPAAAPQEP
 51 DTFEDIINDV EKIIMPGVTH WHSPYFFAYF PTASSYPAML
    ADMLCGAIGC
101 IGFSWAASPA CTELETVMMD WLGKMLELPK AFLNEKAGEG
    GGVIQGSASE
151 ATLVALLAAR TKVIHRLQAA SPELTQAAIM EKLVAYSSDQ
    AHSSVERAGL
201 IGGVKLKAIP SDGNFAMRAS ALQEALERDK AAGLIPFFMV
    ATLGTTTCCS
251 FDNLLEVGPI CNKEDIWLHV DAAYAGSAFI CPEFRHLLNG
    VEFADSFNFN
301 PHKWLLVNFD CSAMWVKKRT DLTGAFRLDP TYLKHSHQDS
    GLITDYRHWQ
351 IPLGRRFRSL KMWFVFRMYG VKGLQAYIRK HVQLSHEFES
    LVRQDPRFEI
401 CVEVILGLVC FRLKGSNKVN EALLQRINSA KKIHLVPCHL
    RDKFVLRFAI
451 CSRTVESAHV QRAWEHIKEL AADVLRAERE *
```

D. Nucleotide sequence of wild type aromatic amino acid decarboxylase (SEQ ID NO:33)

```
atgaacgcaagtgaattccgaaggagagggaaggagatggtggattacgtggccaactacatggaaggcattgag
ggacgccaggtctaccctgacgtggagcccgggtacctgcggccgctgatccctgccgctgcccctcaggagccag
acacgtttgaggacatcatcaacgacgttgagaagataatcatgcctggggtgacgcactggcacagcccctacttct
tcgcctacttccccactgccagctcgtacccggccatgcttgcggacatgctgtgcggggccattggctgcatcggcttc
tcctgggcggcaagcccagcatgcacagagctggagactgtgatgatggactggctcgggaagatgctggaacta
ccaaaggcatttttgaatgagaaagctggagaaggggggaggagtgatccagggaagtgccagtgaagccaccct
ggtggccctgctggccgctcggaccaaagtgatccatcggctgcaggcagcgtccccagagctcacacaggccgc
tatcatggagaagctggtggcttactcatccgatcaggcacactcctcagtggaaagagctgggttaattggtggagtg
aaattaaaagccatcccctcagatggcaacttcgccatgcgtgcgtctgccctgcaggaagccctggagagagaca
aagcggctggcctgattcctttctttatggttgccaccctggggaccacaacatgctgctcctttgacaatctcttagaagt
cggtcctatctgcaacaaggaagacatatggctgcacgttgatgcagcctacgcaggcagtgcattcatctgccctga
gttccggcaccttctgaatggagtggagtttgcagattcattcaactttaatccccacaaatggctattggtgaattttgact
gttctgccatgtgggtgaaaaagagaacagacttaacgggagcctttagactggaccccacttacctgaagcacagc
catcaggattcagggcttatcactgactaccggcattggcagataccactgggcagaagatttcgctctttgaaaatgt
ggtttgtatttaggatgtatggagtcaaaggactgcaggcttatatccgcaagcatgtccagctgtcccatgagtttgagt
cactggtgcgccaggatccccgctttgaaatctgtgtggaagtcattctggggcttgtctgctttcggctaaagggttcca
acaaagtgaatgaagctcttctgcaaagaataaacagtgccaaaaaaatccacttggttccatgtcacctcagggac
aagtttgtcctgcgctttgccatctgttctcgcacggtggaatctgcccatgtgcagcgggcctgggaacacatcaaag
agctggcggccgacgtgctgcgagcagagagggagtag
```

Figure 31


CMV(E)
EIAV R-U5
packaging signal
neo
CMV
truncated TH opti
EMCV IRES
AADC opti
PV IRES
GTP-CH1 opti
W PRE
SIN LTR
Amp

VECTOR SYSTEM

REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 10/408,456, allowed, which is a Continuation-in-Part of PCT/GB01/04433, filed on Oct. 5, 2001, designating the U.S., published on Apr. 11, 2002 as WO 02/29065, and claiming priority from GB 0024550.6, filed on Oct. 6, 2000. All of the above-mentioned applications, as well as all documents cited herein, and documents referenced or cited in documents cited herein, are incorporated by reference.

[0002] The present invention relates to a vector system. In particular, the present invention relates to a lentiviral vector system for the treatment of Parkinson's disease.

BACKGROUND

[0003] Parkinson's Disease

[0004] Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of the nigrostriatal pathway. Although the cause of Parkinson's disease is not known, it is associated with the progressive death of dopaminergic (tyrosine hydroxylase (TH) positive) mesencephalic neurons, inducing motor impairment. The characteristic symptoms of Parkinson's disease appear when up to 70% of TH-positive nigrostriatal neurons have degenerated.

[0005] There is currently no satisfactory cure for Parkinson's disease. Symptomatic treatment of the disease-associated motor impairments involves oral administration of dihydroxyphenylalanine (L-DOPA). L-DOPA is transported across the blood-brain barrier and converted to dopamine, partly by residual dopaminergic neurons, leading to a substantial improvement of motor function. However, after a few years, the degeneration of dopaminergic neurons progresses, the effects of L-DOPA are reduced and side-effects reappear. Better therapy for Parkinson's disease is therefore necessary.

[0006] An alternative strategy for therapy is neural grafting, which is based on the idea that dopamine supplied from cells implanted into the striatum can substitute for lost nigrostriatal cells. Clinical trials have shown that mesencephalic TH positive neurons obtained from human embryo cadavers (aborted foetuses) can survive and function in the brains of patients with Parkinson's disease. However, functional recovery has only been partial, and the efficacy and reproducibility of the procedure is limited. Also, there are ethical, practical and safety issues associated with using tissue derived from aborted human foetuses. Moreover, the large amounts of tissue required to produce a therapeutic effect is likely to prove to be prohibitive. Some attempts have been made to use TH positive neurons from other species (in order to circumvent some of the ethical and practical problems). However, xenotransplantation requires immunosuppressive treatment and is also controversial due to, for example, the possible risk of cross-species transfer of infectious agents. Another disadvantage is that, in current grafting protocols, no more than 5-20% of the expected numbers of grafted TH positive neurons survive. In order to develop a practicable and effective transplantation protocol, an alternative source of TH positive neurons is required.

[0007] A further alternative strategy for therapy is gene therapy. It has been suggested that gene therapy could be used in Parkinson's disease in two ways: to replace dopamine in the affected striatum by introducing the enzymes responsible for L-DOPA or dopamine synthesis (for example, tyrosine hydroxylase); and to introduce potential neuroprotective molecules that may either prevent the TH-positive neurons from dying or stimulate regeneration and functional recovery in the damaged nigrostriatal system (Dunnet S. B. and Björklund A. (1999) Nature 399 A32-A39).

[0008] In vivo, dopamine is synthesised from tyrosine by two enzymes, tyrosine hydroxylase (TH) and aromatic amino acid DOPA-decarboxylase (AADC). Parkinson's disease has been shown to be responsive to treatments that facilitate dopaminergic transmission in caudate-putamen. In experimental animals, genetically modified cells that express tyrosine hydroxylase, and thereby synthesise L-DOPA, induce behavioural recovery in rodent models of PD (Wolff et al. (1989) PNAS (USA) 86:9011-14; Freed et al (1990) Arch. Neurol. 47:505-12; Jiao et al. (1993) Nature 262:4505).

[0009] Functional activity of tyrosine hydroxylase depends on the availability of its cofactor tetrahydrobiopterin (BH_4). The level of cofactor may be insufficient in the denervated striatum, and so it is thought that GTP cyclohydrolase I, the enzyme that catalyses the rate limiting step on the pathway of BH_4-synthesis, may also need to be transduced to obtain s sufficient levels of L-DOPA production in vivo (Bencsics et al (1996) J. Neurosci 16:4449-4456; Leff et al (1998) Exp. Neurol. 151:249-264).

[0010] Although in vivo and ex vivo gene therapy strategies for the treatment of Parkinson's disease have already been proposed (Dunnet and Bjorklund (1999) as above; Raymon et al (1997) Exp. Neurol. 144:82-91; Kang (1998) Mov. Dis. 13: 59-72) significant progress in this technology has been hampered by the limited efficiency of gene transfer and expression in the target cells. One problem in this regard is that the target cells are usually non-dividing cells (i.e. neurones) which are notoriously recalcitrant to transduction.

[0011] Expression of More than One Protein

[0012] WO 98/18934 relates to a a polynucleotide sequence for use in gene therapy, which polynucleotide sequence comprises two or more therapeutic genes operably linked to a promoter, and encodes a fusion protein product of the therapeutic genes. This provides a way of expressing two therapeutic genes from a single "chimeric gene". In a preferred embodiment, the polynucleotide sequence is capable of encoding a fusion protein comprising tyrosine hydroxylase and DOPA decarboxylase in either TH-DD or DD-TH order, linked by a flexible linker.

[0013] As discussed in WO/18924, amongst gene transfer systems, retroviral vectors hold substantial promise for gene therapy. These systems can transfer genes efficiently and new vectors are emerging that are particularly useful for gene delivery to brain cells (Naldini et al., 1996 Science 272, 263). However, it is clear from the literature that retroviral vectors achieve the highest titres and most potent gene expression properties if they are kept genetically simple (PCT/GB96/01230; Bowtell et al., 1988 J. Virol. 62, 2464; Correll et al., 1994 Blood 84, 1812; Emerman and Temin 1984 Cell 39, 459; Ghattas et al., 1991 Mol. Cell. Biol. 11, 5848; Hantzopoulos et al., 1989 PNAS 86, 3519; Hatzoglou

et al., 1991 J. Biol. Chem 266, 8416; Hatzoglou et al., 1988 J. Biol. Chem 263, 17798; Li et al., 1992 Hum. Gen. Ther. 3, 381; McLachlin et al., 1993 Virol. 195, 1; Overell et al., 1988 Mol. Cell Biol. 8, 1803; Scharfman et al., 1991 PNAS 88, 4626; Vile et al., 1994 Gene Ther 1, 307; Xu et al., 1989 Virol. 171, 331; Yee et al., 1987 PNAS 84, 5197). This means using a single transcription unit within the vector genome and orchestrating appropriate gene expression from sequences either within the 5' LTR or from an internal promoter using a self-inactivating LTR, or using the split-intron technology described in the WO99/15683.

[0014] According to WO 98/18934, if there is a need to express two proteins from a single retroviral vector it is preferable to express them as a fusion protein (encoded by a single nucleotide sequence) than to use an internal ribosome entry site (IRES) to initiate translation of the second coding sequence in a poly-cistronic message. This is because, according to WO 98/18934 the efficiency of an IRES is often low and tissue dependent making the strategy undesirable when one is seeking to maximise the efficiency of metabolic conversion of, for example, tyrosine through to dopamine.

[0015] When located between open reading frames in an RNA, an IRES allows translation of the downstream open reading frame by promoting entry of the ribosome at the IRES element followed by downstream initiation of translation. The use of IRES elements in retroviral vectors has been investigated (see, for example, WO 93/0314) but expression of the cDNA situated downstream of the IRES has often been found to be inefficient. This may be due to competition for ribosomes and other cellular factors. The efficiency of translation initiation would therefore be expected to decrease with increasing numbers of IRES elements.

[0016] Expression of Large Heterologous Genes

[0017] Although the concept of using viral vectors to deliver a heterologous gene to a recipient cell is well known (Verma and Somia (1997) Nature 389:239-242), it is widely accepted that there are limits on the size of the heterologous gene which can be successfully transduced (see, for example page 446, Chapter 9 of Coffin et al "Retroviruses" 1997 Cold Spring Harbour Laboratory Press). If incorporation of the heterologous gene and associated regulatory elements dramatically increases the size of the viral genome, then there is a significant risk that it will no longer be able to be successfully packaged, or at least that packaging efficiency will be significantly reduced.

[0018] Despite the apparent prejudice in the art, the present inventors have shown that lentiviral vectors expressing a bicistronic cassette (encoding TH and GTP-CH1) and even a tricistronic cassette (encoding TH, AADC and GTP-CH1) can yield expression of the appropriate enzymes in heterologous cells in culture and in vivo. Incorporation of the tricistronic cassette into the lentiviral vector causes an increase in the size of the RNA genome of approximately 10%-30% (over the wild-type RNA genome) but surprisingly, gene transfer efficiency is not markedly affected. Integration efficiencies are comparable and efficient gene transfer to neurons is demonstrated. Moreover, the inventors have shown that such vectors may be used to increase the levels of certain catecholamines in denervated tissue and therefore correct rodent and primate models of Parkinson's disease.

SUMMARY OF THE INVENTION

[0019] The first aspect of the invention relates to viral vector genomes. In a first embodiment of the first aspect of the invention there is provided a retroviral vector genome comprising two or more NOIs (nucleotide sequences of interest) operably linked by one or more Internal Ribosome Entry Site(s). Preferably the genome comprises three or more NOIs operably linked by two or more Internal Ribosome Entry Site(s). Preferably each NOI is useful in the treatment of a neurodegenerative disorder. Preferably the genome is a lentiviral vector genome.

[0020] In a second embodiment of the first aspect of the invention there is provided a lentiviral vector genome comprising two or more NOIs suitable for treating a neurodegenerative disorder. Preferably the genome comprises three or more NOIs suitable for treating a neurodegenerative disorder. Preferably the NOIs are operably linked by one or more Internal Ribosome Entry Sites(s).

[0021] Preferably the NOIs of these first and second embodiments of the invention are capable of encoding a protein selected from the following group: Tyrosine Hydroxylase, GTP-cyclohydrolase I, Aromatic Amino Acid Dopa Decarboxylase and Vesicular Monoamine Transporter 2 (VMAT2). More preferably the NOIs are capable of encoding Tyrosine Hydroxylase, GTP-cyclohydrolase I and optionally Aromatic Amino Acid Dopa Decarboxylase or Aromatic Amino Acid Dopa Decarboxylase and Vesicular Monoamine Transporter 2. The NOIs of the embodiments may also encode proteins such as growth factors and antibodies.

[0022] In a third embodiment of the first aspect of the invention there is provided a lentiviral vector genome capable of encoding tyrosine hydroxylase and GTP-cyclohydrolase I. Preferably the genome is also capable of encoding Aromatic Amino Acid Dopa Decarboxylase or Aromatic Amino Acid Dopa Decarboxylase and Vesicular Monoamine Transporter 2. Preferably the enzymes are encoded by NOIs, which are operably linked by one or more Internal Ribosome Entry sites.

[0023] The second aspect of the invention relates to vector systems.

[0024] In a first embodiment of the second aspect of the invention there is provided a vector system comprising a genome according to the first aspect of the invention.

[0025] In a second embodiment of the second aspect of the invention there is provided a lentiviral vector system which is capable of delivering an RNA genome to a recipient cell, wherein the genome is longer than the wild type genome of the lentivirus. Preferably the lentiviral vector system is an EIAV vector system.

[0026] According to further aspects of the invention, there is provided:

[0027] a method for producing a lentiviral particle which comprises introducing such a viral genome into a producer cell;

[0028] a viral particle produced by such a system or method;

[0029] a pharmaceutical composition comprising such a genome, system or particle;

[0030] the use of such a genome, system or particle in the manufacture of a pharmaceutical composition to treat and/or prevent a disease;

[0031] a cell which has been transduced with such a system;

[0032] a method of treating and/or preventing a disease by using such a genome, system, viral particle or cell;

[0033] According to a yet further aspect there is provided a bicistronic cassette comprising a nucleotide sequence capable of encoding tyrosine hydroxylase and a nucleotide sequence capable of encoding GTP-cyclohydrolase I operably linked by one or more IRES(s). There is also provided a bicistronic cassette encoding Aromatic Amino Acid Dopa Decarboxylase and Vesicular Monoamine Transporter 2.

[0034] According to a yet further aspect there is provided a tricistronic cassette comprising a nucleotide sequence capable of encoding tyrosine hydroxylase, a nucleotide sequence capable of encoding GTP-cyclohydrolase I and a nucleotide sequence capable of encoding Aromatic Amino Acid Dopa Decarboxylase operably linked by two or more IRES(s).

DETAILED DESCRIPTION OF THE INVENTION

[0035] The first aspect of the invention relates to retroviral and lentiviral vector genomes.

[0036] Retroviruses

[0037] The concept of using viral vectors for gene therapy is well known (Verma and Somia (1997) Nature 389:239-242).

[0038] There are many retroviruses. For the present application, the term "retrovirus" includes: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV) and all other retroviridiae including lentiviruses.

[0039] A detailed list of retroviruses may be found in Coffin et al ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: J M Coffin, S M Hughes, H E Varmus pp 758-763).

[0040] Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells (Lewis et al (1992) EMBO J. 3053-3058).

[0041] The lentivirus group can be split into "primate" and "non-primate". Examples of primate lentiviruses include the human immunodeficiency virus (HIV), the causative agent of human acquired immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV).

[0042] The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

[0043] Details on the genomic structure of some lentiviruses may be found in the art. By way of example, details on HIV and EIAV may be found from the NCBI Genbank database (i.e. Genome Accession Nos. AF033819 and AF033820 respectively), maintained by the National Institutes of Health. Details of HIV variants may also be found at in the HIV databases maintained by Los Alamos National Laboratory. Details of EIAV variants may be found at the NCBI website.

[0044] During the process of infection, a retrovirus initially attaches to a specific cell surface receptor. On entry into the susceptible host cell, the retroviral RNA genome is then copied to DNA by the virally encoded reverse transcriptase which is carried inside the parent virus. This DNA is transported to the host cell nucleus where it subsequently integrates into the host genome. At this stage, it is typically referred to as the provirus. The provirus is stable in the host chromosome during cell division and is transcribed like other cellular genes. The provirus encodes the proteins and other factors required to make more virus, which can leave the cell by a process sometimes called "budding".

[0045] Each retroviral genome comprises genes called gag, pol and env which code for virion proteins and enzymes. These genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration, and transcription. They also serve as enhancer-promoter sequences. In other words, the LTRs can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

[0046] The LTRs themselves are identical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5'end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

[0047] For the viral genome, the site of transcription initiation is at the boundary between U3 and R in the left hand side LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the right hand side LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses have any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: tat, rev, tax and rex.

[0048] With regard to the structural genes gag, pol and env themselves, gag encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The pol gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The env gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor

proteins. This interaction leads ultimately to infection by fusion of the viral membrane with the cell membrane.

[0049] Retroviruses may also contain "additional" genes which code for proteins other than gag, pol and env. Examples of additional genes include in HIV, one or more of vif, vpr, vpx, vpu, tat, rev and nef. EIAV has, for example, the additional genes S2 and dUTPase.

[0050] Proteins encoded by additional genes serve various functions, some of which may be duplicative of a function provided by a cellular protein. In EIAV, for example, tat acts as a transcriptional activator of the viral LTR. It binds to a stable, stem-loop RNA secondary structure referred to as TAR. Rev regulates and co-ordinates the expression of viral genes through rev-response elements (RRE). The mechanisms of action of these two proteins are thought to be broadly similar to the analogous mechanisms in the primate viruses. The function of S2 is unknown. In addition, an EIAV protein, Ttm, has been identified that is encoded by the first exon of tat spliced to the env coding sequence at the start of the transmembrane protein.

[0051] Delivery Systems

[0052] Retroviral vector systems have been proposed as a delivery system for inter alia the transfer of a NOI to one or more sites of interest. The transfer can occur in vitro, ex vivo, in vivo, or combinations thereof. Retroviral vector systems have even been exploited to study various aspects of the retrovirus life cycle, including receptor usage, reverse transcription and RNA packaging (reviewed by Miller, 1992 Curr Top Microbiol Immunol 158:1-24).

[0053] A recombinant retroviral vector particle is capable of transducing a recipient cell with an NOI. Once within the cell the RNA genome from the vector particle is reverse transcribed into DNA and integrated into the DNA of the recipient cell.

[0054] As used herein, the term "vector genome" refers to both to the RNA construct present in the retroviral vector particle and the integrated DNA construct. The term also embraces a separate or isolated DNA construct capable of encoding such an RNA genome. A retroviral or lentiviral genome should comprise at least one component part derivable from a retrovirus or a lentivirus. The term "derivable" is used in its normal sense as meaning a nucleotide sequence or a part thereof which need not necessarily be obtained from a virus such as a lentivirus but instead could be derived therefrom. By way of example, the sequence may be prepared synthetically or by use of recombinant DNA techniques. Preferably the genome comprises a psi region (or an analogous component which is capable of causing encapsidation).

[0055] The viral vector genome is preferably "replication defective" by which we mean that the genome does not comprise sufficient genetic information alone to enable independent replication to produce infectious viral particles within the recipient cell. In a preferred embodiment, the genome lacks a functional env, gag or pol gene.

[0056] The viral vector genome may comprise some or all of the long terminal repeats (LTRs). Preferably the genome comprises at least part of the LTRs or an analogous sequence which is capable of mediating proviral integration, and transcription. The sequence may also comprise or act as an enhancer-promoter sequence.

[0057] The viral vector genome of the first aspect of the invention may be provided as a kit of parts. For example, the kit may comprise (i) a plasmid or plasmids containing the NOIs and IRES sequence(s); and (ii) a retroviral genome construct with suitable restriction enzyme recognition sites for cloning the NOIs and IRES(s) into the viral genome.

[0058] It is known that the separate expression of the components required to produce a retroviral vector particle on separate DNA sequences cointroduced into the same cell will yield retroviral particles carrying defective retroviral genomes that carry therapeutic genes (e.g. Reviewed by Miller 1992). This cell is referred to as the producer cell (see below).

[0059] There are two common procedures for generating producer cells. In one, the sequences encoding retroviral Gag, Pol and Env proteins are introduced into the cell and stably integrated into the cell genome; a stable cell line is produced which is referred to as the packaging cell line. The packaging cell line produces the proteins required for packaging retroviral RNA but it cannot bring about encapsidation due to the lack of a psi region. However, when a vector genome according to the first aspect of the invention (having a psi region) is introduced into the packaging cell line, the helper proteins can package the psi-positive recombinant vector RNA to produce the recombinant virus stock. This can be used to transduce the NOI into recipient cells. The recombinant virus whose genome lacks all genes required to make viral proteins can infect only once and cannot propagate. Hence, the NOI is introduced into the host cell genome without the generation of potentially harmful retrovirus. A summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: J M Coffin, S M Hughes, H E Varmus pp 449).

[0060] The present invention also provides a packaging cell line comprising a viral vector genome of the first aspect of the invention. For example, the packaging cell line may be transduced with a viral vector system comprising the genome or transfected with a plasmid carrying a DNA construct capable of encoding the RNA genome. The present invention also provides a retroviral (or lentiviral) vector particle produced by such a cell.

[0061] The second approach is to introduce the three different DNA sequences that are required to produce a retroviral vector particle i.e. the env coding sequences, the gag-pol coding sequence and the defective retroviral genome containing one or more NOIs into the cell at the same time by transient transfection and the procedure is referred to as transient triple transfection (Landau & Littman 1992; Pear et al 1993). The triple transfection procedure has been optimised (Soneoka et al 1995; Finer et al 1994). WO 94/29438 describes the production of producer cells in vitro using this multiple DNA transient transfection method.

[0062] The components of the viral system which are required to complement the vector genome may be present on one or more "producer plasmids" for transfecting into cells.

[0063] The present invention also provides a vector system, comprising

[0064] (i) a viral genome according to the first aspect of the invention;

[0065] (ii) a nucleotide sequence coding for lentiviral gag and pol proteins;

[0066] (iii) nucleotide sequences encoding other essential viral packaging components not encoded by the nucleotide sequence of ii). In a preferred embodiment, the nucleotide sequence of (iii) is capable of encoding an env protein. The present invention also provides a cell transfected with such a vector system and a retroviral vector particle produced by such a cell. Preferably the gag-pol sequence is codon optimised for use in the particular producer cell (see below).

[0067] The env protein encoded by the nucleotide sequence of iii) may be a homologous retroviral or lentiviral env protein. Alternatively, it may be a heterologous env, or an env from a non-retro or lentivirus (see below under "pseudotyping").

[0068] The term "viral vector system" is used generally to mean a kit of parts which can be used when combined with other necessary components for viral particle production to produce viral particles in host cells. For example, the retroviral vector genome may lack one or more of the genes needed for viral replication. This may be combined in a kit with a further complementary nucleotide sequence or sequences, for example on one or more producer plasmids. By cotransfection of the genome together with the producer plasmid(s), the necessary components should be provided for the production of infectious viral particles.

[0069] Alternatively, the complementary nucleotide sequence(s) may be stably present within a packaging cell line that is included in the kit.

[0070] The present invention also relates to a lentiviral vector system which is capable of delivering an RNA genome to a recipient cell, wherein the genome is longer than the wild type genome of the lentivirus. The vector system may, for example, be an EIAV vector system.

[0071] Preferably the RNA genome of the vector system has up to 5%, more preferably up to 10% or even up to 30% more bases than the wild-type genome. Preferably the RNA genome is about 10% longer than the wild-type genome. For example, wild type EIAV comprises an RNA genome of approximately 8 kb. An EIAV vector system of the present invention may have an RNA genome of up to (preferably about) 8.8 kb.

[0072] Preferably the retroviral vector system of the present invention is a self-inactivating (SIN) vector system.

[0073] By way of example, self-inactivating retroviral vector systems have been constructed by deleting the transcriptional enhancers or the enhancers and promoter in the U3 region of the 3' LTR. After a round of vector reverse transcription and integration, these changes are copied into both the 5' and the 3' LTRs producing a transcriptionally inactive provirus. However, any promoter(s) internal to the LTRs in such vectors will still be transcriptionally active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes. Such effects include increased transcription or suppression of transcription. This strategy can also be used to eliminate downstream transcription from the 3' LTR into genomic DNA. This is of particular concern in human gene therapy where it may be important to prevent the adventitious activation of an endogenous oncogene. Yu et al., (1986) PNAS 83: 3194-98; Marty et al., (1990) Biochimie 72: 885-7; Naviaux et al., (1996) J. Virol. 70: 5701-5; Iwakuma et al., (1999) Virol. 261: 120-32; Deglon et al., (2000) Human Gene Therapy 11: 179-90.

[0074] Preferably a recombinase assisted mechanism is used which facilitates the production of high titre regulated lentiviral vectors from the producer cells of the present invention.

[0075] As used herein, the term "recombinase assisted system" includes but is not limited to a system using the Cre recombinase/loxP recognition sites of bacteriophage P1 or the site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs).

[0076] The site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs) has been configured into DNA constructs in order to generate high level producer cell lines using recombinase-assisted recombination events (Karreman et al (1996) NAR 24:1616-1624). A similar system has been developed using the Cre recombinase/loxP recognition sites of bacteriophage P1 (Vanin et al (1997) J. Virol 71:7820-7826). This was configured into a lentiviral genome such that high titre lentiviral producer cell lines were generated.

[0077] By using producer/packaging cell lines, it is possible to propagate and isolate quantities of retroviral vector particles (e.g. to prepare suitable titres of the retroviral vector particles) for subsequent transduction of, for example, a site of interest (such as adult brain tissue). Producer cell lines are usually better for large scale production or vector particles.

[0078] Transient transfection has numerous advantages over the packaging cell method. In this regard, transient transfection avoids the longer time required to generate stable vector-producing cell lines and is used if the vector genome or retroviral packaging components are toxic to cells. If the vector genome encodes toxic genes or genes that interfere with the replication of the host cell, such as inhibitors of the cell cycle or genes that induce apoptosis, it may be difficult to generate stable vector-producing cell lines, but transient transfection can be used to produce the vector before the cells die. Also, cell lines have been developed using transient infection that produce vector titre levels that are comparable to the levels obtained from stable vector-producing cell lines (Pear et al 1993, PNAS 90:8392-8396).

[0079] Producer cells/packaging cells can be of any suitable cell type. Producer cells are generally mammalian cells but can be, for example, insect cells.

[0080] As used herein, the term "producer cell" or "vector producing cell" refers to a cell which contains all the elements necessary for production of retroviral vector particles.

[0081] Preferably, the producer cell is obtainable from a stable producer cell line.

[0082] Preferably, the producer cell is obtainable from a derived stable producer cell line.

[0083] Preferably, the producer cell is obtainable from a derived producer cell line.

[0084] As used herein, the term "derived producer cell line" is a transduced producer cell line which has been screened and selected for high expression of a marker gene. Such cell lines support high level expression from the retroviral genome. The term "derived producer cell line" is used interchangeably with the term "derived stable producer cell line" and the term "stable producer cell line.

[0085] Preferably the derived producer cell line includes but is not limited to a retroviral and/or a lentiviral producer cell.

[0086] Preferably the derived producer cell line is an HIV or EIAV producer cell line, more preferably an EIAV producer cell line.

[0087] Preferably the envelope protein sequences, and nucleocapsid sequences are all stably integrated in the producer and/or packaging cell. However, one or more of these sequences could also exist in episomal form and gene expression could occur from the episome.

[0088] As used herein, the term "packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant virus which are lacking in the RNA genome. Typically, such packaging cells contain one or more producer plasmids which are capable of expressing viral structural proteins (such as codon optimised gag-pol and env) but they do not contain a packaging signal.

[0089] The term "packaging signal" which is referred to interchangeably as "packaging sequence" or "psi" is used in reference to the non-coding, cis-acting sequence required for encapsidation of retroviral RNA strands during viral particle formation. In HIV-1, this sequence has been mapped to loci extending from upstream of the major splice donor site (SD) to at least the gag start codon.

[0090] Packaging cell lines suitable for use with the above-described vector constructs may be readily prepared (see also WO 92/05266), and utilised to create producer cell lines for the production of retroviral vector particles. As already mentioned, a summary of the available packaging lines is presented in "Retroviruses" (as above).

[0091] Also as discussed above, simple packaging cell lines, comprising a provirus in which the packaging signal has been deleted, have been found to lead to the rapid production of undesirable replication competent viruses through recombination. In order to improve safety, second generation cell lines have been produced wherein the 3'LTR of the provirus is deleted. In such cells, two recombinations would be necessary to produce a wild type virus. A further improvement involves the introduction of the gag-pol genes and the env gene on separate constructs so-called third generation packaging cell lines. These constructs are introduced sequentially to prevent recombination during transfection.

[0092] Preferably, the packaging cell lines are second generation packaging cell lines.

[0093] Preferably, the packaging cell lines are third generation packaging cell lines.

[0094] In these split-construct, third generation cell lines, a further reduction in recombination may be achieved by changing the codons. This technique, based on the redundancy of the genetic code, aims to reduce homology between the separate constructs, for example between the regions of overlap in the gag-pol and env open reading frames.

[0095] The packaging cell lines are useful for providing the gene products necessary to encapsidate and provide a membrane protein for a high titre vector particle production. The packaging cell may be a cell cultured in vitro such as a tissue culture cell line. Suitable cell lines include but are not limited to mammalian cells such as murine fibroblast derived cell lines or human cell lines. Preferably the packaging cell line is a primate or human cell line, such as for example: HEK293, 293-T, TE671, HT1080.

[0096] Alternatively, the packaging cell may be a cell derived from the individual to be treated such as a monocyte, macrophage, blood cell or fibroblast. The cell may be isolated from an individual and the packaging and vector components administered ex vivo followed by re-administration of the autologous packaging cells.

[0097] It is highly desirable to use high-titre virus preparations in both experimental and practical applications. Techniques for increasing viral titre include using a psi plus packaging signal as discussed above and concentration of viral stocks.

[0098] As used herein, the term "high titre" means an effective amount of a retroviral vector or particle which is capable of transducing a target site such as a cell.

[0099] As used herein, the term "effective amount" means an amount of a regulated retroviral or lentiviral vector or vector particle which is sufficient to induce expression of the NOIs at a target site.

[0100] A high-titre viral preparation for a producer/packaging cell is usually of the order of 10^5 to 10^7 retrovirus particles per ml. For transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^9 t.u./ml, more preferably at least 10^9 t.u./ml. (The titer is expressed in transducing units per ml (t.u./ml) as titred on a standard D17 cell line—see Example 9). Other methods of concentration such as ultrafiltration or binding to and elution from a matrix may be used.

[0101] The expression products encoded by the NOIs may be proteins which are secreted from the cell. Alternatively the NOI expression products are not secreted and are active within the cell. For some applications, it is preferred for the NOI expression product to demonstrate a bystander effect or a distant bystander effect; that is the production of the expression product in one cell leading to the modulation of additional, related cells, either neighbouring or distant (e.g. metastatic), which possess a common phenotype. Zennou et al., (2000) Cell 101: 173; Folleuzi et al., (2000) Nat. Genetics 25: 217; Zennou et al., (2001) Nat. Biotechnol. 19: 446.

[0102] The presence of a sequence termed the central polypurine tract (cPPT) may improve the efficiency of gene delivery to non-dividing cells. This cis-acting element is located, for example, in the EIAV polymerase coding region element. Preferably the genome of the present invention comprises a cPPT sequence.

[0103] Preferably the viral genome comprises a post-translational regulatory element. For example, the genome may comprise an element such as the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). Zufferey et al., (1999) J. Virol. 73: 2886; Barry et al., (2001) Human Gene Therapy 12: 1103.

[0104] In addition, or in the alternative, the viral genome may comprise a translational enhancer.

[0105] The NOIs may be operatively linked to one or more promoter/enhancer elements. Transcription of one or more NOIs may be under the control of viral LTRs or alternatively promoter-enhancer elements. Preferably the promoter is a strong viral promoter such as CMV, or is a cellular constitutive promoter such as PGK, beta-actin or EF1alpha. The promoter may be regulated or tissue-specific. Such promoters may be selected from genes such as neurofilaments, nestin, parkin, dopamine receptors, tyrosine hydroxylase. Such promoters may also contain neurorestrictive suppressor sequences such as that found in the mu-opoid receptor gene. In a preferred embodiment, the promoter may be glial-specific or neuron-specific. The control of expression can also be achieved by using such systems as the tetracycline system that switches gene expression on or off in response to outside agents (in this case tetracycline or its analogues).

[0106] Pseudotyping

[0107] In the design of retroviral vector systems it is desirable to engineer particles with different target cell specificities to the native virus, to enable the delivery of genetic material to an expanded or altered range of cell types. One manner in which to achieve this is by engineering the virus envelope protein to alter its specificity. Another approach is to introduce a heterologous envelope protein into the vector particle to replace or add to the native envelope protein of the virus.

[0108] The term pseudotyping means incorporating in at least a part of, or substituting a part of, or replacing all of, an env gene of a viral genome with a heterologous env gene, for example an env gene from another virus. Pseudotyping is not a new phenomenon and examples may be found in WO 99/61639, WO-A-98/05759, WO-A-98/05754, WO-A-97/17457, WO-A-96/09400, WO-A-91/00047 and Mebatsion et al 1997 Cell 90, 841-847.

[0109] In a preferred embodiment of the present invention the vector system is pseudotyped with a gene encoding at least part of the rabies G protein. In a further preferred embodiment of the present invention the vector system is pseudotyped with a gene encoding at least part of the VSV-G protein.

[0110] It has been demonstrated that a lentivirus minimal system can be constructed from HIV, SIV, FIV, and EIAV viruses. Such a system requires none of the additional genes vif, vpr, vpx, vpu, tat, rev and nef for either vector production or for transduction of dividing and non-dividing cells. It has also been demonstrated that an EIAV minimal vector system can be constructed which does not require S2 for either vector production or for transduction of dividing and non-dividing cells. The deletion of additional genes is highly advantageous. Firstly, it permits vectors to be produced without the genes associated with disease in lentiviral (e.g. HIV) infections. In particular, tat is associated with disease. Secondly, the deletion of additional genes permits the vector to package more heterologous DNA. Thirdly, genes whose function is unknown, such as S2, may be omitted, thus reducing the risk of causing undesired effects. Examples of minimal lentiviral vectors are disclosed in WO-A-99/32646 and in WO-A-98/17815.

[0111] Thus, preferably, the delivery system used in the invention is devoid of at least tat and S2 (if it is an EIAV vector system), and possibly also vif, vpr, vpx, vpu and nef. More preferably, the systems of the present invention are also devoid of rev. Rev was previously thought to be essential in some retroviral genomes for efficient virus production. For example, in the case of HIV, it was thought that rev and RRE sequence should be included. However, it has been found that the requirement for rev and RRE can be reduced or eliminated by codon optimisation (see below) or by replacement with other functional equivalent systems such as the MPMV system. As expression of the codon optimised gag-pol is REV independent, RRE can be removed from the gag-pol expression cassette, thus removing any potential for recombination with any RRE contained on the vector genome.

[0112] In a preferred embodiment the viral genome of the first aspect of the invention lacks the Rev response element (RRE).

[0113] In a preferred embodiment, the system used in the present invention is based on a so-called "minimal" system in which some or all of the additional genes have be removed.

[0114] Condon Optimisation

[0115] Codon optimisation has previously been described in WO99/41397. Different cells differ it their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available.

[0116] Many viruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms.

[0117] Codon optimisation has a number of other advantages. By virtue of alterations in their sequences, the nucleotide sequences encoding the packaging components of the viral particles required for assembly of viral particles in the producer cells/packaging cells have RNA instability sequences (INS) eliminated from them. At the same time, the amino acid sequence coding sequence for the packaging components is retained so that the viral components encoded by the sequences remain the same, or at least sufficiently similar that the function of the packaging components is not compromised. Codon optimisation also overcomes the Rev/RRE requirement for export, rendering optimised sequences Rev independent. Codon optimisation also reduces homolo-

gous recombination between different constructs within the vector system (for example between the regions of overlap in the gag-pol and env open reading frames). The overall effect of codon optimisation is therefore a notable increase in viral titre and improved safety.

[0118] In one embodiment only codons relating to INS are codon optimised. However, in a much more preferred and practical embodiment, the sequences are codon optimised in their entirety, with the exception of the sequence encompassing the frameshift site.

[0119] The gag-pol gene comprises two overlapping reading frames encoding gag and pol proteins respectively. The expression of both proteins depends on a frameshift during translation. This frameshift occurs as a result of ribosome "slippage" during translation. This slippage is thought to be caused at least in part by ribosome-stalling RNA secondary structures. Such secondary structures exist downstream of the frameshift site in the gag-pol gene. For HIV, the region of overlap extends from nucleotide 1222 downstream of the beginning of gag (wherein nucleotide 1 is the A of the gag ATG) to the end of gag (nt 1503). Consequently, a 281 bp fragment spanning the frameshift site and the overlapping region of the two reading frames is preferably not codon optimised. Retaining this fragment will enable more efficient expression of the gag-pol proteins.

[0120] For EIAV the beginning of the overlap has been taken to be nt 1262 (where nucleotide 1 is the A of the gag ATG). The end of the overlap is at 1461 bp. In order to ensure that the frameshift site and the gag-pol overlap are preserved, the wild type sequence has been retained from nt 1156 to 1465.

[0121] Derivations from optimal codon usage may be made, for example, in order to accommodate convenient restriction sites, and conservative amino acid changes may be introduced into the gag-pol proteins.

[0122] In a highly preferred embodiment, codon optimisation was based on highly expressed mammalian genes. The third and sometimes the second and third base may be changed.

[0123] Due to the degenerate nature of the Genetic Code, it will be appreciated that numerous gag-pol sequences can be achieved by a skilled worker. Also there are many retroviral variants described which can be used as a starting point for generating a codon optimised gag-pol sequence. Lentiviral genomes can be quite variable. For example there are many quasi-species of HIV-1 which are still functional. This is also the case for EIAV. These variants may be used to enhance particular parts of the transduction process. Examples of HIV-1 variants may be found at in the HIV databases maintained by Los Alamos National Laboratory. Details of EIAV clones may be found at the NCBI database.

[0124] The strategy for codon optimised gag-pol sequences can be used in relation to any retrovirus. This would apply to all lentiviruses, including EIAV, FIV, BIV, CAEV, VMR, SIV, HIV-1 and HIV-2. In addition this method could be used to increase expression of genes from HTLV-1, HTLV-2, HFV, HSRV and human endogenous retroviruses (HERV), MLV and other retroviruses.

[0125] Codon optimisation can render gag-pol expression Rev independent. In order to enable the use of anti-rev or RRE factors in the retroviral vector, however, it would be necessary to render the viral vector generation system totally Rev/RRE independent. Thus, the genome also needs to be modified. This is achieved by optimising vector genome components. Advantageously, these modifications also lead to the production of a safer system absent of all additional proteins both in the producer and in the transduced cell.

[0126] As described above, the packaging components for a retroviral vector include expression products of gag, pol and env genes. In addition, efficient packaging depends on a short sequence of 4 stem loops followed by a partial sequence from gag and env (the "packaging signal"). Thus, inclusion of a deleted gag sequence in the retroviral vector genome (in addition to the full gag sequence on the packaging construct) will optimise vector titre. To date efficient packaging has been reported to require from 255 to 360 nucleotides of gag in vectors that still retain env sequences, or about 40 nucleotides of gag in a particular combination of splice donor mutation, gag and env deletions. It has surprisingly been found that a deletion of all but the N-termnial 360 or so nucleotides in gag leads to an increase in vector titre. Thus, preferably, the retroviral vector genome includes a gag sequence which comprises one or more deletions, more preferably the gag sequence comprises about 360 nucleotides derivable from the N-terminus.

[0127] NOIs

[0128] In the present invention, the term NOI (nucleotide sequence of interest) includes any suitable nucleotide sequence, which need not necessarily be a complete naturally occurring DNA or RNA sequence. Thus, the NOI can be, for example, a synthetic RNA/DNA sequence, a codon optimised RNA/DNA sequence, a recombinant RNA/DNA sequence (i.e. prepared by use of recombinant DNA techniques), a cDNA sequence or a partial genomic DNA sequence, including combinations thereof. The sequence need not be a coding region. If it is a coding region, it need not be an entire coding region. In addition, the RNA/DNA sequence can be in a sense orientation or in an anti-sense orientation. Preferably, it is in a sense orientation. Preferably, the sequence is, comprises, or is transcribed from cDNA.

[0129] The NOI(s), also referred to as "heterologous sequence(s)", "heterologous gene(s)" or "transgene(s)", may be any one or more of, for example, a selection gene(s), marker gene(s) and therapeutic gene(s).

[0130] The NOI may be a candidate gene which is of potential significance in a disease process. Thus the vector system of the present invention may, for example, be used for target validation purposes.

[0131] The NOI may have a therapeutic or diagnostic application. Suitable NOIs include, but are not limited to: sequences encoding enzymes, cytokines, chemokines, hormones, antibodies, anti-oxidant molecules, engineered immunoglobulin-like molecules, a single chain antibody, fusion proteins, immune co-stimulatory molecules, immunomodulatory molecules, anti-sense RNA, a transdominant negative mutant of a target protein, a toxin, a conditional toxin, an antigen, a tumour suppresser protein and growth factors, membrane proteins, vasoactive proteins and peptides, anti-viral proteins and ribozymes, and derivatives thereof (such as with an associated reporter group). The NOIs may also encode pro-drug activating enzymes.

[0132] Preferably the NOI is useful in the treatment of a neurodegenerative disorder.

[0133] More preferably the NOI is useful in the treatment of Parkinson's disease.

[0134] The NOI may encode an enzyme involved in dopamine synthesis or storage. For example, the enzyme may be one of the following: Tyrosine Hydroxylase, GTP-cyclohydrolase I and/or Aromatic Amino Acid Dopa Decarboxylase. The sequences of all three genes are available: Accession Nos. X05290, U19523 and M76180 respectively.

[0135] Alternatively the NOI may encode the vesicular monoamine transporter 2 (VMAT2, Accession number L23205.1). In a preferred embodiment the viral genome comprises an NOI encoding Aromatic Amino Acid Dopa Decarboxylase and an NOI encoding VMAT 2. Such a genome may be used in the treatment of Parkinson's disease, in particular in conjunction with peripheral administration of L-DOPA.

[0136] Alternatively the NOI may encode a growth factor capable of blocking or inhibiting degeneration in the nigrostriatal system. An example of such a growth factor is a neurotrophic factor. For example the NOI may encode glial cell-line derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), persephin growth factor, artemin growth factor, or neurturin growth factor, cilliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), pantropic neurotrophin, and other related or unrelated neurotrophic factors. WO99/14235; WO00/18799; U.S. Pat. No. 6,090,778; U.S. Pat. No. 5,834,914; WO97/08196; U.S. Pat. No. 6,090,778; U.S. Pat. No. 5,288,622; WO92/05254; U.S. Pat. No. 6,037,320; WO95/33829; Baumgartner, B J and Shine, H D, J. Neurosci. 17: 6504-11 (1997). In a preferred embodiment, a lentiviral vector comprises one or more of these NOIs encoding neurotrophic factors.

[0137] Alternatively the NOI may encode a neuroprotective factor. In particular, the NOI(s) may encode molecules which prevent TH-positive neurons from dying or which stimulate regeneration and functional recovery in the damaged nigrostriatal system.

[0138] The NOI may encode all or part of the protein of interest ("POI"), or a mutant, homologue or variant thereof. For example, the NOI may encode a fragment of the POI which is capable of functioning in vivo in an analogous manner to the wild-type protein.

[0139] In a highly preferred embodiment, one of the NOIs comprises a truncated form of the TH gene, lacking the regulatory domain. Such an NOI avoids feed-back inhibition by dopamine which may limit expression of the full-length enzyme.

[0140] The term "mutant" includes POIs which include one or more amino acid variations from the wild-type sequence. For example, a mutant may comprise one or more amino acid additions, deletions or substitutions. A mutant may arise naturally, or may be created artificially (for example by site-directed mutagenesis).

[0141] Here, the term "homologue" means an entity having a certain homology with the NOI, or which encodes a protein having a degree of homology with the POI. Here, the term "homology" can be equated with "identity".

[0142] In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

[0143] In the present context, an homologous sequence is taken to include a nucleotide sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical to the subject sequence. Typically, the homologues will comprise the same sequences that code for the active sites etc. as the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

[0144] Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

[0145] % homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

[0146] Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

[0147] However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible—reflecting higher relatedness between the two compared sequences—will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Besffit package the default gap penalty for amino acid sequences is −12 for a gap and −4 for each extension.

[0148] Calculation of maximum % homology therefore firstly requires the production of an optimal alignment,

taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Besffit package (University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid—Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

[0149] Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix—the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

[0150] Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

[0151] The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

[0152] Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P I L V
	Polar - uncharged	C S T M N Q
	Polar - charged	D E K R
AROMATIC		H F W Y

[0153] The present invention also encompasses homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another.

[0154] Preferably the NOI encodes a single POI or a mutant, homologue or variant thereof. In a highly preferred embodiment, the NOI does not encode a fusion protein. As used herein, the term "fusion protein" is used in its conventional sense to mean an entity which comprises two or more protein activities, joined together by a peptide bond to form a single chimeric protein. A fusion protein is encoded by a single polynucleotide driven by a single promoter.

[0155] Internal Ribosome Entry Site (IRES)

[0156] The viral genome of the first aspect of the invention comprises two or more NOIs. In order for both of the NOIs to be expressed, there may be two or more transcription units within the vector genome, one for each NOI. However, it is clear from the literature that retroviral vectors achieve the highest titres and most potent gene expression properties if they are kept genetically simple (PCT/GB96/01230; Bowtell et al., 1988 J. Virol. 62, 2464; Correll et al., 1994 Blood 84, 1812; Emerman and Temin 1984 Cell 39, 459; Ghattas et al., 1991 Mol. Cell. Biol. 11, 5848; Hantzopoulos et al., 1989 PNAS 86, 3519; Hatzoglou et al., 1991 J. Biol. Chem 266, 8416; Hatzoglou et al., 1988 J. Biol. Chem 263, 17798; Li et al., 1992 Hum. Gen. Ther. 3, 381; McLachlin et al., 1993 Virol. 195, 1; Overell et al., 1988 Mol. Cell Biol. 8, 1803; Scharfman et al., 1991 PNAS 88, 4626; Vile et al., 1994 Gene Ther 1, 307; Xu et al., 1989 Virol. 171, 331; Yee et al., 1987 PNAS 84, 5197) and so it is preferable to use an internal ribosome entry site (IRES) to initiate translation of the second (and subsequent) coding sequence(s) in a polycistronic message (Adam et al 1991 J. Virol. 65, 4985).

[0157] Insertion of IRES elements into retroviral vectors is compatible with the retroviral replication cycle and allows expression of multiple coding regions from a single promoter (Adam et al (as above); Koo et al (1992) Virology 186:669-675; Chen et al 1993 J. Virol 67:2142-2148). IRES elements were first found in the non-translated 5' ends of picomaviruses where they promote cap-independent translation of viral proteins (Jang et al (1990) Enzyme 44: 292-309). When located between open reading frames in an RNA, IRES elements allow efficient translation of the downstream open reading frame by promoting entry of the ribosome at the IRES element followed by downstream initiation of translation.

[0158] A review on IRES is presented by Mounfford and Smith (TIG May 1995 vol 11, No 5:179-184). A number of different IRES sequences are known including those from encephalomyocarditis virus (EMCV) (Ghattas, I. R., et al., *Mol. Cell.* Biol., 11:5848-5859 (1991); BiP protein [Macejak and Sarnow, Nature 353:91 (1991)]; the *Antennapedia* gene of *Drosophila* (exons d and e) [Oh, et al., *Genes & Development,* 6:1643-1653 (1992)] as well as those in polio virus (PV) [Pelletier and Sonenberg, *Nature* 334: 320-325 (1988); see also Mounfford and Smith, TIG 11, 179-184 (1985)].

[0159] According to WO-A-97/14809, IRES sequences are typically found in the 5' non-coding region of genes. In

addition to those in the literature they can be found empirically by looking for genetic sequences that affect expression and then determining whether that sequence affects the DNA (i.e. acts as a promoter or enhancer) or only the RNA (acts as an IRES sequence).

[0160] IRES elements from PV, EMCV and swine vesicular disease virus have previously been used in retroviral vectors (Coffin et al, as above).

[0161] The term "IRES" includes any sequence or combination of sequences which work as or improve the function of an IRES.

[0162] The IRES(s) may be of viral origin (such as EMCV IRES, PV IRES, or FMDV 2A-like sequences) or of cellular origin (such as FGF2 IRES, NRF IRES, Notch 2 IRES or EIF4 IRES). Examples of IRES elements and references in which they are described are set forth below.

Virus/gene type	Virus/gene	Reference
Viral RNAs		
Picornaviruses	Poliovirus (PV)	Pelletier & Sonenberg (1988)
	Encephalomyocarditis virus (EMCV)	Jang et al. (1988)
	Foot-and-mouth disease virus (FMDV)	Kühn et al. (1990)
Flavivirus	Hepatitus C virus (HCV)	Reynolds et al. (1995)
Pestivirus	Classical swine fever virus (CSFV)	Pestova et al. (1998)
Retrovirus	Murine leukemia virus (MLV)	Berlioz & Darlix (1995)
Lentivirus	Simian immunodeficiency virus (SIV)	Ohlmann et al. (2000)
Cellular mRNAs		
Translation initiation factors	eIF4G	Johannes & Sarnow (1998)
	DAP5	Henis-Korenblit et al. (2000)
Transcription factors	c-Myc	Stoneley et al. (2000)
	NE-κB-repressing factor (NRF)	Oumrad et al. (2000)
Growth factors	Vascular endothelial growth facter (VEGF)	Huez et al. (1998)
	Fibroblast growth factor 2 (FGF-2)	Vagner et al. (1995)
	Platelet-derived growth factor B (PDGF B)	Bernstein et al. (1997)
Homeotic genes	*Antennapedia*	Oh et al. (1992)
Survival proteins	Apaf-1	Coldwell et al. (2000)
Miscellaneous	BiP	Macejak & Samow (1991)

[0163] In order for the IRES to be capable of initiating translation of each NOI, it should be located between or prior to NOIs in the vector genome. For example, for a multicistronic sequence containing n NOIs, the genome may be as follows:

[0164] $[(NOI_1\text{-}IRES_1] \ldots NOI_n \; n=1 \rightarrow n$

[0165] For bi and tricistronic sequences, the order may be as follows:

[0166] $NOI_1\text{-}IRES_1\text{-}NOI_2$

[0167] $NOI_1\text{-}IRES_1\text{-}NOI_2\text{-}IRES_2\text{-}NOI_3$

[0168] Alternative configurations of IRESs and NOIs can also be utilised. For example transcripts containing the IRESs and NOIs need not be driven from the same promoter.

[0169] An example of this arrangement may be:

[0170] $IRES_1\text{-}NOI_1\text{-promoter-}NOI_2\text{-}IRES_2\text{-}NOI_3$.

[0171] In a preferred embodiment, in any construct utilising an internal cassette having more than one IRES and NOI, the IRESs may be of different origins, that is, heterologous to one another. For example, one IRES may be from EMCV and the other IRES may be from polio virus.

[0172] Other Methods of Expressing Multiple Genes from One Vector

[0173] Although IRESs are an efficient way to co-express multiple genes from one vector, other methods are also useful, and may be used alone or in conjunction with IRESs. These include the use of multiple internal promoters in the vector (Overell et al., Mol Cell Biol. 8: 1803-8 (1988)), or the use of alternate splicing patterns leading to multiple RNA species derived from the single viral genome that expresses the different genes. This strategy has previously been used by itself for two genes (Cepko et al. Cell 37: 1053 (1984)).

[0174] Transduced Cells

[0175] The present invention also relates to a cell which has been transduced with a vector system comprising a viral genome according to the first aspect of the invention.

[0176] Transduction with the vector system of the present invention may confer or increase the ability of the cell to produce catecholamines. It may, for example, confer or increase the ability of the cell to convert tyrosine to L-dopa and/or L-dopa to dopamine. Release of catecholamines can be measured by techniques known in the art, for example by using an electrochemical detector connected to an analytical cell. In addition of the catecholamines themselves, biproducts associated with catecholamine release (such as DOPAC, a specific degradation product of dopamine) may also be detected.

[0177] The cell may be transduced in vivo, in vitro or ex vivo. For example, if the cell is a cell from a mammalian subject, the cell may be removed from the subject and transduced ready for reimplantation into the subject (ex vivo transduction). Alternatively the cell may be transduced by direct gene transfer in vivo, using the vector system of the

present invention in accordance with standard techniques (such as via injection of vector stocks expressing the NOIs). If the cell is part of a cell line which is stable in culture (i.e. which can survive numerous passages and can multiple in vitro) then it may be transduced in vitro by standard techniques, for example by exposure of the cell to viral supernatants comprising vectors expressing the NOIs.

[0178] The cell may be any cell which is susceptible to transduction. If the vector system is capable of transducing non-dividing cells (for example if it is a lentiviral system) then the cell may be a non-dividing cell such as a neuron.

[0179] In a preferred embodiment the transduced cell forms part of a genetically modified neuronal cell line. Such a cell line may, for example, be transplanted into the brain for the treatment of Parkinson's disease.

[0180] In a further embodiment the cell is a neuronal stem cell. Such a cell line may, for example, be transplanted into the brain for the treatment of Parkinson's disease.

[0181] In a further embodiment the cell is a cell in the striatum of a subject, such as a neuron or glial cell. Direct gene transfer in vivo to such a cell may, for example, convert it into a dopamine-producer cell.

[0182] Cassettes

[0183] The present invention also provides multicistronic cassettes comprising two or more NOIs operably linked by an IRES. These cassettes may be used in a method for producing the vector genome in a producer cell.

[0184] The present invention also provides an expression vector comprising such a cassette. Transfection of a suitable cell with such an expression vector should result I-n a cell which expresses each POI encoded by the NOI in the cassette. The present invention also provides such a transfected cell.

[0185] Cloning of the cassette into an expression vector and transfection of cells with the vector (to give expression of the cassette) can be carried out by techniques well known in the art (such as those described in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks).

[0186] Preferably the cassette comprises a promoter. In a highly preferred embodiment the cassette is bicistronic or tricistronic and comprises the following elements:

[0187] Promoter-(NOI_1)-($IRES_1$)-(NOI_2)

[0188] Promoter-(NOI_1)-($IRES_1$)-(NOI_2)-($IRES_2$)-(NOI_3)

[0189] In a particularly preferred embodiment the cassette is bicistronic and comprises an NOI encoding tyrosine hydroxylase (or a mutant, variant or homologue thereof) and an NOI encoding GTP-cyclohydrolase I (or a mutant, variant or homologue thereof) in either order. In another particularly preferred embodiment the cassette is bicistronic and comprises an NOI encoding Aromatic Amino Acid Dopa Decarboxylase and an NOI encoding Vesicular Monoamine Transporter 2, in either order.

[0190] In another particularly preferred embodiment the cassette is tricistronic and comprises an NOI encoding tyrosine hydroxylase (or a mutant, variant or homologue thereof), an NOI encoding GTP-cyclohydrolase I (or a mutant, variant or homologue thereof) and an NOI encoding Aromatic Amino Acid Dopa Decarboxylase (or a mutant, variant or homologue thereof) in any order.

[0191] Pharmaceutical Compositions

[0192] The present invention also provides the use of a retroviral vector genone as defined in the first aspect of the invention in the manufacture of a pharmaceutical composition. The pharmaceutical composition may be used for treating an individual by gene therapy, wherein the composition comprises a therapeutically effective amount of a retroviral vector particle according to the present invention.

[0193] The pharmaceutical composition may be used to treat a human or animal subject. Preferably the subject is a mammalian subject. More preferably the subject is a human. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

[0194] The composition may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent, any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase the viral entry into the target site (such as for example a lipid delivery system).

[0195] Where appropriate, the pharmaceutical compositions can be administered by any one or more of: inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

[0196] Preferably the viral vector particles of the present invention are administered by injection into the caudate putamen.

[0197] Diseases

[0198] The retroviral vector genome and vector particles of the present invention are particularly useful for the treatment and/or prevention of neurodegenerative diseases.

[0199] Diseases which may be treated include, but are not limited to: Parkinson's disease; motor neuron disease, Huntington's disease and disorders of movement which are responsive to L-dopa, such as distonias.

[0200] In particular, the present invention is useful in treating and/or preventing Parkinson's disease.

[0201] Treatment by gene therapy with vectors capable of delivering, for example, TH, GTP-CH1 and optionally AADC or AADC and VMAT2, is likely to be particularly useful for the late stages of PD patients which do not respond significantly to L-dopa treatment. Treatment using AADC or AADC and VMAT2, in combination with L-dopa administered peripherally may also be useful for late stage PD patients.

[0202] The present invention will now be described only by way of example, in which reference will be made to the following Figures and Tables

[0203] FIG. **1**: Oligonucleotide sequences of the primers (SEQ ID NOs: 8-11) used for cloning the human Tyrosine Hydroxylase Type 2 cDNA (Accession number X05290). Restriction endonuclease recognition sites (BamHI and HindIII) appear underlined, consensus Kozak sequence in italic, and c-myc epitope in bold.

[0204] FIG. **2**: Plasmid map of pNE4. A mammalian expression plasmid derived from pcDNA3.1/Zeo that expresses the c-myc tagged human Tyrosine Hydroxylase Type 2 (cmyc-hTH).

[0205] FIG. **3**: Oligonucleotide sequences of the primers (SEQ ID NOs: 12 and 13) used for cloning the human Aromatic Amino Acid Dopa Decarboxylase cDNA (Accession number M76180 M30772). Restriction endonuclease recognition sites (BgI II and HindIII) appear underlined, consensus Kozak sequence in italic, and HA epitope in bold.

[0206] FIG. **4**: Plasmid map of pNE2. A mammalian expression plasmid derived from pcDNA3.1/Neo that expresses the HA tagged human Aromatic Amino Acid Dopa Decarboxylase (HA-hAADC).

[0207] FIG. **5**: Oligonucleotide sequences of the primers (SEQ ID NOs: 14 and 15) used for cloning the human GTP-cyclohydrolase 1 cDNA (Accession number U19523). Restriction endonuclease recognition sites (BgI II and HindIII) appear underlined, consensus Kozak sequence in italic, and FLAG epitope in bold.

[0208] FIG. **6**: Plasmid map of pNE6. A mammalian expression plasmid derived from pcDNA3.1/Hygro that expresses the FLAG tagged human GTP-cyclohydrolase 1 (FLAG-hGTP).

[0209] FIG. **7**: Oligonucleotide sequences of the primers (SEQ ID NOs: 16 and 17) used for cloning a truncated form of the human Tyrosine hydroxylase Type 2. Restriction endonuclease recognition sites (BamHI, HindIII and EcoRI) appear underlined, consensus Kozak sequence in italic, and c-myc epitope in bold.

[0210] FIG. **8**: Plasmid map of phTHt-1. A mammalian expression plasmid derived from pcDNA3.1/Zeo that expresses the truncated form of hTH tagged with the c-myc epitope (cmyc-hTHt).

[0211] FIG. **9**: Plasmid map of pneo2. A mammalian expression plasmid derived from BL-EP (Science (1995) 269:847) that expresses cmyc-hTHt and FLAG-hGTP as a bicistronic cassette. The polio virus IRES is located downstream the cmyc-hTHt gene.

[0212] FIG. **10**: Plasmid map of ptricis. A mammalian expression plasmid derived from BL-EP (Science (1995) 269:847) that expresses HA-hAADC, cmyc-hTHt and FLAG-hGTP as a tricistronic cassette. The EMCV IRES is located downstream the HA-hAADC gene and polio virus IRES downstream the cmyc-hTHt gene.

[0213] FIG. **11**: Transient expression of the Bicistronic and Tricistronic cassettes in HEK 293T cells. Western Blot probed with specific mouse monoclonal antibodies. The tagged proteins bound to the antibodies are detected with a horse radish peroxidase (HRP) conjugated anti mouse rabbit IgG. Lanes; 1, Mock; 2, phTHt; 3, Bicistronic plasmid (pneo2); 4, Tricistronic plasmid (ptricis) and 5, the three monocistronic plasmids (phTHt, pNE2 and pNE6).

[0214] FIG. **12**: A schematic diagram of EIAV minimal vectors.

[0215] FIG. **13**: A schematic diagram of EIAV BIC and EIAV TRIC vectors.

[0216] FIG. **14**: A schematic diagram of EIAV TRIC vectors containing the central polypurine tract (cppt).

[0217] FIG. **15**: PERT and viral RNA content of EIAV vectors

[0218] FIG. **16**: Expression of EIAV BIC and EIAV TRIC vectors in D17 cells transduced at different MOIs (MOI). Western Blot probed with specific mouse monoclonal antibodies. The tagged proteins bound to the antibodies are detected with a horse radish peroxidase (HRP) conjugated anti mouse rabbit IgG. Lanes: a, pONY8G (100×); b, pONY8.1Z (100×); c, pONY8.1BIC (100×); d, pONY8.1BIC (10×); e, pONY8BIC (1×); f, untransduced cells; h, pONY8.1TRIC (100×); i, pONY8.1TRIC (10×); j, pONY8.1TRIC (1×); k, pONY8TRIC (100×); I, pONY8TRIC (10×); m, pONY8TRIC (1×); n, pONY8.1TRIC-B(100×); o, pONY8.1TRIC-B (10×); p, pONY8.1TRIC-B (1×); and g, HEK 293T cells transfected with the monocistronic plasmids (see FIG. **11**).

[0219] FIG. **17**: Expression of EIAV TRIC vectors in D17 cells transduced at an MOI~-100. (A) Immunostaining of D17 cells transduced with EIAVlacZ or EIAV TRIC vectors using rabbit polyclonal anti LacZ or mouse monoclonal anti-HA, respectively. The antibody bound to the native proteins was detected with Alexa 488 (green) conjugated goat anti-rabbit or goat anti-mouse IgG (Magnification ~10×). (B) D17 cells transduced with EIAV TRIC vectors. Immunostaining as in (A)+propidium iodide (red) that stains the nuclei (Magnification ~40×).

[0220] FIG. **18**: Catecholamines (pg/10^6cells) produced by HEK 293T cells transduced with EIAV TRIC vectors.

[0221] FIG. **19**: Transduction of the adult rat striatum with EIAV lacZ vectors. Panels A-C correspond to 3 independent 50 μm coronal sections stained with X-gal. About fifty of such sections are stained per animal, indicating that the transduction spans the rat striatum. Panels D-H represent higher magnification of the section in C showing that many of the cells transduced have neuronal morphology both within striatum (D-F) and in nucleus accumbens (G-H).

[0222] FIG. **20**: Transduction of the adult rat striatum with EIAV TRIC vectors. Panel A represents 50 μm coronal sections stained with mouse monoclonal HA antibody. Immunofluorescent detection with a FITC secondary antibody indicates expression of AADC. Panel C represents 50 μm coronal sections stained with mouse monoclonal FLAG

antibody. Immunofluorescent detection with Alexa 488 indicates expression of GTP-CH1. No expression is detected on the contralateral striatum (Panels B and D). Panel E represents staining with mouse monoclonal c-myc antibody detected with DAB immunohistochemistry. The results indicate that TH is expressed in the ipsilateral but not in the contralateral striatum (panel F). The cell specificty of the expression of these proteins in the transduced side is further confirmation of effective transduction.

[0223] FIG. **21**: Plasmid map of pONY8G

[0224] FIG. **22**: Plasmid map of pONY8.1G

[0225] FIG. **23**: Plasmid map of pONY8Z

[0226] FIG. **24**. (A) Histogram showing the change in turns/minute induced by apomorphine stimulation (0.05 mg/kg) in 6-OHDA lesioned rats after injection of pONY8.1Z or pONY8.1T. pONY8.1Z n=5, pONY8.1T n=2. (B) Apomorphine-induced rotational behavior in the 6-OHDA lesioned rats following injection of pONY8.1 Z (n=4) or pONY8.1T (n=7).

[0227] FIG. **25**. Tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra (A) and the striatum (B) of 6-OHDA lesioned rats injected with EIAV TRIC vectors. Note that there is no TH immunoreactivity on the ipsilateral side, compared with the contralateral side (control), indicating that the 6-OHDA has affected the dopaminergic neurons in substantia nigra pars reticulata (SNr).

[0228] FIG. **26**: Catecholamines (ng/mg wet tissue) content in the normal and denervated striata of 6-OHDA lesioned rats injected with EIAV TRIC vectors. The amount of catecholamines detected in the denervated striatum confirm that the 6-OHDA lesion has affected most of the dopaminergic neurons of the nigra. The amount of dopamine produced by EIAV TRIC varies between 5-8% compared to the unlesioned striatum.

[0229] FIG. **27**: DOPAC/Dopaine ratios in the normal and denervated striata of 6-OHDA lesioned rats injected with EIAV TRIC vectors. Note that the injected animals that had more pronounced reduction in drug-induced rotations are the animals where the DOPAC/Dopamine ratio (dopamine turnover) in the denervated striatum was lower.

[0230] FIG. **28**A shows the amino acid sequence of codon-optimised GTP-cyclohydrolase I (SEQ ID NO:18). FIG. **28**B shows the nucleotide sequence of codon-optimised GTP-cyclohydrolase I (SEQ ID NO:19). FIG. **28**C shows the amino acid sequence of wild type GTP-cyclohydrolase I (SEQ ID NO:20). FIG. **28**D shows the nucleotide sequence of wild type GTP-cyclohydrolase I (SEQ ID NO:21).

[0231] FIG. **29**A shows the amino acid sequence of codon-optimised truncated tyrosine hydroxylase, type 2, from 8.9.4 MV opti Y (SEQ ID NO:22). The tyrosine hydroxylase sequence starts at position 3. MV is a "leader" required for efficient translation. FIG. **29**B shows the nucleotide sequence of codon-optimised truncated tyrosine hydroxylase, type 2, from 8.9.4 MV opti Y (SEQ ID NO:23). FIG. **29**C shows the amino acid sequence of codon-optimised truncated tyrosine hydroxylase, type 2 with serine at position 211 (SEQ ID NO:24). FIG. **29**D shows the nucleotide sequence of codon-optimised tyrosine hydroxylase, type 2 with serine encoded by nucleotides 631-633 (SEQ ID NO:25). FIG. **29**E shows the amino acid sequence of wild type truncated tyrosine hydroxylase, type 2 with tyrosine at position 211 (SEQ ID NO:26). FIG. **29**F shows the nucleotide sequence of truncated wild type tyrosine hydroxylase, type 2, with tyrosine encoded by nucleotides 631-633 (SEQ ID NO:27). FIG. **29**G shows the amino acid sequence of full-length tyrosine hydroxylase, type 2, with tyrosine at position 374 (SEQ ID NO:28). FIG. **29**H shows the nucleotide sequence of full-length tyrosine hydroxylase, type 2, which encodes tyrosine with nucleotides 1120-1122 (SEQ ID NO:29).

[0232] FIG. **30**A shows the amino acid sequence of codon-optimised aromatic amino acid decarboxylase in pONY8.9.4 MV opti Y (SEQ ID NO:30). FIG. **30**B shows the nucleotide sequence of codon-optimised aromatic amino acid decarboxylase in pONY8.9.4 MV opti Y (SEQ ID NO:31). FIG. **30**C shows the amino acid sequence of wild type aromatic amino acid decarboxylase (SEQ ID NO:32). FIG. **30**D shows the nucleotide sequence of wild type aromatic amino acid decarboxylase (SEQ ID NO:33).

[0233] FIG. **31** shows a plasmid map of pONY8.9.4 MV opti Y.

[0234] Table 1: Catecholamines (ng/hour/10^6 cells) released by HEK 293T cells transfected with either the monocistronic, bicistronic or tricistronic plasmids (n.d., no detectable).

[0235] Table 2: Catecholamines (ng/10^6 cells) produced by HEK 293T cells transfected with either the monocistronic, bicistronic or tricistronic plasmids (n.d., no detectable).

[0236] Table 3: Integration efficiency of EIAV vectors.

[0237] Experimental

EXAMPLE 1

Cloning of the Human Tyrosine Hydroxylase 1 Type 2 cDNA

[0238] The human Tyrosine Hydroxylase 1 Type 2 cDNA (Accession number X05290) is amplified by RT-PCR from human Substantia nigra poly A^+ mRNA (Clontech) and epitope tagged with the c-myc epitope using the primers described in FIG. **1**. A 169 bp fragment corresponding to the 5' end of the gene is amplified using 5'hTH2 and 3'hTH2 primers (FIG. **1**) whilst the 1418 bp 3' end fragment of the tyrosine hydroxylase cDNA is obtained using primers 5'hTH3 and 3'hTH1 (FIG. **1**).

[0239] Titan One Tube RT-PCR kit (Boehringer) was used to perform the RT-PCR reaction. Typically the reaction is composed of two solutions.

[0240] Solution A

[0241] Contains 0.2 kg of human substantia nigra poly A+ RNA, 32 μM each dNTPs, 10 mM DTT, 1 μl RNAse Inhibitor (RNAsin, Promega), ~100 ng each primer and water, up to 25 μl.

[0242] Solution B

[0243] Contains 10 μl of 5×RT-PCR Buffer, 1 μl Enzyme mix, and water up to 25 μl.

[0244] Solutions A and B are mixed and the RT-PCR conditions set.

[0245] 1.1. Amplification of the 169 bp product is carried out at 50° C., 30 min, to allow the RT reaction to take place, followed by 2 min at 94° C., and 35 cycles of 30 sec at 94° C., 30 sec at 60° C. and 45 sec at 68° C.

[0246] 1.2. Amplification of the 1418 bp product is performed at 50° C., 30 min, to allow the RT reaction to take place, followed by 2 min at 94° C., and 35 cycles of 1 min at 94° C., 1 min at 60° C. and 2 min at 68° C.

[0247] Both fragments are purified and used as template in a third PCR reaction to obtain the full length Tyrosine Hydroxylase (TH) cDNA. The recombinant PCR reaction is carried out using primers 5'hTH2 and 3'hTH1 (FIG. **1**) and a KlenTaq kit (Clontech) according to the instructions of the manufacturer. The PCR conditions are set up as follows: 35 cycles of 1 min at 94° C., 1 min at 60° C. and 2 min at 68° C. The recombinant PCR product is cloned into pGEM-Teasy vector (Promega) to create pNE3.

[0248] The TH cDNA is then excised from pNE3 as a BamHI-EcoRI 1.57 kb fragment and ligated to pcDNA3.1/Zeo (Invitrogen) previously digested with the same enzymes. The newly generated mammalian expression plasmid is called pNE4 (FIG. **2**).

EXAMPLE 2

Cloning of the Human Aromatic Amino Acid Dopa Decarboxylase 1 cDNA

[0249] The human Aromatic Amino Acid Dopa Decarboxylase (AADC) cDNA (Accession number M76180 M30772) is amplified from a human liver cDNA expression library (Clontech) and epitope tagged with the HA epitope using the primers 5'hAADC and 3'hAADC, described in FIG. **3**. The PCR reaction was performed using a KlenTaq kit (Clontech) following the instructions of the manufacturer. The reaction contains 4 μl human liver cDNA and 1 μM of each primer, in a final volume of 50 μl. The PCR conditions are as follows: A first step, 30 sec at 94° C.; a second step, 5 cycles of 30 sec at 94° C., 30 sec at 58° C. and 2 min at 68° C. and a third step, 30 cycles of 30 sec at 94° C., 30 sec at 55° C. and 2 min at 68° C.

[0250] The PCR amplifies the two expected bands, 1.485 kb and 1.36 kb, corresponding to the two transcripts of the Aromatic Amino Acid Dopa Decarboxylase (AADC). The 1.485 kb band is purified and cloned into pGEM-Teasy vector (Promega) to generate the plasmid called pNE1. The human AADC full-length cDNA is excised from pNE1 as a ~1.5 kb BglII-SalI fragment and ligated to pcDNA3.1/Neo digested previously with BamHI and XhoI enzymes. The new plasmid generated is called pNE2 (FIG. **4**).

EXAMPLE 3

Cloning of the Human GTP-Cyclohydrolase 1 cDNA

[0251] The human GTP-cyclohydrolase I (GTP-CH1) cDNA (Accession number U19523) is amplified from Poly A+ mRNA from human Substantia nigra and epitope tagged with the FLAG epitope using the primers 5'hGTP and 3'hGTP (FIG. **5**). Titan One Tube RT-PCR kit (Boehringer) was used to perform the RT-PCR reaction. Typically the reaction is composed of two solutions, as described above in Example 1. Solutions A and B are mixed and the RT-PCR conditions set as follows: 50° C., 30 min, to allow the RT reaction to take place, followed by 30 sec at 94° C., and 35 cycles of 30 sec at 94° C., 30 sec at 60° C. and 1 min at 68° C.

[0252] The RT-PCR product (~0.75 kb) is purified and cloned into pGEM-Teasy vector (Promega) to generate plasmid pNE5. The GTP-CH cDNA is excised from pNE5 as a ~0.75 kb BglII-NotI and ligated to pcDNA3.1/Hygro digested with BamHI and NotI enzymes to generate pNE6 (FIG. **6**).

EXAMPLE 4

Cloning of a Truncated form of the Human Tyrosine Hydroxylase I Type 2

[0253] To avoid feed-back inhibition by dopamine on the TH enzyme it was decided to use the truncated form of TH type 2 lacking the regulatory domain. TH is activated by phosphorylation at sites located in this N-terminal domain and undergoes feedback end-product inhibition mediated by at least one of this phosphorylation sites (J. Biol. Chem. (1992) 267:25754-25758; Adv. Exp. Med. & Biol. (1993) 338:87-92). This truncated TH (hTHt) is epitope tagged with the c-myc epitope and amplified by PCR using the primers 5'hTHt and 3'hTHt (FIG. **7**) and plasmid pNE4 as template. The PCR reaction is carried out using Pfu I Polymerase (Stratagene) at 95° C., 1 min and 30 cycles of 1 min at 95° C., 1 min at 60° C. and 1 min at 72° C. A ~1.04 kb band is amplified, digested with BamHI and EcoRI enzymes and ligated to pcDNA3.1/Zeo previously digested with the same enzymes. The new plasmid generated is called phTHt (FIG. **8**).

EXAMPLE 5

Cloning of TH, AADC and GTP-CH into a Mammalian Expression Vector

[0254] The hTHt cDNA is cloned into BL-EP plasmid (Science, 269:847 (1995)) downstream the EMCV IRES. To achieve this, CMVp-hTHt fragments from phTHt is excised as a BgIII-EcoRV and cloned into BLEP digested with BamHI-EcoRV to generate pneo1. The CMVp-DC fragment is excised from pNE2 as a BgIII-EcoRV and ligated to BLEP cut with SmaI-BamHI to generate BLEP-CMV-DC.

[0255] To create a mammalian expression cassette comprising the hTHt and GTP-CH1 genes (bicistronic cassette), the GTP-CH1 cDNA is cloned downstream of the polio IRES as follows. The GTP-CH1 cDNA is excised from pNE6 as a ~0.75 kb NheI-XbaI fragment and cloned into BLEP-THt digested with the same enzymes. The new plasmid is called BLEP-THt-CH1. The CMVp-THt fragment is excised from pneo1 as a XbaI-EcoRV fragment and ligated to BLEP-hTHt-CH1 digested with the same enzymes to generate pneo2 (pbicis) (FIG. **9**).

[0256] To create a tricistronic cassette comprising the hTHt, GTP-CH1 and AADC genes, BLEP-CMVp-DC and BLEP-hTHt-CH1 are digested with BlnI-ClaI to generate ptricis (FIG. **10**). This creates a cassette which has the CMV promoter, DC, hTHt and GTP-CH1 in that order.

EXAMPLE 6

Transient Expression from the Bicistronic and Tricistronic Cassettes in Heterologous Human Cells

[0257] Human embryonic kidney 293T (HEK293T) cells do not synthesise any catecholamines and they do not

express any catecholaminergic enzymes. They are chosen to determine if the bicistronic and tricistronic expression cassettes are functional. HEK 293T cells are seeded in a 6× well plates at a density of ~2-3×10^5 cells/well. Twenty-four hours post-plating the cells are transfected with 2 μg of plasmid DNA using Fugene™ (Roche) in serum-free medium, following the instructions of the manufacturer. As control of transfection 0.2 μg ($1/10^{th}$) of the GFP expressing plasmid pEGFP-C1 (Clontech) is added to the DNA-Fugene™ mix.

[0258] Approximately 48 h post-transfection the cells are washed in Phosphate Buffer Saline (PBS) and harvested. Total cell extracts are prepared using Lysis buffer (Promega). Approximately 10 μg of total protein are loaded onto three 10% SDS-PAGE gel and the proteins are separated and transferred to a nitrocellulose ECL-western membrane (Amersham-Pharmacia). The membranes are probed with $1/1000^{th}$ dilution of either mouse anti-HA (Roche), mouse anti-cmyc (Roche) or mouse anti-FLAG (Sigma) antibodies. The secondary antibody was a $1/2000^{th}$ dilution of HRP-labelled rabbit anti-mouse (Dako). The antibodies bound to the membranes are detected using an ECL-Western detection kit.

[0259] Proteins of the appropriate apparent molecular weight are detected in the transfected cells and not in the mock control: HA-hAADC, ~53 kDa; cmyc-hTHt, ~42 kDa and FLAG-GTP/CH1, ~30 kDa. The bicistronic and tricistronic cassettes express two or three of the enzymes, respectively (FIG. **11**).

EXAMPLE 7

Production of Catecholamines in Transiently Transfected Human Cells

[0260] As described in example 6, HEK 293T cells are seeded in a 6× well plates at a density of ~2-3×10^5 cells/well. Twenty-four hours post-plating the cells are transfected with 2 μg of plasmid DNA using Fugene™ (Roche) in serum-free medium, following the instructions of the manufacturer. As control of transfection 0.2 μg ($1/10^{th}$) of the GFP expressing plasmid pEGFP-C1 (Clontech) is added to the DNA-Fugene™ mix.

[0261] Approximately 48 h post-transfection the cells are washed in Phosphate Buffer Saline (PBS). To measure the catecholamines release into the medium, 0.5 ml of 'Release Buffer' (Hank's Balanced Salt Solution, 25 mM Hepes pH7.4, 0.25% BSA and 1 nM tyrosine) is added to the transfected cells. These cells are incubated at 37° C. for 30 min. No tetra-hydrobiopterin (BH_4), the TH cofactor, is added to the cells in this experiment. The catecholamines present in the buffer are extracted with the same volume of 0.8M perchloric acid (PCA) and 0.2 mM EDTA. Cell debris is removed by spinning down in a microfuge at 4° C., 10,000 rpm, for 15 min. The release step can be repeated for another 30 min. The catecholamines produced in the cells are extracted in 0.5 ml 0.4M PCA and 0.1 mM EDTA.

[0262] The catecholamines are separated in a reverse phase C18 column (ESA Analytical) by HPLC (Dionex) using Cat-A-Phase mobile phase (ESA Analytical) at a flow rate of 1.5 ml/min for 15 min. Approximately 20 μl are injected in the system. The catecholamines are detected in an electrochemical detector (ESA Analytical) connected to an Analytical cell (model 5144, ESA Analytical) with input potentials as follows: Guard cell, +250 mV; Channel 1, 10 mV and Channel 2, −250 mV. The amount of catecholamines in the samples is calculated by integrating the area of the peaks to known standards separated following the same protocol. This method allows the detection of L-dopa, Dopamine and DOPAC, a specific degradation product of dopamine.

[0263] The detection of catecholamines released (Table 1) and/or produced (Table 2) by heterologous cells independent of BH_4 confirms that the enzymes are functional. As expected, L-dopa is produced by mono-, bi- and tricistronic expression cassettes whilst dopamine is only produced by the tricistronic cassette. The bicistronic makes far greater amount of L-dopa than TH alone confirming the utility of GTP-CH1 for providing BH_4 in these cells. Dopamine is also produced by the bicistronic in combination with AADC. DOPAC, the specific degradation product of dopamine is only detected when high amounts of dopamine are produced.

EXAMPLE 8

Construction of Lentiviral Vector Expressing the Bicistronic and Tricistronic Cassettes

[0264] Lentiviral vectors are particularly useful for gene transfer to non-dividing cells. Amongst many important non-dividing target cells are the neurons of the human brain. These cells might be target cells for the delivery of TH; AADC and GTP-CH1 for the treatment of Parkinson's disease. Here we describe the construction of minimal EIAV based vectors that will deliver and express TH, AADC and GTP-CH1 and will be capable of producing the neurotransmitter (dopamine) missing in the severely affected Parkinsonian brain. This therapy will be appropriate for late stages of PD patients that do not respond to L-DOPA treatment. The structure of the general minimal EIAV vectors is shown in FIG. **12**.

[0265] pONY8G Construction

[0266] pONY8G was derived from pONY8.0Z by exchange of the LacZ reporter gene for the enhanced green fluorescent protein (GFP) gene. This was done by transferring the SacII-KpnI fragment corresponding to the GFP gene and flanking sequences from pONY2.13GFP (WO99/32646) into pONY8.0Z cut with the same enzymes. pONY8.0Z was derived from pONY4.0Z (WO99/32646) by introducing mutations which 1) prevented expression of TAT by an 83 nt deletion in the exon 2 of tat) prevented S2 ORF expression by a 51 nt deletion 3) prevented REV expression by deletion of a single base within exon 1 of rev and 4) prevented expression of the N-terminal portion of gag by insertion of T in ATG start codons, thereby changing the sequence to ATTG from ATG. With respect to the wild type EIAV sequence Acc. No. U01866 these correspond to deletion of nt 5234-5316 inclusive, nt 5346-5396 inclusive and nt 5538. The insertion of T residues was after nt 526 and 543.

[0267] The Bicistronic cassette expressing the human THt and GTP-CH1 genes is excised from pneo2 as a XhoI-XbaI fragment and ligated to pONY8G (SEQ ID NO 1, FIG. **21**), the construction of which is described above, digested with the same enzymes. In this case the CMVp-GFP cassette is replaced by the CMVp-hTHt-CH1 cassette. The new plasmid is called pONY8-BIC (SEQ ID No 4).

[0268] The Tricistronic cassette expressing the human AADC, THt and GTP-CH1 genes is excised from pTricis as a XhoI-XbaI fragment and ligated to the backbone of pONY8G (SEQ ID NO 1, FIG. **21**), the construction of which is described above. The new plasmid is called pONY8TRIC (SEQ ID NO 5). The resulting vector RNA genome size of this vector is 8.8 kb and therefore 10% longer than that of the 8 kb EIAV RNA genome.

[0269] pONY8.1Z and pONY8.1G Construction

[0270] pONY8.1Z was obtained directly from pONY8.0Z by digestion with SaII and partial digestion with SapI. Following restriction the overhanging termini of the DNA were made blunt ended by treatment with T4 DNA polymerase. The resulting DNA was then religated. This manipulation results in a deletion of sequence between the LacZ reporter gene and just upstream of the 3'PPT. The 3' border of the deletion is nt 7895 with respect to wild type EIAV, Acc. No. U01866. Thus pONY8.1Z does not contain sequences corresponding to the EIAV RREs. pONY8.1G was derived from pONY8G using the same strategy.

[0271] Both the Bicistronic and Tricistronic cassettes are excised as NsiI-XhoI fragments from pONY8BIC (SEQ ID NO: 4) or pONY8TRIC (SEQ ID NO: 5) respectively and ligated to the backbone of pONY8.1G (construction described above, SEQ ID NO 2, FIG. **22**) digested with the same enzymes. The two new plasmids are called pONY8.1BIC and pONY8.1TRIC (FIG. **13**).

[0272] The presence of a sequence termed the central polypurine tract (cPPT) may improve the efficiency of gene delivery to non-dividing cells. This cis-acting element is located in the EIAV polymerase coding region element and can be obtained as a functional element by using PCR amplification using any plasmid that contains the EIAV polymerase coding region (for example pONY3.1, which is described in WO 99/32646 (eg. see example 9, FIG. **6**)) as follows. The PCR product includes the cPPT and the central termination sequences (CTS). The oligonucleotide primers used in the PCR reaction were:

[0273] EIAV cPPT PD POS:

[0274] 5'-CGG ATC AGA TCT TGA TCA CTG CAG GCT CTC ATT ACT TGT AAC AAA GGG AG-3' (SEQ ID NO: 6)

[0275] EIAV cPPT PD NEG:

[0276] 5'-AG CTC GGA TCC CTG CAG CAT GTT CAC CAG GGA TTT TG-3' (SEQ ID NO: 7)

[0277] The recognition site for BglII is underlined, for BclI in italic, for BamHI in bold italic and PstI in bold. The introduction of the cPPT/CTS into a position upstream of the EMCV IRES or PV IRES was achieved by subcloning the unique BclI-BssHII fragment of pONY8TRIC into pSL-1180 (Pharmacia) using the same sites in the vector. This was termed pSL-1180-PD. Digestion of the cPPT/CTS PCR product with BglII and BamHI allowed the insertion into the BclI site upstream of the EMCV IRES or with PstI, into the unique PstI site upstream of the polio IRES, to generate pSL-1180-PD-5'cPPT or pSL-1180-PD-3'cPPT, respectively. The orientation of the fragment cloned into pSL-1180-PD was confirmed by DNA sequencing. The BclI-BssHII fragment from these two clones was ligated into pONY8TRICdelCTS, a modified form of pONY8TRIC. PONY8TRICdelCTS was constructed by ligating the SaII-PinAI fragment from pONY8ZdelCTS (described below) into pONY8TRIC digested with XhoI and PinAI. The two new vector genomes are called pONY8TRIC5'cPPT and pONY8TRIC3'cPPT. A schematic representation of these vector genomes is shown in FIG. **14**.

[0278] Construction of pONY8ZdelCTS

[0279] pONY8Z (SEQ ID NO 3, FIG. **23**) is modified to remove the CTS which already is present the pONY8Z vector. This is achieved by subcloning the SaII to ScaI fragment encompassing the CTS and RRE region from pONY8Z into pSP72, prepared for ligation by digestion with SaII and EcoRV. The CTS region is then removed by digestion with KpnI and PpuMI, the overhanging ends 'blunted' by T4 DNA polymerase treatment and then the ends religated. The modified EIAV vector fragment is then excised using SaII and NheI and ligated into pONY8Z prepared for ligation by digestion with the same enzymes. This new EIAV vector is termed pONY8Z delCTS.

[0280] Construction of pONY 8.9.4 MV opti Y pONY8.9.4 MV opti Y (SEQ ID NO:34, FIG. **31**) is a derivative of pONY8Z (SEQ ID NO:3, FIG. **23**) obtained as a result of multiple routine molecular biological manipulations. The features of the plasmid are as follows:

[0281] 1) Immediate early promoter of human cytomegalovirus: nucleotides (nt) 1-1108;

[0282] **2**) EIAV R-U5-packaging signal region: nt 1109-1748. This sequence corresponds to 268 to 897 from EIAV Gen bank Accession No. U01866 except that there is an additional C residue present after nucleotides 270 and 8178. These correspond to the 4^{th} position of the R region, and these additional residues are incorporated into the genome of pONY8.9.4 MV opti Y. The vector sequence also contains alterations in the EIAV Gag encoding region, which alter all ATG codons to ATTG;

[0283] 3) A linker region including a Kozak consensus sequence for efficient initiation of translation: nt 1749-1791;

[0284] 4) Neomycin phosphotransferase gene: nt 1792-2586;

[0285] **5**) A linker region: nt 2587-2663;

[0286] **6**) Immediate early promoter of human cytomegalovirus (CMV): nt 2664-3389, corresponding to human herpesvirus 5 strain AD169, complete genome. BK000394 nt 175388-174652;

[0287] 7) 5'untranslated region, which includes a Kozak consensus sequence for efficient initiation of translation: nt 3390-3508;

[0288] 8) Codon-optimised truncated tyrosine hydroxylase (TH) gene (SEQ ID NO:23): nt 3509-4525, including stop codon. The sequence of the gene differs from that of the wild type TH type 2 (SEQ ID NO:27);

[0289] 9) Linker region: nt 4526-4549;

[0290] 10) Encephalomyocarditis virus (EMCV) sequence which acts as an internal ribosome entry site (IRES): nt 4550-5126;

[0291] 11) Linker: nt 5127-5128. The last two bases of the EMCV IRES before AUG 11 (AT) are changed to (CC) to introduce an NcoI site;

[0292] 12) Codon-optimised aromatic amino acid decarboxylase gene (SEQ ID NO:31): nt 5129-6571, which includes the stop codon;

[0293] 13) Linker: nt 6572-6675;

[0294] 14) Poliovirus sequence which acts as an internal ribosome entry site: nt 6676-7407. This sequence is derived from poliovirus type 11 (Lansing strain), however IRESs from other polioviruses could be substituted;

[0295] 15) Linker: nt 7408-7428;

[0296] 16) Codon-optimised GTP-cyclohydrolase I sequence (SEQ ID NO:19): nt 7429-8181, which includes the stop codon;

[0297] 17) Linker: nt 8182-8205;

[0298] 18) Modified form of the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE): 8206-8795. The modifications ablate expression of the X-protein of the hepatitis virus and any peptides derived from it. This element boosts expression 2-3 fold in 293T cells and is optional;

[0299] 19) Linker: nt 8796-8809;

[0300] 20) EIAV sequence including the 3'PPT and approximately 25 nucleotides from the 5' end of the U3 region: nt 8809-8902;

[0301] 21) EIAV sequence including approximately 5 nucleotides from the extreme 3' end of the U3 sequence and the R-U5 sequence: nt 8910-9030; and

[0302] 22) Sequences derived from the backbone of pBS II KS+: nt 9031-11622.

EXAMPLE 9

Production of Lentiviral Vector Stocks Expressing Therapeutic Genes

[0303] The three plasmids transfection method as described previously (Soneoka et al., 1995) was used to generate pseudotyped lentiviral vectors. Transfections are carried out in HEK 293T cell line (Soneoka et al., 1995) to produce the vector virions. Culture supernatants were harvested 48 h post-transfection and filtered through 0.45 μm pore-size filters (Millipore). The viral supernatant is concentrated 100-1000 fold by ultracentrifugation (Bums et al., 1993 PNAS 90:8033-8037) and resuspended in PBS.

[0304] The number of particles in the viral stocks were titered by Performance Enhanced Reverse Transcriptase (PERT) assays and compared to a standard pONY8G viral prep with known biological titer. The biological titter is evaluated by transducing D17cells, a dog osteosarcoma cell line. The titer is expressed in transducing units per ml (t.u./ml). For this purpose, cells were seeded into 12×well tissue culture plates the day before infection at 1×10^5 cells per well. Viral supernatants prepared by transfecting 293T cells with appropiate plasmids, as described above, are added to the target cells. Polybrene (8 μg/ml) is added to each well at the time of transduction into 0.5 ml of the culture supernatant used for infection. Approximately 2-5 hours post-transduction, the culture supernatant is replaced by fresh medium. Cells expressing GFP (green) are viewed under UV light and counted.

[0305] The PERT assay uses real time quantitative RT-PCR technology to detect a specific PCR product from MS2 RNA and the retroviral reverse transcriptase present in the viral particles (in this case EIAV RT). Briefly, the viral particles are disrupted by mixing 1:1 volumes of viral vector stocks and disruption buffer (40 mM Tris-HCl pH7.5, 50 mM KCl, 20 mM DTT and 0.2% NP-40). Serial dilutions of the disrupted particles are carried out prior to adding them to the RT-PCR TaqMan reaction mix (Perkin-Elmer). In addition, the reaction mix contains $1/10^{th}$ volume of disrupted viral particles, 300 nM PERT forward primer, 300 nM PERT reverse primer, 150 nM PERT probe, $1/10^{th}$ of 0.8 mg/ml MS2 RNA. The RT-PCR conditions are as follows: Hold, 48° C. for 30 min; hold, 95° C. for 10 min; forty cycles, 95° C. for 15 sec and 60° C. for 1 min. The data is analysed using ABI PRISM^R Sequence Detection System (Perkin-Elmer).

[0306] Similarly, the RNA content of the viral preps is also estimated by RT-PCR comparing to a standard pONY8G viral prep. Viral RNA is isolated from the viral stocks using a Qiagen viral RNA kit (Qiagen) and DNAse I treated (Ambion). Serial dilutions of the viral RNA are used as template in the RT-PCR reaction. Two reaction mixtures are prepared, +RT and −RT, containing $1/10^{th}$ volume of viral RNA template and the specific forward and reverse primers and probe. The RT-PCR conditions are as follows: Hold, 48° C. for 30 min; hold, 95° C. for 10 min; forty cycles, 95° C. for 15 sec and 60° C. for 1 min. The data is analysed using ABI PRISM^R Sequence Detection System (Perkin-Elmer). FIG. **15** shows the PERT assay results and the viral RNA content of EIAV TRIC and EIAV GFP vectors. EIAV TRIC vectors seem to have similar number of particles per prep, but ~4 times less RNA than EIAV GFP.

[0307] The efficiency of integration of the EIAV-TRIC vector genomes is measured by quantitative real-time PCR of total genomic DNA from transduced cells. For this purpose, target cells such as D17 or HT1080 cells are transduced with EIAV-TRIC or EIAV-GFP at different MOI(s) as described previously. The transduced cells are split at least three times prior to isolating total DNA from them. Approximately 100 ng of total DNA is used as template in the PCR reaction. Amplification of the EIAV packaging signal fragment is quantified by comparing to the amplification of a house-keeping gene, such as beta-actin or GAPDH. Real time quantitative PCR conditions are as follows: hold, 95° C. for 10 min; forty cycles, 95° C. for 15 sec and 60° C. for 1 min. The data is analysed using ABI PRISM^R Sequence Detection System (Perkin-Elmer). Table 3 shows the integration efficiency of EIAV vectors.

EXAMPLE 10

EIAV-BIC and -TRIC Vectors Yield Expression of TH, AADC and GTP-CH1 in Heterologous Cells in Culture

[0308] Heterologous cells, such as D17 or HEK 293T cells are transduced with EIAV-TRIC vectors at different multiplicity of infection (MOI). Viral supernatants are prepared by transfecting 293T cells with the appropriate plasmids and added to the target cells as described in previous examples. The cells are split at least three times before analysing them to ensure that there is no pseudotransduction. Expression of the TH, AADC and GTP-CH1 genes is analysed by Western blot (FIG. **16**) and immunocytochemistry (FIG. **17**). Bands

of the appropriate apparent molecular weight are detected in cell extracts of transduced D17 cells: HA-hAADC, ~53 kDa; cmyc-hTHt, ~42 kDa and FLAG-GTP/CH1, ~30 kDa. Mouse monoclonal antibodies that recognise the tagged proteins have been used as described before. The antibodies bound to the proteins are detected with an HRP conjugated rabbit anti-mouse IgG. The bicistronic and tricistronic cassettes express two or three of the enzymes, respectively (FIG. **16**).

[**0309**] The transduction of D17 cells is determined by immunocytochemistry using mouse monoclonal HA antibody (Roche) and Alexa 488 conjugated goat anti-mouse IgG (Molecular Probes) (FIG. **17**). As control, D17 cells were transduced with EIAV lacZ.

[**0310**] The catecholamines produced in the transduced cells are extracted in 0.5 ml 0.4M PCA and 0.1 mM EDTA, separated by HPLC and detected electrochemically as previously described in the above examples. L-dopa, Dopamine and DOPAC are produced by HEK 293T cells transduced with EIAV TRIC vectors (FIG. **18**).

EXAMPLE 11

EIAV Vectors Yield Expression of TH, AADC and GTP-CH1 in the Caudate Nucleus of Adult Rats

[**0311**] Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of the nigrostriatal pathway and is responsive to treatments that facilitate dopaminergic transmission in caudate-putamen. In experimental animals, genetically modified cells that express tyrosine hydroxylase, and thereby synthesize dihydroxyphenylalanine (L-dopa), induce behavioural recovery in rodent models of PD (Wolff et al. (1989) PNAS (USA) 86:9011-14; Freed et al (1990) Arch. Neurol. 47:505-12; Jiao et al. (1993) Nature 262:4505). An alternative approach is that of direct in vivo somatic cell gene transfer whereby the cells of the striatum are converted into dopamine producer cells by transduction with a vector expressing TH, AADC and GTP-CH1.

[**0312**] In order to examine virally encoded gene expression EIAV-TRIC and EIAVlacZ are stereotaxically microinjected into the adult rat striatum as follows. Rats are anesthesized with hypnorm and hypnovel (Wood et al., (1994) Gene Therapy 1:283-291) and injected with 2×1 μl of viral stocks (for EIAV lacZ is typically $1\text{-}5\times10^9$ t.u./ml) into the striatum, at coordinates: Bregma 3.5 mm lateral, 4.75 mm vertical from dura, and 1 mm rostral, 3.5 mm lateral 4.75 mm vertical using a fine drawn glass micropippette over a period of 2 min. The pippette was pulled up 1 mm and left for another 2 min before retracting slowly to the surface. Animals are analysed 1 and 2 weeks following injection. Rats are perfused with 4% paraformaldehyde (PFA) containing 2 mM $MgCl_2$ and 5 mM ethylene glycol bis (beta-aminoethylether)-N,N,N',N'-tetraacetic acid. Brains are removed and placed in fixative overnight, submersed in 30% sucrose at 4° C. overnight and frozen on Tissue-Tech OCT embedding compound (Miles Ind. USA). Fifty-micrometer sections are cut on a freezing microtome and floated briefly in PBS-2 mM $MgCl_2$ at 4° C. as a wash. Expression of lacZ is determined by placing the sections in X-gal staining solution for 3-5 hours. EIAV TRIC is injected into the rat striatum using the same coordinates as described above. In addition two more injection sites at Bregma 2.5 mm lateral, 4.75 mm vertical and 1.8 mm rostral, 2.5 mm lateral and 5 mm vertical were performed. Expression of AADC, TH and GTP-CH1 is detected by immunohistochemistry using mouse monoclonal antibodies raised against the epitope tags, HA, c-myc and FLAG respectively. These antibodies will distinguish between the rat and the human proteins. Brains sections are incubated with mouse anti-HA (Santa Cruz), anti-c-myc (Santa Cruz) or anti-FLAG (Sigma) antibodies (1:100 th dilutions) at 4° C. overnight in PBS-10% goat serum and 0.5% TritonX-100. Sections are washed with PBS and then incubated with Alexa 488 (Molecular Probes) or FITC (Jackson Laboratories) conjugated goat anti-mouse or anti-rabbit IgG ($\frac{1}{1000}^{th}$ dilutions) at room temperature for 2-3 hours. After washing the sections are examined under a fluorescence microscope. For DAB staining sections were developed using the avidin-biotin system (Vectastain kit (Vactor Laboratories)).

[**0313**] TH is not expressed within either neurons or glia of the rat striatum (Chatterjee et al. (1992) Science 258:1485-88). Endogenous TH immunoreactivity (TH-IR) within the striatum is limited to the dopaminergic terminals of afferent fibers from substantia nigra. To determine whether the cells transduced are neurons or glial-cells a TH antibody is used in conjuction with antibodies that recognise either neuronal (NeuN) or glial (GFAP) markers. Double immunostaining is carried out on brain sections. Sections are incubated with rabbit polyclonal TH antibody ($\frac{1}{100}^{th}$; Affinitti) and mouse monoclonal neurofilament (NeuN) antibody ($\frac{1}{50}^{th}$; Chemicon), or mouse monoclonal GFAP ($\frac{1}{50}^{th}$; Chemicon) at 4° C. overnight in PBS-10% goat serum and 0.5% TritonX-100. Sections are washed with PBS and then incubated with Alexa 488 conjugated goat anti rabbit IgG (**½00th**; Molecular Probes) or CY3 conjugated goat anti-mouse IgG ($\frac{1}{200}^{th}$; Jackson Laboratories) at room temperature for 2-3 hours. After washing the sections are examined under a fluorescence microscope.

[**0314**] FIG. **19** shows transduction of the adult rat striatum with pONY8Z seven days following injection. FIG. **20** shows transduction of the rat striatum with pONY8TRIC two weeks following injection.

EXAMPLE 12

Efficacy of EIAV-TRIC Vectors in a Rodent Model of Parkinson's Disease: Apomorphine-Induced Rotational Behavior

[**0315**] The aim of the present study is to replace dopamine in the striatum of animal model of Parkinson's disease. Rats receive 6-OHDA lesions of the right medial forebrain bundle (MFB). Stereotaxic injections are performed under anesthesia using 10 μl Hamilton syringe with 33-gauge blunt tip needle. Each rat receives 4 μl of 4 μg/μl 6-OHDA HCl (Sigma) dissolved in 2 mg/ml ascorbate-saline (0.2% ascorbic acid, 0.9% NaCl). The solution is slowly infused at the speed of 0.5 μl/min. Three weeks following 6-OHDA lesion, rats are tested for amphetamine-induced rotation. Animals are injected i.p. with 2.5 mg/kg D-amphetamine (Sigma). Amphetamine is diluted in PBS. Rotational asymmetry is monitored over 90 minutes. Only rats with >7 turns per minute are used for the following experiment. For apomorphine-induced rotation, animals are tested twice on 0.05 mg/kg sc 4 days apart. Fifteen rats show good homogeneity

as to the extent of the 6-OHDA lesions. Two experiments are performed with EIAV-TRIC vectors. Three weeks after 6-OHDA lesions, EIAV-based lentiviral vectors carrying the genes involved in the dopamine synthesis are unilaterally injected into the striatum (ipsilateral to the lesion). Two groups of animals are included in each study: in the first experiment pONY8.1Z n=5; pONY8.1T n=4; in the second study pONY8.1Z n=4; pONY8.1T n=7. In order to assess a possible functional benefit of the treatment, apomorphine-induced rotation is tested weekly after the viral injection (FIG. **24**.A). Two pONY8.1T-injected animals (C3R5 & C5R4) showed reduction in contralateral rotation than the pre-apo2 rotation during the whole experiment period, reaching a 65 and 70% decrease 3 weeks after viral injection (The present inventors suggest that the 70% is probably an artefact since one rat slipped out of the hamess during this rotation). A 60 and 35% decrease is observed 10 weeks following injection of the viral solution for these two rats. In the second study, dopamine replacement did reduce the number of apomorphine-induced rotations experienced in 6 animals (from 7 rats) injected with pONY8.1T (FIG. **24**.B). The average of reduction in rotations 6 weeks after viral injection is about 45% compared to pre-apomorphine 2.

[**0316**] At the end of each experiment, rats are perfused with ice-cold PBS containing 0.02% ascorbic acid and 5000 units of heparin followed by 4% paraformaldehyde solution. The brains are dissected and placed overnight in 4% paraformaldehyde solution followed by the cryoprotection in 30% sucrose solution. TH-immunohistochemical labeling is performed on nigral and striatal sections to test the extension of the lesion. TH-immunostaining is performed using polyclonal Rabbit anti-TH antibodies on nigral (FIG. **25**.A) and striatal (FIG. **25**.B) sections. Catecholamines produced by EIAV TRIC vectors in the denervated striata of 6-OHDA rats are determined by HPLC and electrochemical detection, as described in the previous examples. The results are shown in FIGS. **26** and **27**.

EXAMPLE 13

EIAV-TRIC Vectors Used for Correcting the 6-OHDA Primate Model of Parkinson's Disease

[**0317**] This model comprises unilateral injection of 6-hydroxydopamine (6-OHDA) into the nigrostriatal bundle of the small New World monkey the common marmoset (*Callithrix jacchus*). As in the rodent model, the asymmetry caused by the toxin in receptor sensitivity between the denervated and intact striatum results in rotational behaviour upon i.m. administration of domapinergic factors, such as apomorphine (Annett et al., (1997). The rate of amphetamine-induced rotations is directly related to the striatal dopaminergic dysfunction and is used to evaluate the therapeutic efficacy of different treatments for PD (Annett et al. (1994) Exp Neurol. 125:228-246; Annett et al. (1992) Brain, 115:825-856). Marmosets aged 18-24 months are lesioned under anesthesia by delivery of 4 mg/ml free base weight 6-OHDA (Sigma) disolved in 0.01% ascorbate-saline. 6-OHDA was injected stereotaxically into five sites in the nigrostriatal bundle on one side of the brain (coordinates: AP+6.5; L±1.2, V+6 and +7: L±2.2, V+6.5 and V+7.5, L±3.2, V+7.5, as described in Stephan et al. (1980) Berlin: Springer-Verlag). Three microlitres are injected in the most lateral site and two microlitres in the other four sites. The 6-OHDA lesioned animals are examined for rotational behaviour prior to the lesion, after the lesion before the viral vectors injection and one month after the vectors are injected. Rotations are recorded during 30 min sessions starting 30 min after the injection of the drug. The marmosets are filmed while in a transparent Perspex box and the number of complete turns are counted.

[**0318**] Four 6-OHDA lesioned animals are injected with 30 μl of EIAV-TRIC or EIAVlacZ viral stocks into the Caudate Putamen at 6 sites (5 μl/site). Behavioural assessment of the monkeys on reaching tasks and apomorphine-induced rotations tests will be made one month post-injection and at regular interval for several months for long-term follow-up. Animals are sacrificed and brain tissue sections are analysed for TH immunoreactivity as described previously. The level of catecholamines in the denervated striatum is determined by HPLC and electrochemical detection (as described above).

EXAMPLE 14

EIAV-TRIC Vectors Used for Correcting the MPTP Primate Model of Parkinson's Disease

[**0319**] The primate model of Parkinson's disease is considered the gold-standard model for evaluation of potential therapies prior to entering human clinical trials. This model is originally developed from the observation in the early 1980s that groups of younger people are developing a neurodegenerative disorder strikingly similar to idiopathic Parkinson's disease. The source of this disorder is traced to the use of a street drug, and specifically to the chemical known as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston (1985) Trends in Pharmacol. Sci. 6:375-378). When MPTP is given to primates, the animals developed a parkinsonian disorder that has become the principle model for testing anti-parkinsonian agents. Peripherally administered MPTP will cross the blood brain barrier, whereupon is converted into MPP+ by monoamine oxidase B (MAO-B). MPP+ is a potent neurotoxin that eventually causes the degeneration of nigro-striatal dopamine pathway, as seen in Parkinson's disease.

[**0320**] Cynomolgus monkeys (*Macaca fascicularis*) are rendered parkinsonian by weekly intravenous injections of 0.5-1 mg/kg MPTP for ten consecutive months. Animals are trained to perform fine motor tasks prior to the administration of MPTP. The parkinsonian monkeys are tested for marked reduction of spontaneous activity, bilateral action tremor, freezing and posture and balance impairment to assess the efficacy of the lesion. Motor deficits are assessed according to a nonhuman primate disability rating scale (Herrero et al., (1993) Neuroscience 56:965-72). In addition, apomorphine (0.1 mg/kg, i.m.) is also given every two weeks to test the appearance of circling behaviour. The monkeys are allowed to recover from the last MPTP administration for 3 months prior to the intrastriatal transduction. Animals are anesthesized with a mixture of ketamine (10 mg/kg) and midazolan (1 mg/kg) and placed in the stereotactic frame. A hole is drilled in the skull at the level of the right frontal ventricle according to the atlas of Szabo and Cowan (Szabo and Cowan (1984) J. Comp. Neurol. 222:265-300), and a ventriculography is performed by injecting 0.4 ml of Omnigrass into the right ventricle. The

intercommisural line (AC-PC line) is measured and the coordinates for the putamen nucleus are adjusted according to the atlas.

[0321] EIAV-TRIC and EIAVlacZ viral vectors (5 μl of ~1-5×10^9 t.u./ml) are stereotaxically injected unilaterally into the left putamen in two sites along the rostrocaudal axis using a Hamilton syringe. Briefly, 2×5 μl of ~1-5×10^9 t.u./ml are injected into the putamen nucleus as follows: rostral putamen, AP+3.4 mm from the midpoint of the AC-PC line; ML 12 mm from the longitudinal sinus, and VD 15 mm below dura mater. Animal receive antibiotics (ampicillin 250 mg/day, i.m.) prophylactically for two weeks and analgesia with nonsteroidal anti-inflammatory drugs (flunixin, 2.5 mg/kg). Animals are followed periodically (every two weeks) for 3-5 months in order to determine whether the therapeutic vectors improve the parkinsonian behaviour (During et al. (1994)). They are tested for motor deficits as described above. At the end of the experimental period, animals are transcardially perfused with 4% PFA in PBS. The brains are fixed ovemight in the same fixative at 4° C. and then immersed in 30% sucrose in PBS. Coronal brain sections (30 μm thick) were cut on a freezing microtome and collected in PBS. TH immunoreactivity and levels of catecholamines in the denervated putamen are analysed as described previously.

[0322] All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology or related fields are intended to be within the scope of the following claims.

TABLE 1

DNA	L-DOPA	DOPAC	DA
Mock	n.d.	n.d.	n.d.
TRIC	0.745 +/− 0.047	n.d.	0.545 +/− 0.055
BIC + AADC	0.729 +/− 0.045	0.531 +/− 0.10	11.31 +/− 1.01
BIC	58.55 +/− 6.20	n.d.	n.d.
hTHt	0.845	n.d.	n.d.

[0323]

TABLE 2

DNA	L-DOPA	DOPAC	DA
Mock	n.d.	n.d.	n.d.
TRIC	0.745 +/− 0.047	n.d.	0.545 +/− 0.055
BIC + AADC	0.729 +/− 0.045	0.531 +/− 0.10	11.31 +/− 1.01
BIC	58.55 +/− 6.20	n.d.	n.d.
hTHt	0.845	n.d.	n.d.

[0324]

TABLE 3

Relative Integration Efficiency of EIAV Vectors

MOI	pONY8G	pONY8T-1	pONY8T-2	pONY8.1Z	pONY8.1T	Integrase-
100X	10.61	11.44	10.6	11.6	11.6	2.1
10X	7	6.16	5.7	8.67	6.63	2.4
1X	4.37	4.8	n.d.	7.09	4.6	n.d.

D17 cells have been transduced at different MOIs with EIAV vectors. The dCT values represent the ratio of β-actin/EIAV genomes in 100 ng of total DNA (dCt = βactin Ct-CMVp Ct). The PCR reaction amplifies the CMV promoter region present in the integrated EIAV genome. The dCT values of untransduced cells was ~1.85. Similar results are obtained using the EIAV packaging signal.

[0325]

SEQUENCE LISTING

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<211> LENGTH: 8531
<212> TYPE: DNA
<213> ORGANISM: Equine Infectious Anemia Virus

<400> SEQUENCE: 1

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gactaaattc atgtcgcgcg atagtggtgt ttatcgccga tagagatggc gatattggaa 120

-continued

aaattgatat ttgaaaatat ggcatattga aaatgtcgcc gatgtgagtt tctgtgtaac 180

tgatatcgcc atttttccaa aagtgatttt tgggcatacg cgatatctgg cgatagcgct 240

tatatcgttt acgggggatg gcgatagacg actttggtga cttgggcgat tctgtgtgtc 300

gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg ccgatagagg 360

cgacatcaag ctggcacatg gccaatgcat atcgatctat acattgaatc aatattggcc 420

attagccata ttattcattg gttatatagc ataaatcaat attggctatt ggccattgca 480

tacgttgtat ccatatcgta atatgtacat ttatattggc tcatgtccaa cattaccgcc 540

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caatcaaatg ctatagaatg ctggggttcc ttcccggggt gtagaccatt tcaaaattac 5580

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taaagacttc tggcataact cctttaccta tttcttctga agctaacact ggactaatta 5760

gacataagag agattttggt ataagtgcaa tagtggcagc tattgtagcc gctactgcta 5820

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tacaaaatca tacttttgag gtagaaaata gtactctaaa tggtatggat ttaatagaac 5940

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agagctcgtt tagtgaac 11058

<210> SEQ ID NO 6
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Equine Infectious Anemia Virus

<400> SEQUENCE: 6

cggatcagat cttgatcact gcaggctctc attacttgta acaaagggag 50

<210> SEQ ID NO 7
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Equine Infectious Anemia Virus

<400> SEQUENCE: 7

agctcggatc cctgcagcat gttcaccagg gattttg 37

<210> SEQ ID NO 8
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 8

gcggatccgc caccatggaa aaactcatct cagaagagga tctgcccacc cccgacgcca 60

ccacg 65

<210> SEQ ID NO 9
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 9

gaaccgcggg gactgccctc ttacc 25

<210> SEQ ID NO 10
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 10

ggtaaagagg gcagtccccg cggttc 26

<210> SEQ ID NO 11
<211> LENGTH: 35
<212> TYPE: DNA

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<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 11

cgaagcttct agccaatggc actcagcgca tgggc 35

<210> SEQ ID NO 12
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 12

cgagatctgc caccatgtac ccctacgacg tgcccgacta cgccaacgca agtgaattcc 60

gaagg 65

<210> SEQ ID NO 13
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 13

cgaagcttct actccctctc tgctcgc 27

<210> SEQ ID NO 14
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 14

cgagatctgc caccatggac tacaaggacg acgatgacga gaagggccct gtgcggcg 58

<210> SEQ ID NO 15
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 15

cgaagctttc agctcctaat gagagtcagg aa 32

<210> SEQ ID NO 16
<211> LENGTH: 77
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 16

cgaagcttgg atccgccacc atggaacaaa aactcatctc agaagaggat ctgaaggtcc 60

cctggttccc aagaaaa 77

<210> SEQ ID NO 17
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 17

cggaattcct agccaatggc actcagcgca tgggc 35

<210> SEQ ID NO 18
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

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<400> SEQUENCE: 18

Met Glu Lys Gly Pro Val Arg Ala Pro Ala Glu Lys Pro Arg Gly Ala
1 5 10 15

Arg Cys Ser Asn Gly Phe Pro Glu Arg Asp Pro Pro Arg Pro Gly Pro
20 25 30

Ser Arg Pro Ala Glu Lys Pro Pro Arg Pro Glu Ala Lys Ser Ala Gln
35 40 45

Pro Ala Asp Gly Trp Lys Gly Glu Arg Pro Arg Ser Glu Glu Asp Asn
50 55 60

Glu Leu Asn Leu Pro Asn Leu Ala Ala Ala Tyr Ser Ser Ile Leu Ser
65 70 75 80

Ser Leu Gly Glu Asn Pro Gln Arg Gln Gly Leu Leu Lys Thr Pro Trp
85 90 95

Arg Ala Ala Ser Ala Met Gln Phe Phe Thr Lys Gly Tyr Gln Glu Thr
100 105 110

Ile Ser Asp Val Leu Asn Asp Ala Ile Phe Asp Glu Asp His Asp Glu
115 120 125

Met Val Ile Val Lys Asp Ile Asp Met Phe Ser Met Cys Glu His His
130 135 140

Leu Val Pro Phe Val Gly Lys Val His Ile Gly Tyr Leu Pro Asn Lys
145 150 155 160

Gln Val Leu Gly Leu Ser Lys Leu Ala Arg Ile Val Glu Ile Tyr Ser
165 170 175

Arg Arg Leu Gln Val Gln Glu Arg Leu Thr Lys Gln Ile Ala Val Ala
180 185 190

Ile Thr Glu Ala Leu Arg Pro Ala Gly Val Gly Val Val Val Glu Ala
195 200 205

Thr His Met Cys Met Val Met Arg Gly Val Gln Lys Met Asn Ser Lys
210 215 220

Thr Val Thr Ser Thr Met Leu Gly Val Phe Arg Glu Asp Pro Lys Thr
225 230 235 240

Arg Glu Glu Phe Leu Thr Leu Ile Arg Ser
245 250

<210> SEQ ID NO 19
<211> LENGTH: 753
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 19

atggagaagg gccctgtgcg cgccccggcc gagaagccgc gcggcgcccg ctgcagcaat 60

gggttccccg agcgcgaccc gccgcgcccc gggcccagca ggccggccga gaagcccccg 120

cgccccgagg ccaagagcgc gcagcccgcg gacggctgga agggcgagcg cccccgcagc 180

gaggaggaca acgagctgaa cctccctaac ctggccgccg cctactcctc catcctgagc 240

tcgctgggcg agaaccccca gcggcagggg ctgctcaaga ccccctggag ggcggcctcg 300

gccatgcagt tcttcaccaa gggctaccag gagaccatct cagacgtcct gaacgacgct 360

atcttcgacg aagatcacga tgagatggtg atcgtgaagg acatagacat gttctccatg 420

tgcgagcacc acctggtgcc atttgtggga aaggtccata tcggctacct gcctaacaag 480

caggtcctgg gcctcagcaa gctggcgagg attgtggaaa tctatagtag aagactacag 540

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gttcaggagc gccttaccaa acaaattgct gtggcaatca cggaagcctt gcggcctgct 600

ggagtcgggg tcgtggtgga agcaacacac atgtgtatgg tgatgcgagg tgtacagaaa 660

atgaacagca aaaccgtgac cagcacaatg ctgggtgtgt tccgggagga tccaaagact 720

cgggaagagt tcctgactct catcaggagc tga 753

<210> SEQ ID NO 20
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 20

Met Glu Lys Gly Pro Val Arg Ala Pro Ala Glu Lys Pro Arg Gly Ala
1 5 10 15

Arg Cys Ser Asn Gly Phe Pro Glu Arg Asp Pro Pro Arg Pro Gly Pro
20 25 30

Ser Arg Pro Ala Glu Lys Pro Pro Arg Pro Glu Ala Lys Ser Ala Gln
35 40 45

Pro Ala Asp Gly Trp Lys Gly Glu Arg Pro Arg Ser Glu Glu Asp Asn
50 55 60

Glu Leu Asn Leu Pro Asn Leu Ala Ala Ala Tyr Ser Ser Ile Leu Ser
65 70 75 80

Ser Leu Gly Glu Asn Pro Gln Arg Gln Gly Leu Leu Lys Thr Pro Trp
85 90 95

Arg Ala Ala Ser Ala Met Gln Phe Phe Thr Lys Gly Tyr Gln Glu Thr
100 105 110

Ile Ser Asp Val Leu Asn Asp Ala Ile Phe Asp Glu Asp His Asp Glu
115 120 125

Met Val Ile Val Lys Asp Ile Asp Met Phe Ser Met Cys Glu His His
130 135 140

Leu Val Pro Phe Val Gly Lys Val His Ile Gly Tyr Leu Pro Asn Lys
145 150 155 160

Gln Val Leu Gly Leu Ser Lys Leu Ala Arg Ile Val Glu Ile Tyr Ser
165 170 175

Arg Arg Leu Gln Val Gln Glu Arg Leu Thr Lys Gln Ile Ala Val Ala
180 185 190

Ile Thr Glu Ala Leu Arg Pro Ala Gly Val Gly Val Val Val Glu Ala
195 200 205

Thr His Met Cys Met Val Met Arg Gly Val Gln Lys Met Asn Ser Lys
210 215 220

Thr Val Thr Ser Thr Met Leu Gly Val Phe Arg Glu Asp Pro Lys Thr
225 230 235 240

Arg Glu Glu Phe Leu Thr Leu Ile Arg Ser
245 250

<210> SEQ ID NO 21
<211> LENGTH: 753
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 21

atggagaagg gccctgtgcg ggcaccggcg gagaagccgc ggggcgccag gtgcagcaat 60

gggttccccg agcgggatcc gccgcggccc gggcccagca ggccggcgga gaagcccccg 120

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cggcccgagg ccaagagcgc gcagcccgcg gacggctgga agggcgagcg gccccgcagc 180

gaggaggata acgagctgaa cctccctaac ctggcagccg cctactcgtc catcctgagc 240

tcgctgggcg agaaccccca gcggcaaggg ctgctcaaga cgccctggag ggcggcctcg 300

gccatgcagt tcttcaccaa gggctaccag gagaccatct cagatgtcct aaacgatgct 360

atatttgatg aagatcatga tgagatggtg attgtgaagg acatagacat gttttccatg 420

tgtgagcatc acttggttcc atttgttgga aaggtccata ttggttatct tcctaacaag 480

caagtccttg gcctcagcaa acttgcgagg attgtagaaa tctatagtag aagactacaa 540

gttcaggagc gccttacaaa acaaattgct gtagcaatca cggaagcctt gcggcctgct 600

ggagtcgggg tagtggttga agcaacacac atgtgtatgg taatgcgagg tgtacagaaa 660

atgaacagca aaactgtgac cagcacaatg ttgggtgtgt tccgggagga tccaaagact 720

cgggaagagt tcctgactct cattaggagc tga 753

<210> SEQ ID NO 22
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 22

Met Val Lys Val Pro Trp Phe Pro Arg Lys Val Ser Glu Leu Asp Lys
1 5 10 15

Cys His His Leu Val Thr Lys Phe Asp Pro Asp Leu Asp Leu Asp His
20 25 30

Pro Gly Phe Ser Asp Gln Val Tyr Arg Gln Arg Arg Lys Leu Ile Ala
35 40 45

Glu Ile Ala Phe Gln Tyr Arg His Gly Asp Pro Ile Pro Arg Val Glu
50 55 60

Tyr Thr Ala Glu Glu Ile Ala Thr Trp Lys Glu Val Tyr Thr Thr Leu
65 70 75 80

Lys Gly Leu Tyr Ala Thr His Ala Cys Gly Glu His Leu Glu Ala Phe
85 90 95

Ala Leu Leu Glu Arg Phe Ser Gly Tyr Arg Glu Asp Asn Ile Pro Gln
100 105 110

Leu Glu Asp Val Ser Arg Phe Leu Lys Glu Arg Thr Gly Phe Gln Leu
115 120 125

Arg Pro Val Ala Gly Leu Leu Ser Ala Arg Asp Phe Leu Ala Ser Leu
130 135 140

Ala Phe Arg Val Phe Gln Cys Thr Gln Tyr Ile Arg His Ala Ser Ser
145 150 155 160

Pro Met His Ser Pro Glu Pro Asp Cys Cys His Glu Leu Leu Gly His
165 170 175

Val Pro Met Leu Ala Asp Arg Thr Phe Ala Gln Phe Ser Gln Asp Ile
180 185 190

Gly Leu Ala Ser Leu Gly Ala Ser Asp Glu Glu Ile Glu Lys Leu Ser
195 200 205

Thr Leu Tyr Trp Phe Thr Val Glu Phe Gly Leu Cys Lys Gln Asn Gly
210 215 220

Glu Val Lys Ala Tyr Gly Ala Gly Leu Leu Ser Ser Tyr Gly Glu Leu
225 230 235 240

Leu His Cys Leu Ser Glu Glu Pro Glu Ile Arg Ala Phe Asp Pro Glu

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               245                 250                 255

Ala Ala Ala Val Gln Pro Tyr Gln Asp Gln Thr Tyr Gln Ser Val Tyr
            260                 265                 270

Phe Val Ser Glu Ser Phe Ser Asp Ala Lys Asp Lys Leu Arg Ser Tyr
        275                 280                 285

Ala Ser Arg Ile Gln Arg Pro Phe Ser Val Lys Phe Asp Pro Tyr Thr
    290                 295                 300

Leu Ala Ile Asp Val Leu Asp Ser Pro Gln Ala Val Arg Arg Ser Leu
305                 310                 315                 320

Glu Gly Val Gln Asp Glu Leu Asp Thr Leu Ala His Ala Leu Ser Ala
               325                 330                 335

Ile Gly
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<210> SEQ ID NO 23
<211> LENGTH: 1014
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 23

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gtcaccaagt tcgaccccga cctggacttg gaccaccccg gcttctcgga ccaggtgtac    120

cgccagcgca ggaagctgat cgctgagatc gccttccagt acaggcacgg cgacccgatc    180

ccccgtgtgg agtacaccgc cgaggagatc gccacctgga aggaggtcta caccaccctg    240

aagggcctct acgccaccca cgcctgcggg gagcacctgg aggcctttgc tttgctggag    300

cgcttcagcg gctaccggga agacaacatc ccccagctgg aggacgtctc ccgcttcctg    360

aaggagcgca caggcttcca gctgcggccc gtggccggcc tgctgtccgc ccgggacttc    420

ctggccagcc tggccttccg cgtgttccag tgcacccagt atatccgcca cgcgtcctcg    480

cccatgcact cccctgagcc ggactgctgc cacgagctgc tggggcacgt gcccatgctg    540

gccgaccgca ccttcgcgca gttcagccag gacatcggcc tggcgtccct gggggccagc    600

gatgaggaaa tcgagaagct gtccactctg tactggttca cggtggagtt cgggctgtgt    660

aagcagaacg gggaggtgaa ggcctatggt gccgggctgc tgtcctccta cggggagctc    720

ctgcactgcc tgtctgagga gcctgagatc cgggccttcg accctgaggc tgcggccgtg    780

cagccctacc aagaccagac gtaccagtca gtctacttcg tgtctgagag cttcagcgac    840

gccaaggaca agctcaggag ctatgccagc cgcatccagc gccccttctc cgtgaagttc    900

gacccgtaca ccctggccat cgacgtgctg gacagccccc aggccgtgcg gcgctccctg    960

gagggtgtcc aggatgagct ggacaccctt gcccatgcgc tgagcgccat cggc         1014
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<210> SEQ ID NO 24
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 24

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Met Val Lys Val Pro Trp Phe Pro Arg Lys Val Ser Glu Leu Asp Lys
1               5                   10                  15

Cys His His Leu Val Thr Lys Phe Asp Pro Asp Leu Asp Leu Asp His
            20                  25                  30

Pro Gly Phe Ser Asp Gln Val Tyr Arg Gln Arg Arg Lys Leu Ile Ala
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        35                  40                  45

Glu Ile Ala Phe Gln Tyr Arg His Gly Asp Pro Ile Pro Arg Val Glu
    50                  55                  60

Tyr Thr Ala Glu Glu Ile Ala Thr Trp Lys Glu Val Tyr Thr Thr Leu
65                  70                  75                  80

Lys Gly Leu Tyr Ala Thr His Ala Cys Gly Glu His Leu Glu Ala Phe
                85                  90                  95

Ala Leu Leu Glu Arg Phe Ser Gly Tyr Arg Glu Asp Asn Ile Pro Gln
            100                 105                 110

Leu Glu Asp Val Ser Arg Phe Leu Lys Glu Arg Thr Gly Phe Gln Leu
        115                 120                 125

Arg Pro Val Ala Gly Leu Leu Ser Ala Arg Asp Phe Leu Ala Ser Leu
    130                 135                 140

Ala Phe Arg Val Phe Gln Cys Thr Gln Tyr Ile Arg His Ala Ser Ser
145                 150                 155                 160

Pro Met His Ser Pro Glu Pro Asp Cys Cys His Glu Leu Leu Gly His
                165                 170                 175

Val Pro Met Leu Ala Asp Arg Thr Phe Ala Gln Phe Ser Gln Asp Ile
            180                 185                 190

Gly Leu Ala Ser Leu Gly Ala Ser Asp Glu Glu Ile Glu Lys Leu Ser
        195                 200                 205

Thr Leu Ser Trp Phe Thr Val Glu Phe Gly Leu Cys Lys Gln Asn Gly
    210                 215                 220

Glu Val Lys Ala Tyr Gly Ala Gly Leu Leu Ser Ser Tyr Gly Glu Leu
225                 230                 235                 240

Leu His Cys Leu Ser Glu Glu Pro Glu Ile Arg Ala Phe Asp Pro Glu
                245                 250                 255

Ala Ala Ala Val Gln Pro Tyr Gln Asp Gln Thr Tyr Gln Ser Val Tyr
            260                 265                 270

Phe Val Ser Glu Ser Phe Ser Asp Ala Lys Asp Lys Leu Arg Ser Tyr
        275                 280                 285

Ala Ser Arg Ile Gln Arg Pro Phe Ser Val Lys Phe Asp Pro Tyr Thr
    290                 295                 300

Leu Ala Ile Asp Val Leu Asp Ser Pro Gln Ala Val Arg Arg Ser Leu
305                 310                 315                 320

Glu Gly Val Gln Asp Glu Leu Asp Thr Leu Ala His Ala Leu Ser Ala
                325                 330                 335

Ile Gly


<210> SEQ ID NO 25
<211> LENGTH: 1017
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 25

atggtgaagg taccctggtt cccaagaaaa gtgtcagagc tggacaagtg tcatcacctg     60

gtcaccaagt tcgaccccga cctggacttg gaccaccccg gcttctcgga ccaggtgtac    120

cgccagcgca ggaagctgat cgctgagatc gccttccagt acaggcacgg cgacccgatc    180

ccccgtgtgg agtacaccgc cgaggagatc gccacctgga aggaggtcta caccaccctg    240

aagggcctct acgccaccca cgcctgcggg gagcacctgg aggcctttgc tttgctggag    300
```

-continued

cgcttcagcg gctaccggga agacaacatc ccccagctgg aggacgtctc ccgcttcctg 360

aaggagcgca caggcttcca gctgcggccc gtggccggcc tgctgtccgc ccgggacttc 420

ctggccagcc tggccttccg cgtgttccag tgcacccagt atatccgcca cgcgtcctcg 480

cccatgcact cccctgagcc ggactgctgc cacgagctgc tggggcacgt gcccatgctg 540

gccgaccgca ccttcgcgca gttcagccag gacatcggcc tggcgtccct gggggccagc 600

gatgaggaaa tcgagaagct gtccactctg tcatggttca cggtggagtt cgggctgtgt 660

aagcagaacg gggaggtgaa ggcctatggt gccgggctgc tgtcctccta cggggagctc 720

ctgcactgcc tgtctgagga gcctgagatc cgggccttcg accctgaggc tgcggccgtg 780

cagccctacc aagaccagac gtaccagtca gtctacttcg tgtctgagag cttcagcgac 840

gccaaggaca agctcaggag ctatgccagc cgcatccagc gccccttctc cgtgaagttc 900

gacccgtaca ccctggccat cgacgtgctg gacagccccc aggccgtgcg gcgctccctg 960

gagggtgtcc aggatgagct ggacaccctt gcccatgcgc tgagcgccat cggctga 1017

<210> SEQ ID NO 26
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 26

Met Val Lys Val Pro Trp Phe Pro Arg Lys Val Ser Glu Leu Asp Lys
1 5 10 15

Cys His His Leu Val Thr Lys Phe Asp Pro Asp Leu Asp Leu Asp His
20 25 30

Pro Gly Phe Ser Asp Gln Val Tyr Arg Gln Arg Arg Lys Leu Ile Ala
35 40 45

Glu Ile Ala Phe Gln Tyr Arg His Gly Asp Pro Ile Pro Arg Val Glu
50 55 60

Tyr Thr Ala Glu Glu Ile Ala Thr Trp Lys Glu Val Tyr Thr Thr Leu
65 70 75 80

Lys Gly Leu Tyr Ala Thr His Ala Cys Gly Glu His Leu Glu Ala Phe
85 90 95

Ala Leu Leu Glu Arg Phe Ser Gly Tyr Arg Glu Asp Asn Ile Pro Gln
100 105 110

Leu Glu Asp Val Ser Arg Phe Leu Lys Glu Arg Thr Gly Phe Gln Leu
115 120 125

Arg Pro Val Ala Gly Leu Leu Ser Ala Arg Asp Phe Leu Ala Ser Leu
130 135 140

Ala Phe Arg Val Phe Gln Cys Thr Gln Tyr Ile Arg His Ala Ser Ser
145 150 155 160

Pro Met His Ser Pro Glu Pro Asp Cys Cys His Glu Leu Leu Gly His
165 170 175

Val Pro Met Leu Ala Asp Arg Thr Phe Ala Gln Phe Ser Gln Asp Ile
180 185 190

Gly Leu Ala Ser Leu Gly Ala Ser Asp Glu Glu Ile Glu Lys Leu Ser
195 200 205

Thr Leu Tyr Trp Phe Thr Val Glu Phe Gly Leu Cys Lys Gln Asn Gly
210 215 220

Glu Val Lys Ala Tyr Gly Ala Gly Leu Leu Ser Ser Tyr Gly Glu Leu
225 230 235 240

-continued

```
Leu His Cys Leu Ser Glu Glu Pro Glu Ile Arg Ala Phe Asp Pro Glu
                245                 250                 255

Ala Ala Ala Val Gln Pro Tyr Gln Asp Gln Thr Tyr Gln Ser Val Tyr
            260                 265                 270

Phe Val Ser Glu Ser Phe Ser Asp Ala Lys Asp Lys Leu Arg Ser Tyr
        275                 280                 285

Ala Ser Arg Ile Gln Arg Pro Phe Ser Val Lys Phe Asp Pro Tyr Thr
    290                 295                 300

Leu Ala Ile Asp Val Leu Asp Ser Pro Gln Ala Val Arg Arg Ser Leu
305                 310                 315                 320

Glu Gly Val Gln Asp Glu Leu Asp Thr Leu Ala His Ala Leu Ser Ala
                325                 330                 335

Ile Gly
```

<210> SEQ ID NO 27
<211> LENGTH: 1017
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 27

```
atggtgaagg taccctggtt cccaagaaaa gtgtcagagc tggacaagtg tcatcacctg     60

gtcaccaagt tcgaccctga cctggacttg gaccacccgg gcttctcgga ccaggtgtac    120

cgccagcgca ggaagctgat tgctgagatc gccttccagt acaggcacgg cgacccgatt    180

ccccgtgtgg agtacaccgc cgaggagatt gccacctgga aggaggtcta caccacgctg    240

aagggcctct acgccacgca cgcctgcggg gagcacctgg aggcctttgc tttgctggag    300

cgcttcagcg gctaccggga agacaatatc ccccagctgg aggacgtctc ccgcttcctg    360

aaggagcgca cgggcttcca gctgcggcct gtggccggcc tgctgtccgc ccgggacttc    420

ctggccagcc tggccttccg cgtgttccag tgcacccagt atatccgcca cgcgtcctcg    480

cccatgcact cccctgagcc ggactgctgc cacgagctgc tggggcacgt gcccatgctg    540

gccgaccgca ccttcgcgca gttctcgcag gacattggcc tggcgtccct gggggcctcg    600

gatgaggaaa ttgagaagct gtccacgctg tactggttca cggtggagtt cgggctgtgt    660

aagcagaacg gggaggtgaa ggcctatggt gccgggctgc tgtcctccta cggggagctc    720

ctgcactgcc tgtctgagga gcctgagatt cgggccttcg accctgaggc tgcggccgtg    780

cagccctacc aagaccagac gtaccagtca gtctacttcg tgtctgagag cttcagtgac    840

gccaaggaca agctcaggag ctatgcctca cgcatccagc gccccttctc cgtgaagttc    900

gacccgtaca cgctggccat cgacgtgctg gacagccccc aggccgtgcg gcgctccctg    960

gagggtgtcc aggatgagct ggacaccctt gcccatgcgc tgagtgccat tggctag      1017
```

<210> SEQ ID NO 28
<211> LENGTH: 501
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 28

```
Met Pro Thr Pro Asp Ala Thr Thr Pro Gln Ala Lys Gly Phe Arg Arg
1               5                   10                  15

Ala Val Ser Glu Leu Asp Ala Lys Gln Ala Glu Ala Ile Met Val Arg
            20                  25                  30
```

-continued

```
Gly Gln Ser Pro Arg Phe Ile Gly Arg Arg Gln Ser Leu Ile Glu Asp
        35                  40                  45

Ala Arg Lys Glu Arg Glu Ala Ala Val Ala Ala Ala Ala Ala Ala Val
    50                  55                  60

Pro Ser Glu Pro Gly Asp Pro Leu Glu Ala Val Ala Phe Glu Glu Lys
65                  70                  75                  80

Glu Gly Lys Ala Val Leu Asn Leu Leu Phe Ser Pro Arg Ala Thr Lys
                85                  90                  95

Pro Ser Ala Leu Ser Arg Ala Val Lys Val Phe Glu Thr Phe Glu Ala
            100                 105                 110

Lys Ile His His Leu Glu Thr Arg Pro Ala Gln Arg Pro Arg Ala Gly
        115                 120                 125

Gly Pro His Leu Glu Tyr Phe Val Arg Leu Glu Val Arg Arg Gly Asp
    130                 135                 140

Leu Ala Ala Leu Leu Ser Gly Val Arg Gln Val Ser Glu Asp Val Arg
145                 150                 155                 160

Ser Pro Ala Gly Pro Lys Val Pro Trp Phe Pro Arg Lys Val Ser Glu
                165                 170                 175

Leu Asp Lys Cys His His Leu Val Thr Lys Phe Asp Pro Asp Leu Asp
            180                 185                 190

Leu Asp His Pro Gly Phe Ser Asp Gln Val Tyr Arg Gln Arg Arg Lys
        195                 200                 205

Leu Ile Ala Glu Ile Ala Phe Gln Tyr Arg His Gly Asp Pro Ile Pro
    210                 215                 220

Arg Val Glu Tyr Thr Ala Glu Glu Ile Ala Thr Trp Lys Glu Val Tyr
225                 230                 235                 240

Thr Thr Leu Lys Gly Leu Tyr Ala Thr His Ala Cys Gly Glu His Leu
                245                 250                 255

Glu Ala Phe Ala Leu Leu Glu Arg Phe Ser Gly Tyr Arg Glu Asp Asn
            260                 265                 270

Ile Pro Gln Leu Glu Asp Val Ser Arg Phe Leu Lys Glu Arg Thr Gly
        275                 280                 285

Phe Gln Leu Arg Pro Val Ala Gly Leu Leu Ser Ala Arg Asp Phe Leu
    290                 295                 300

Ala Ser Leu Ala Phe Arg Val Phe Gln Cys Thr Gln Tyr Ile Arg His
305                 310                 315                 320

Ala Ser Ser Pro Met His Ser Pro Glu Pro Asp Cys Cys His Glu Leu
                325                 330                 335

Leu Gly His Val Pro Met Leu Ala Asp Arg Thr Phe Ala Gln Phe Ser
            340                 345                 350

Gln Asp Ile Gly Leu Ala Ser Leu Gly Ala Ser Asp Glu Glu Ile Glu
        355                 360                 365

Lys Leu Ser Thr Leu Tyr Trp Phe Thr Val Glu Phe Gly Leu Cys Lys
    370                 375                 380

Gln Asn Gly Glu Val Lys Ala Tyr Gly Ala Gly Leu Leu Ser Ser Tyr
385                 390                 395                 400

Gly Glu Leu Leu His Cys Leu Ser Glu Glu Pro Glu Ile Arg Ala Phe
                405                 410                 415

Asp Pro Glu Ala Ala Ala Val Gln Pro Tyr Gln Asp Gln Thr Tyr Gln
            420                 425                 430
```

-continued

```
Ser Val Tyr Phe Val Ser Glu Ser Phe Ser Asp Ala Lys Asp Lys Leu
        435                 440                 445

Arg Ser Tyr Ala Ser Arg Ile Gln Arg Pro Phe Ser Val Lys Phe Asp
    450                 455                 460

Pro Tyr Thr Leu Ala Ile Asp Val Leu Asp Ser Pro Gln Ala Val Arg
465                 470                 475                 480

Arg Ser Leu Glu Gly Val Gln Asp Glu Leu Asp Thr Leu Ala His Ala
                485                 490                 495

Leu Ser Ala Ile Gly
            500
```

<210> SEQ ID NO 29
<211> LENGTH: 1506
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 29

```
atgcccaccc ccgacgccac cacgccacag gccaagggct tccgcagggc cgtgtctgag     60

ctggacgcca agcaggcaga ggccatcatg gtaagagggc agtccccgcg gttcattggg    120

cgcaggcaga gcctcatcga ggacgcccgc aaggagcggg aggcggcggt ggcagcagcg    180

gccgctgcag tcccctcgga gcccggggac cccctggagg ctgtggcctt tgaggagaag    240

gaggggaagg ccgtgctaaa cctgctcttc tccccgaggg ccaccaagcc ctcggcgctg    300

tcccgagctg tgaaggtgtt tgagacgttt gaagccaaaa tccaccatct agagacccgg    360

cccgcccaga ggccgcgagc tgggggcccc cacctggagt acttcgtgcg cctcgaggtg    420

cgccgagggg acctggccgc cctgctcagt ggtgtgcgcc aggtgtcaga ggacgtgcgc    480

agccccgcgg ggcccaaggt cccctggttc ccaagaaaag tgtcagagct ggacaagtgt    540

catcacctgg tcaccaagtt cgaccctgac ctggacttgg accacccggg cttctcggac    600

caggtgtacc gccagcgcag gaagctgatt gctgagatcg ccttccagta caggcacggc    660

gacccgattc cccgtgtgga gtacaccgcc gaggagattg ccacctggaa ggaggtctac    720

accacgctga agggcctcta cgccacgcac gcctgcgggg agcacctgga ggcctttgct    780

ttgctggagc gcttcagcgg ctaccgggaa gacaatatcc cccagctgga ggacgtctcc    840

cgcttcctga aggagcgcac gggcttccag ctgcggcctg tggccggcct gctgtccgcc    900

cgggacttcc tggccagcct ggccttccgc gtgttccagt gcacccagta tatccgccac    960

gcgtcctcgc ccatgcactc ccctgagccg gactgctgcc acgagctgct ggggcacgtg   1020

cccatgctgg ccgaccgcac cttcgcgcag ttctcgcagg acattggcct ggcgtccctg   1080

ggggcctcgg atgaggaaat tgagaagctg tccacgctgt actggttcac ggtggagttc   1140

gggctgtgta agcagaacgg ggaggtgaag gcctatggtg ccgggctgct gtcctcctac   1200

ggggagctcc tgcactgcct gtctgaggag cctgagattc gggccttcga ccctgaggct   1260

gcggccgtgc agccctacca agaccagacg taccagtcag tctacttcgt gtctgagagc   1320

ttcagtgacg ccaaggacaa gctcaggagc tatgcctcac gcatccagcg ccccttctcc   1380

gtgaagttcg acccgtacac gctggccatc gacgtgctgg acagccccca ggccgtgcgg   1440

cgctccctgg agggtgtcca ggatgagctg gacacccttg cccatgcgct gagtgccatt   1500

ggctag                                                              1506
```

-continued

<210> SEQ ID NO 30
<211> LENGTH: 480
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 30

Met Asp Ala Ser Glu Phe Arg Arg Arg Gly Lys Glu Met Val Asp Tyr
1 5 10 15

Val Ala Asn Tyr Met Glu Gly Ile Glu Gly Arg Gln Val Tyr Pro Asp
20 25 30

Val Glu Pro Gly Tyr Leu Arg Pro Leu Ile Pro Ala Ala Ala Pro Gln
35 40 45

Glu Pro Asp Thr Phe Glu Asp Ile Ile Asn Asp Val Glu Lys Ile Ile
50 55 60

Met Pro Gly Val Thr His Trp His Ser Pro Tyr Phe Phe Ala Tyr Phe
65 70 75 80

Pro Thr Ala Ser Ser Tyr Pro Ala Met Leu Ala Asp Met Leu Cys Gly
85 90 95

Ala Ile Gly Cys Ile Gly Phe Ser Trp Ala Ala Ser Pro Ala Cys Thr
100 105 110

Glu Leu Glu Thr Val Met Met Asp Trp Leu Gly Lys Met Leu Glu Leu
115 120 125

Pro Lys Ala Phe Leu Asn Glu Lys Ala Gly Glu Gly Gly Gly Val Ile
130 135 140

Gln Gly Ser Ala Ser Glu Ala Thr Leu Val Ala Leu Leu Ala Ala Arg
145 150 155 160

Thr Lys Val Ile His Arg Leu Gln Ala Ala Ser Pro Glu Leu Thr Gln
165 170 175

Ala Ala Ile Met Glu Lys Leu Val Ala Tyr Ser Ser Asp Gln Ala His
180 185 190

Ser Ser Val Glu Arg Ala Gly Leu Ile Gly Gly Val Lys Leu Lys Ala
195 200 205

Ile Pro Ser Asp Gly Asn Phe Ala Met Arg Ala Ser Ala Leu Gln Glu
210 215 220

Ala Leu Glu Arg Asp Lys Ala Ala Gly Leu Ile Pro Phe Phe Met Val
225 230 235 240

Ala Thr Leu Gly Thr Thr Thr Cys Cys Ser Phe Asp Asn Leu Leu Glu
245 250 255

Val Gly Pro Ile Cys Asn Lys Glu Asp Ile Trp Leu His Val Asp Ala
260 265 270

Ala Tyr Ala Gly Ser Ala Phe Ile Cys Pro Glu Phe Arg His Leu Leu
275 280 285

Asn Gly Val Glu Phe Ala Asp Ser Phe Asn Phe Asn Pro His Lys Trp
290 295 300

Leu Leu Val Asn Phe Asp Cys Ser Ala Met Trp Val Lys Lys Arg Thr
305 310 315 320

Asp Leu Thr Gly Ala Phe Arg Leu Asp Pro Thr Tyr Leu Lys His Ser
325 330 335

His Gln Asp Ser Gly Leu Ile Thr Asp Tyr Arg His Trp Gln Ile Pro
340 345 350

Leu Gly Arg Arg Phe Arg Ser Leu Lys Met Trp Phe Val Phe Arg Met
355 360 365

-continued

```
Tyr Gly Val Lys Gly Leu Gln Ala Tyr Ile Arg Lys His Val Gln Leu
    370                 375                 380

Ser His Glu Phe Glu Ser Leu Val Arg Gln Asp Pro Arg Phe Glu Ile
385                 390                 395                 400

Cys Val Glu Val Ile Leu Gly Leu Val Cys Phe Arg Leu Lys Gly Ser
                405                 410                 415

Asn Lys Val Asn Glu Ala Leu Leu Gln Arg Ile Asn Ser Ala Lys Lys
            420                 425                 430

Ile His Leu Val Pro Cys His Leu Arg Asp Lys Phe Val Leu Arg Phe
        435                 440                 445

Ala Ile Cys Ser Arg Thr Val Glu Ser Ala His Val Gln Arg Ala Trp
    450                 455                 460

Glu His Ile Lys Glu Leu Ala Ala Asp Val Leu Arg Ala Glu Arg Glu
465                 470                 475                 480
```

<210> SEQ ID NO 31
<211> LENGTH: 1443
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 31

```
atggacgcca gtgagttccg aaggcgcggc aaggagatgg tggactacgt ggccaactac     60

atggaaggca tcgagggccg ccaagtctac cccgacgtgg agcccggcta cctgcgcccg    120

ctgatccccg ccgctgcccc tcaggagccc gacaccttcg aggacatcat caacgacgtg    180

gagaagatca tcatgcctgg cgtgacgcac tggcacagcc cctacttctt cgcctacttc    240

cccaccgcca gctcgtaccc ggccatgctg gcggacatgc tgtgcggggc cattggctgc    300

atcggcttct cctgggcggc gagcccagcg tgcaccgagc tggagaccgt gatgatggac    360

tggctcggga agatgctgga gctcccaaag gcgttcttga acgagaaggc tggcgagggg    420

ggcggcgtga tccagggcag cgccagcgag gccaccctgg tggccctgct ggccgctcgg    480

accaaagtga tccaccggct gcaggcagcg tccccagagc tcacccaggc cgctatcatg    540

gagaagctgg tggcttactc ctccgatcag gcacactcct ccgtggaacg cgctgggctc    600

attggtggag tgaagctcaa ggccatcccc agcgatggca acttcgccat gcgtgcgagc    660

gccctgcagg aagccctgga gagagacaag gcggctggcc tgattccttt cttcatggtg    720

gccaccctgg ggaccacaac atgctgctcc ttcgacaacc tcctcgaagt cggtcctatc    780

tgcaacaagg aagacatctg gctgcacgtt gatgcagcct acgcaggcag cgcattcatc    840

tgccctgagt tccggcacct tctgaacgga gtggagttcg cagatagctt caacttcaat    900

ccccacaagt ggctattggt gaatttcgac tgcagcgcca tgtgggtgaa gaagcgcacc    960

gacctcacgg gagccttccg cctggacccc acttacctga agcacagcca ccaggattca   1020

gggcttatca ctgactaccg gcactggcag atcccactgg gccgcagatt ccgcagcttg   1080

aagatgtggt tcgtattcag gatgtatgga gtcaagggac tgcaggctta tatccgcaag   1140

catgtccagc tgtcccatga gtttgagtca ctggtgcgcc aggatccccg ctttgaaatc   1200

tgtgtggaag tcattctggg gcttgtctgc tttcggctaa agggttccaa caaagtgaat   1260

gaagctcttc tgcaaaggat caacagtgcc aaaaaaatcc acttggttcc atgtcacctc   1320

agggacaagt ttgtcctgcg ctttgccatc tgttctcgca ccgtggaatc tgcccatgtg   1380

cagcgggcct gggaacacat caaagagctg gcggccgacg tgctgcgagc agagagggag   1440
```

-continued

tag 1443

<210> SEQ ID NO 32
<211> LENGTH: 480
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 32

Met Asn Ala Ser Glu Phe Arg Arg Arg Gly Lys Glu Met Val Asp Tyr
1 5 10 15

Val Ala Asn Tyr Met Glu Gly Ile Glu Gly Arg Gln Val Tyr Pro Asp
20 25 30

Val Glu Pro Gly Tyr Leu Arg Pro Leu Ile Pro Ala Ala Ala Pro Gln
35 40 45

Glu Pro Asp Thr Phe Glu Asp Ile Ile Asn Asp Val Glu Lys Ile Ile
50 55 60

Met Pro Gly Val Thr His Trp His Ser Pro Tyr Phe Phe Ala Tyr Phe
65 70 75 80

Pro Thr Ala Ser Ser Tyr Pro Ala Met Leu Ala Asp Met Leu Cys Gly
85 90 95

Ala Ile Gly Cys Ile Gly Phe Ser Trp Ala Ala Ser Pro Ala Cys Thr
100 105 110

Glu Leu Glu Thr Val Met Met Asp Trp Leu Gly Lys Met Leu Glu Leu
115 120 125

Pro Lys Ala Phe Leu Asn Glu Lys Ala Gly Glu Gly Gly Gly Val Ile
130 135 140

Gln Gly Ser Ala Ser Glu Ala Thr Leu Val Ala Leu Leu Ala Ala Arg
145 150 155 160

Thr Lys Val Ile His Arg Leu Gln Ala Ala Ser Pro Glu Leu Thr Gln
165 170 175

Ala Ala Ile Met Glu Lys Leu Val Ala Tyr Ser Ser Asp Gln Ala His
180 185 190

Ser Ser Val Glu Arg Ala Gly Leu Ile Gly Gly Val Lys Leu Lys Ala
195 200 205

Ile Pro Ser Asp Gly Asn Phe Ala Met Arg Ala Ser Ala Leu Gln Glu
210 215 220

Ala Leu Glu Arg Asp Lys Ala Ala Gly Leu Ile Pro Phe Phe Met Val
225 230 235 240

Ala Thr Leu Gly Thr Thr Thr Cys Cys Ser Phe Asp Asn Leu Leu Glu
245 250 255

Val Gly Pro Ile Cys Asn Lys Glu Asp Ile Trp Leu His Val Asp Ala
260 265 270

Ala Tyr Ala Gly Ser Ala Phe Ile Cys Pro Glu Phe Arg His Leu Leu
275 280 285

Asn Gly Val Glu Phe Ala Asp Ser Phe Asn Phe Asn Pro His Lys Trp
290 295 300

Leu Leu Val Asn Phe Asp Cys Ser Ala Met Trp Val Lys Lys Arg Thr
305 310 315 320

Asp Leu Thr Gly Ala Phe Arg Leu Asp Pro Thr Tyr Leu Lys His Ser
325 330 335

His Gln Asp Ser Gly Leu Ile Thr Asp Tyr Arg His Trp Gln Ile Pro
340 345 350

-continued

```
Leu Gly Arg Arg Phe Arg Ser Leu Lys Met Trp Phe Val Phe Arg Met
        355                 360                 365

Tyr Gly Val Lys Gly Leu Gln Ala Tyr Ile Arg Lys His Val Gln Leu
    370                 375                 380

Ser His Glu Phe Glu Ser Leu Val Arg Gln Asp Pro Arg Phe Glu Ile
385                 390                 395                 400

Cys Val Glu Val Ile Leu Gly Leu Val Cys Phe Arg Leu Lys Gly Ser
                405                 410                 415

Asn Lys Val Asn Glu Ala Leu Leu Gln Arg Ile Asn Ser Ala Lys Lys
            420                 425                 430

Ile His Leu Val Pro Cys His Leu Arg Asp Lys Phe Val Leu Arg Phe
        435                 440                 445

Ala Ile Cys Ser Arg Thr Val Glu Ser Ala His Val Gln Arg Ala Trp
    450                 455                 460

Glu His Ile Lys Glu Leu Ala Ala Asp Val Leu Arg Ala Glu Arg Glu
465                 470                 475                 480
```

<210> SEQ ID NO 33
<211> LENGTH: 1443
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 33

```
atgaacgcaa gtgaattccg aaggagaggg aaggagatgg tggattacgt ggccaactac     60

atggaaggca ttgagggacg ccaggtctac cctgacgtgg agcccgggta cctgcggccg    120

ctgatccctg ccgctgcccc tcaggagcca gacacgtttg aggacatcat caacgacgtt    180

gagaagataa tcatgcctgg ggtgacgcac tggcacagcc cctacttctt cgcctacttc    240

cccactgcca gctcgtaccc ggccatgctt gcggacatgc tgtgcggggc cattggctgc    300

atcggcttct cctgggcggc aagcccagca tgcacagagc tggagactgt gatgatggac    360

tggctcggga agatgctgga actaccaaag gcatttttga atgagaaagc tggagaaggg    420

ggaggagtga tccagggaag tgccagtgaa gccaccctgg tggccctgct ggccgctcgg    480

accaaagtga tccatcggct gcaggcagcg tccccagagc tcacacaggc cgctatcatg    540

gagaagctgg tggcttactc atccgatcag gcacactcct cagtggaaag agctgggtta    600

attggtggag tgaaattaaa agccatcccc tcagatggca acttcgccat gcgtgcgtct    660

gccctgcagg aagccctgga gagagacaaa gcggctggcc tgattccttt ctttatggtt    720

gccaccctgg ggaccacaac atgctgctcc tttgacaatc tcttagaagt cggtcctatc    780

tgcaacaagg aagacatatg gctgcacgtt gatgcagcct acgcaggcag tgcattcatc    840

tgccctgagt tccggcacct tctgaatgga gtggagtttg cagattcatt caactttaat    900

ccccacaaat ggctattggt gaattttgac tgttctgcca tgtgggtgaa aaagagaaca    960

gacttaacgg gagcctttag actggacccc acttacctga agcacagcca tcaggattca   1020

gggcttatca ctgactaccg gcattggcag ataccactgg gcagaagatt tcgctctttg   1080

aaaatgtggt ttgtatttag gatgtatgga gtcaaaggac tgcaggctta tatccgcaag   1140

catgtccagc tgtcccatga gtttgagtca ctggtgcgcc aggatccccg ctttgaaatc   1200

tgtgtggaag tcattctggg gcttgtctgc tttcggctaa agggttccaa caaagtgaat   1260

gaagctcttc tgcaaagaat aaacagtgcc aaaaaaatcc acttggttcc atgtcacctc   1320
```

-continued

agggacaagt ttgtcctgcg ctttgccatc tgttctcgca cggtggaatc tgcccatgtg 1380

cagcgggcct gggaacacat caaagagctg gcggccgacg tgctgcgagc agagagggag 1440

tag 1443

<210> SEQ ID NO 34
<211> LENGTH: 11622
<212> TYPE: DNA
<213> ORGANISM: Equine Infectious Anemia Virus

<400> SEQUENCE: 34

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1-71. (canceled)

72. A method for treating Parkinson's disease in a subject in need thereof, comprising administering into the brain of the subject a lentiviral vector comprising three nucleotides of interest (NOIs) operably linked by one or more Internal Ribosome Entry Site(s) (IRES), wherein the NOIs encode tyrosine hydroxylase (TH), GTP-cyclohydrolase I (GTP-CH1) and aromatic amino acid dopa decarboxylase (AADC), and wherein the three NOIs are expressed to stimulate dopamine synthesis in the brain, thereby treating the subject.

73. The method according to claim 72, wherein the lentiviral vector is an HIV lentiviral vector.

74. The method according to claim 72, wherein the lentiviral vector is a non-primate lentiviral vector.

75. The method according to claim 74, wherein the lentiviral vector is an EIAV lentiviral vector.

76. The method according to claim 72, wherein at least one of the NOIs is operably linked to a promoter or promoter elements.

77. The method according to claim 72, wherein the lentiviral vector further comprises a post-transcriptional regulatory element or a translational enhancer.

78. The method according to claim 72, wherein at least one of the NOIs is codon optimized.

79. The method according to claim 72, wherein the lentiviral vector is pseudotyped with at least part of a heterologous env protein.

80. The method according to claim 79, wherein the heterologous env protein is Rabies-G or VSV-G.

81. The method according to claim 72, wherein the IRES is a viral IRES.

82. The method according to claim 81, wherein the viral IRES is from a picornavirus.

83. The method according to claim 82, wherein the picornavirus is encephalomyocarditis virus (EMCV) or poliovirus (PV).

84. The method according to claim 72, wherein the lentiviral vector comprises a self-inactivating lentiviral vector genome.

85. A pharmaceutical composition comprising a lentiviral vector comprising three nucleotides of interest (NOIs) operably linked by one or more Internal Ribosome Entry Site(s) (IRES), wherein the NOIs encode tyrosine hydroxylase (TH), GTP-cyclohydrolase I (GTP-CH1) and aromatic amino acid dopa decarboxylase (AADC).

86. The pharmaceutical composition of claim 85, wherein the lentiviral vector is an HIV lentiviral vector.

87. The pharmaceutical composition of claim 85, wherein the lentiviral vector is a non-primate lentiviral vector.

88. The pharmaceutical composition of claim 87, wherein the lentiviral vector is an EIAV lentiviral vector.

89. The pharmaceutical composition of claim 85, wherein at least one of the NOIs is operably linked to a promoter or promoter elements.

90. The pharmaceutical composition of claim 85, wherein the lentiviral vector further comprises a post-transcriptional regulatory element or a translational enhancer.

91. The pharmaceutical composition of claim 85, wherein at least one of the NOIs is codon optimized.

92. The pharmaceutical composition of claim 85, wherein the lentiviral vector is pseudotyped with at least part of a heterologous env protein.

93. The pharmaceutical composition of claim 92, wherein the heterologous env protein is Rabies-G or VSV-G.

94. The pharmaceutical composition of claim 85, wherein the IRES is a viral IRES.

95. The pharmaceutical composition of claim 94, wherein the viral IRES is from a picornavirus.

96. The pharmaceutical composition of claim 95, wherein the picornavirus is encephalomyocarditis virus (EMCV) or poliovirus (PV).

97. The pharmaceutical composition of claim 85, wherein the lentiviral vector comprises a self-inactivating lentiviral vector genome.

* * * * *