

pGIPZ EG5 and GAPDH lentiviral shRNAmir positive control vectors

RHS 4371, RHS4480

The pGIPZ EG5 and GAPDH lentiviral shRNAmir vectors have been validated as positive controls for RNAi experiments performed using the pGIPZ shRNAmir-containing lentiviral vectors. These shRNAmir have been tested in transduction based experiments and have shown efficient knockdown at both mRNA and protein levels.

Features of the pGIPZ vector that make it a versatile tool for RNAi studies include:

- Ability to perform transfections (transient and stable) or transductions using the replication incompetent lentivirus
- Turbo-GFP(tGFP) and shRNAmir are part of a bicistronic transcript allowing the visual marking of shRNAmir expressing cells
- Amenable to *in vitro* and *in vivo* applications
- Puromycin drug resistance marker for selecting stable cell lines
- Molecular barcodes enable multiplexed screening in pools

Vector shipping and storage

Each vial of the GIPZ lentiviral shRNAmir positive controls EG5 or GAPDH are shipped as bacterial cultures of *E. coli* (prime+) in LB (low salt) broth with 8% glycerol, carbenicillin (100ug/ml) and zeocin (25ug/ml). Individual vials are shipped on wet ice. All GIPZ lentiviral shRNAmir constructs should be stored at -80°C. Open Biosystems checks all cultures for growth prior to shipment.

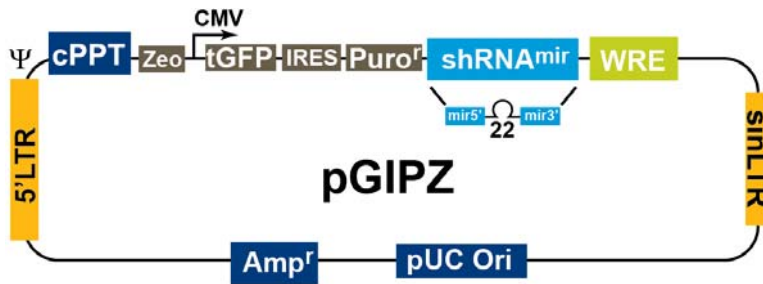


Figure 1: pGIPZ lentiviral vector

Table 1: Features of the pGIPZ Vector

Vector Element	Utility
CMV Promoter	RNA Polymerase II promoter
cPPT	Central polypurine tract helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
GFP	Marker to track shRNAmir expression
IRES-Puro	Mammalian selectable marker
AMP ^r	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in <i>E.coli</i>
SIN-LTR	3' Self inactivating long terminal repeat
RRE	Rev response element
Zeo ^r	Bacterial selectable marker

Table 2: Antibiotic Resistances Conveyed by pGIPZ

Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100µg/ml	Bacterial selection marker
Zeocin	25µg/ml	Bacterial selection marker (vector)
Puromycin	Variable	Mammalian selectable marker

Transduction based validation studies

Hek 293T cells were trypsinized from a healthy, growing cultures, seeded into 24-well plates at $5-8 \times 10^4$ per well and allowed to adhere for 24 hours in DMEM with 10% FCS. DMEM containing serum was replaced with 200 μ l serum-free media and lentiviral particles containing GAPDH or EG5 shRNAmir, non-silencing or non-transduced controls were added to the appropriate wells at three different multiplicity of infections (MOI) and incubated for 6 hours. DMEM containing serum was then added and the transduced cells were further incubated for a total of 48 hours.

RNA Extraction and Validation

At 48 hours post-transduction, transduced cells were lysed and total RNA was extracted using the Qiagen RNAeasy Kit (cat# 74104). The RNA was converted to cDNA using the ABI- High Capacity cDNA RT Kit (cat#4368813), using 500ng total RNA in a 100 μ l reaction. A 1/100 dilution of the cDNA was used in Real Time Quantitative PCR (QPCR). Each gene was validated in triplicate, standardized to a 18s endogenous control and compared to non-silencing or non-transduced experimental controls. Knockdown was calculated as the percentage remaining gene expression normalized to the relevant non-silencing control.

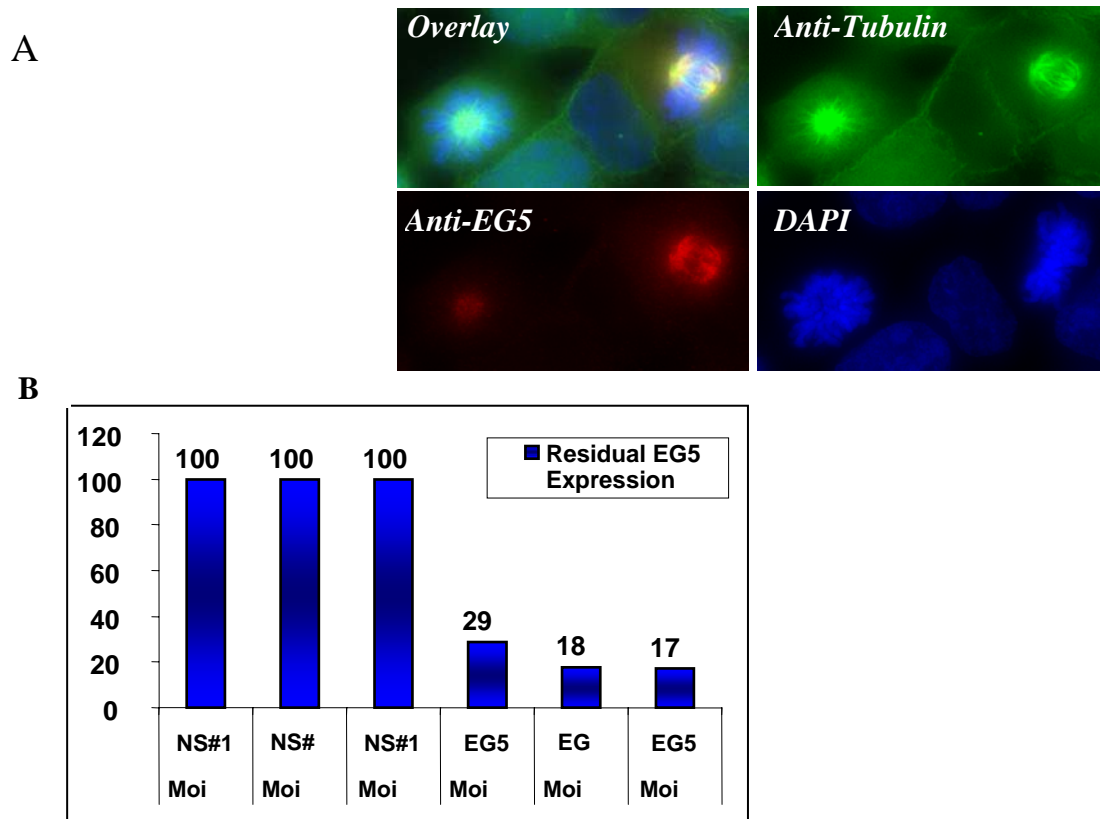


Figure 2. Knockdown of the EG5 (KIF11) gene allowed evaluation of phenotypic evidence of RNAi as well as its molecular manifestation

A. The characteristic phenotype observed by the targeting of the EG5 (KIF11) gene results in the formation of half spindles, mitotic arrest and monoastrial microtubular arrays (green, see the cell on the left). By contrast, normal cells show bipolar spindles and microtubule networks in mitosis and in interphase (see the cell on the right). The

comparative expression of EG5 (red) between the cell on the left and the right shows the extensive knockdown of EG5 in the cell displaying the phenotype (left). The cells were visualized at 100x magnification using a Leica DMIRB fluorescence microscope. HEK293T cells were stained for tubulin (anti-tubulin, green), DNA (DAPI, blue) and EG5 (anti-EG5, red). B. Hek293T cells were transduced with lentiviral particles expressing EG5 or non-silencing shRNAmir at MOIs of 3.5, 8.5 and 17. The graph depicts the residual levels of EG5 relative to its non-silencing control.

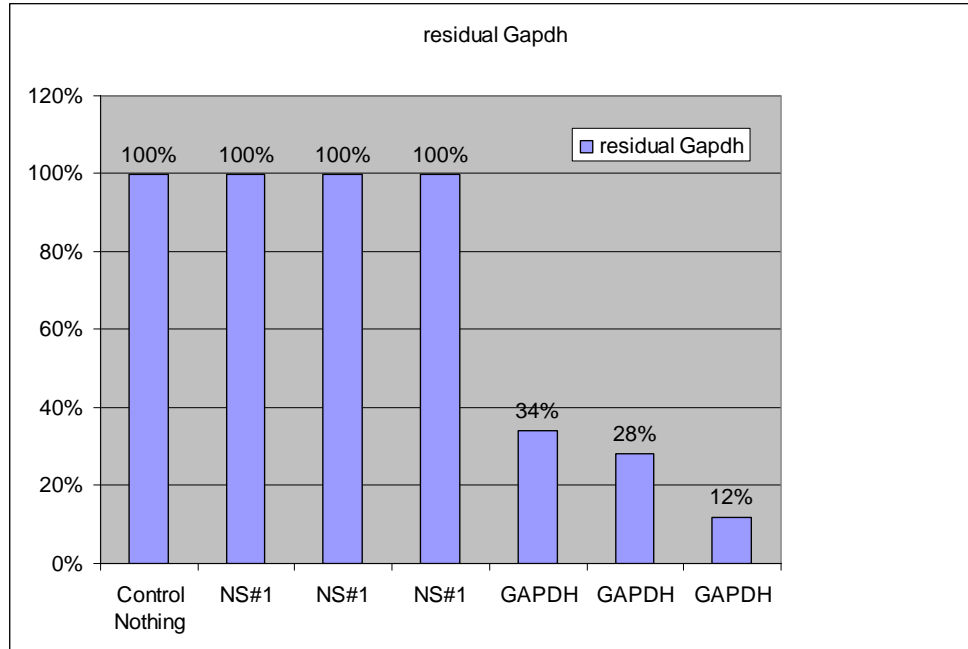


Figure 3. Hek293T cells were transduced with lentiviral particles expressing GAPDH or non-silencing shRNAmir at variable MOIs ranging from 9-48. The graph depicts the residual levels of GAPDH relative to its non-silencing control.

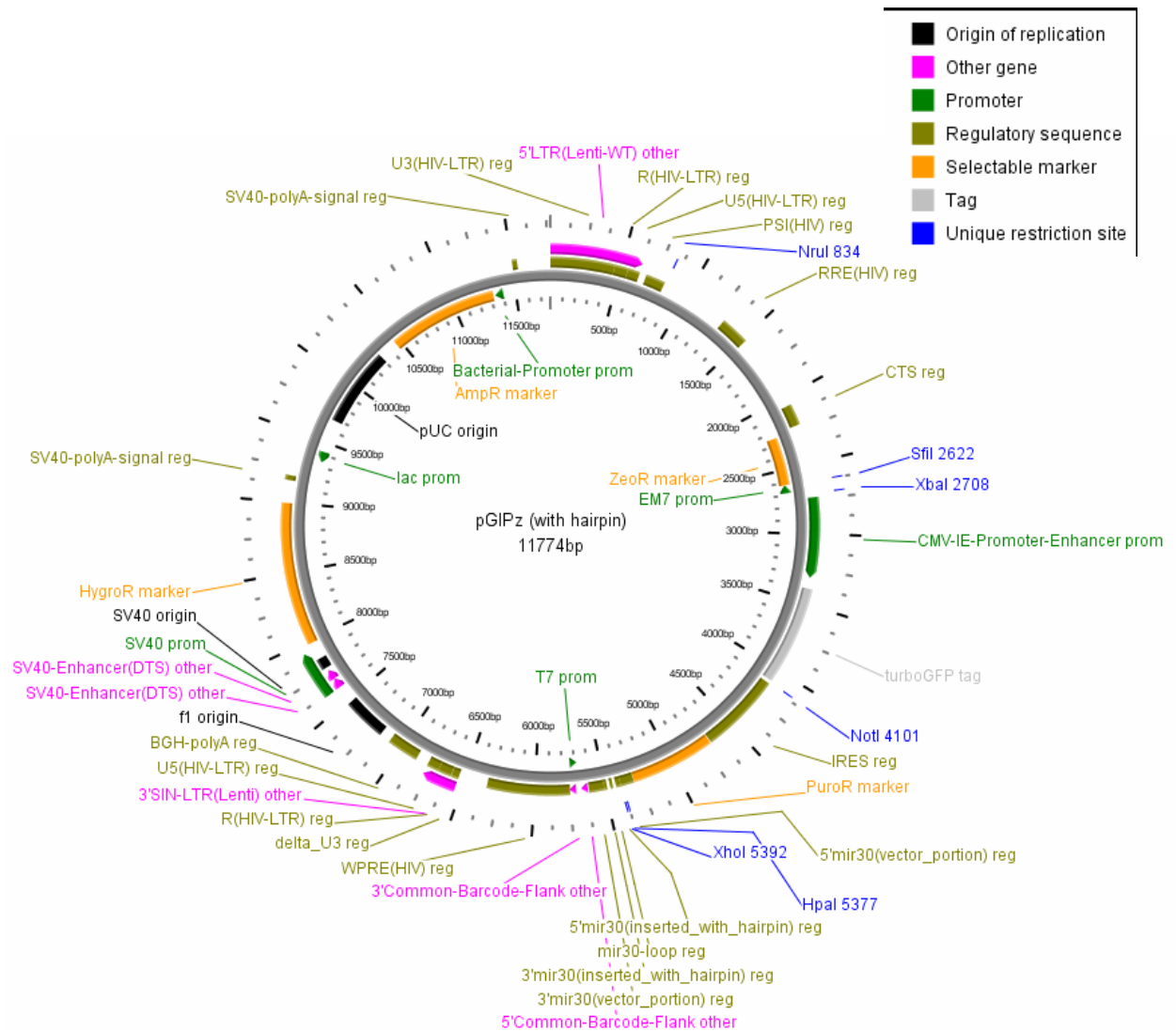


Figure 4: Detailed Vector Map of pGIPZ.

Sequence of pGIPZ lentiviral vector (11774bp)

5'LTR(Lenti-WT) other(1,635)>>>
|
U3(HIV-LTR) reg(1,455)>>>
|

1 tggaaagggctaattcactcccaaagaagacaagatatccttgatctgtggatctaccaca 60
ACCTTCCCGATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT

61 cacaaggctacttccctgattagcagaactacacaccagggccaggggtcagatatccac 120
GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCCAGTCTATAGGTG

121 tgacctttggatgggtgctacaagctagtaccagttgagccagataaggtagaagaggcca 180
ACTGGAACCTACCACGATGTTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT

181 ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgaccgg 240
TATTTCTCTCTTGTGGTTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC

241 agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccag 300
TCTCTCTTCACAATCTCACCTCCAAACTGTGGGCGGATCGTAAAGTAGTGCACCGGGCTC

301 agctgcatccggagtagtacttcaagaactgctgatatcgagcttgctacaagggactttccg 360
TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC

361 ctggggactttccagggagggcgtggcctggggcgggactggggagtggcgagccctcagat 420
GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA

R(HIV-LTR) reg(456,550)>>>
|

421 cctgcatataagcagctgctttttgctgtactgggtctctctggttagaccagatctga 480
GGACGTATATTCGTCGACGAAAAACGGACATGACCCAGAGAGACCAATCTGGTCTAGACT

481 gcctgggagctctctggctaactagggaaaccactgcttaagcctcaataaagcttgct 540
CGGACCCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTGAAACGGA

U5(HIV-LTR) reg(551,635)>>>
|

541 tgagtgtttcaagtagtgtgtgcccgtctgttggtgactctggtaactagagatccctc 600
ACTCACGAAGTTCATCACACACGGGCAGACAACACACTGAGACCATTGATCTCTAGGGAG

601 agacccttttagtcagtggtgaaaatctctagcagtgggcggccgaacagggacttgaaag 660
TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC

PSI(HIV) reg(685,822)>>>
|

661 cgaaagggaaaccagaggagctctctcgacgcaggactcggcttgctgaagcgcgcacgg 720
GCTTTCCCTTTGGTCTCCTCGAGAGAGCTGCGTCTGAGCCGAACGACTTCGCGCGTGCC

721 caagagggcagggggcggcgactggtgagtacgcaaaaattttgactagcggaggctaga 780
GTTCTCCGCTCCCCGCGCTGACCACTCATGCGGTTTTTAAACTGATCGCCTCCGATCT

NruI
|

781 aggagagagatgggtgagagagcgtcagtattaagcgggggagaattagatcgcgatggg 840
TCCTCTCTTACCCACGCTCTCGCAGTCATAATTGCCCCCTCTTAATCTAGCGCTACCC

841 aaaaaattcgggtaaggccagggggaagaaaaatataaattaaaacatatagtatggg 900
TTTTTTAAGCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATACCC

901 caagcagggagctagaacgattcgcagttaatcctggcctgtagaaacatcagaaggct 960
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961 gtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagat 1020
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1021 cattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagaca 1080
GTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTTCTGT

1081 ccaaggaagcttttagacaagatagaggaagagcaaaacaaaagtaagaccaccgcacagc 1140
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1141 aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag 1200
TTCCGCCGGCCGGCGACTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTAACTCTTC

1201 tgaattatataaatataaagtagtaaaaattgaaccattaggagtagcaccaccaaggc 1260
ACTTAATATATTTATATTTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTCCG

RRE (HIV)

reg(1314,1518)>>>

1261 aaagagaagagtggtgcagagagaaaaaagagcagtggggaataggagctttgttccttgg 1320
TTTCTCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCTCGAAACAAGGAACC

1321 gttccttgggagcagcaggaagcactatgggpcgagcgtcaatgacgctgacggtacagggc 1380
CAAGAACCCTCGTCGTCTTTCGTGATACCCGCGTCGCAGTTACTGCGACTGCCATGTCCG

1381 cagacaattattgtctgggtatagtgagcagcagacaatttgctgagggctattgagggc 1440
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1441 gcaacagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaagaatcct 1500
CGTTGTGCTAGACAACGTTGAGTGTGAGACCCCGTAGTTCGTGAGGTCCGTTCTTAGGA

1501 ggctgtggaaagatacctaaaggatcaacagctcctggggatttgggggtgctctggaaa 1560
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1561 actcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctggaaaca 1620
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1621 gatttgggaatcacacgacctggatggagtgaggacagagaaattaacaattacacaagctt 1680
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1681 aatacactccttaattgaagaatcgaaaaccagcaagaaaagaatgaacaagaattatt 1740
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1741 ggaattagataaatgggcaagtttgggaattggtttaacataacaaattggctgtggta 1800
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1801 tataaaattattcataatgatagtaggaggttggtaggtttaagaatagtttttctgtgt 1860
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1861 actttctatagtgaaatagagtttaggcagggatattcaccattatcgtttcagaccacct 1920
TGAAAGATATCACTTATCTCAATCCGTCCCTATAAGTGGTAATAGCAAAGTCTGGGTGGA

1921 cccaacccccgaggggacccgacaggccccgaaggaatagaagaagaaggtggagagagaga 1980
GGGTTGGGGCTCCCCTGGGCTGTCCGGGCTTCTTATCTTCTTCTTCCACCTCTCTCTCT

1981 cagagacagatccattcgattagtgaacggatcggcactgctgctgcgccaattctgcagac 2040
GTCTCTGTCTAGGTAAGCTAATCACTTGCCTAGCCGTGACGCACGCGGTTAAGACGTCTG

CTS reg(2064,2214)>>>

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2101 gggaaagaatagtagacataatagcaacagacatacaaaactaagaattacaaaaacaaa 2160
CCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTAATGTTTTTGT

2161 ttacaaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220
AATGTTTTTAAGTTTTAAAAGCCCAAATAATGTCCCTGTCGTCTCTAGGTCAAACCAATC

ZeoR marker(2245,2619)<<<

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ATGGCCCGGGCGAGATCAGGCCTTAGTCAGGACGAGGAGCCGGTGCTTACGTGCGTCAA

2281 gccggccgggtcgcgcagggcgaactcccgccccacggctgctcgcgatctcggatcat 2340
CGGCCGGCCAGCGCGTCCCGCTTGAGGGCGGGGTGCCGACGAGCGGCTAGAGCCAGTA

2341 ggccggccggaggcgtcccggaagtccgtggacacgacctccgaccactcggcgtacag 2400
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2401 ctcgctcaggccgcgcacccacacccaggccaggggtgtgtcggccaccacctggtcctg 2460
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2461 gaccgcgctgatgaacaggggtcacgtcgtcccggaccacaccggcgaagtgcctccac 2520
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2521 gaagtcccgggagaaacccgagccgggtcgggtccagaactcgaccgctccggcgacgtcgcg 2580
CTTCAGGGCCCTCTTGGGCTCGGCCAGCCAGGTCTTGAGCTGGCGAGGCCGCTGCAGCGC

SfiI

EM7 prom(2620,2683)<<<

2581 cgcggtgagcaccggaacggcactggtcaacttggccatggtggccctcctatagtgagt 2640
GCGCCACTCGTGGCCTTGCCGTGACCAGTTGAACCGGTACCACCGGGAGGATATCACTCA

2641 cgtattatactatgccgatatactatgccgatgattaattgtcaacacgtgctgcaggtc 2700
GCATAATATGATACGGCTATATGATACGGCTACTAATTAACAGTTGTGCACGACGTCCAG

XbaI

CMV-IE-Promoter-Enhancer

prom(2738,3311)>>>

2701 cgaggttctagacgtattaccgceatgcattagttattaatagtaatcaattacgggggtc 2760
GCTCCAAGATCTGCATAATGGCGGTACGTAATCAATAATTATCATTAGTTAATGCCCCAG

2761 attagttcatagcccatatatggagttccgcggttacataacttacggtaaatggcccgc 2820
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2821 tggctgaccgccccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagt 2880
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 turboGFP tag(3390,4088)>>>
 |
 3361 acaccgactctactagaggatctgccaccatggagagcgacgagagcggcctgcccgcca 3420
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 3421 tggagatcgagtgccgcatcaccggcaccctgaacggcgtggagttcgagctgggtggcg 3480
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 3661 ccaacaccgcatcgagaagtacgaggacggcggcgtgctgcacgtgagcttcagctacc 3720
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 3901 gctactacagctccgtgggtggacagccacatgcacttcaagagcggccatccaccccagca 3960

6661 ttaagcctcaataaagcttgcccttgagtgcttcaagtagtggtgcccgtctggtgtgtg 6720
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6721 actctggtaactagagatccctcagacccttttagtcagtggtgaaaatctctagcagta 6780
TGAGACCATTGATCTCTAGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCAT

6781 gtagttcatgtcatcttattattcagtatattataacttgcaaagaaatgaatatcagaga 6840
CATCAAGTACAGTAGAATAATAAGTCATAAATATTGAACGTTTCTTTACTTATAGTCTCT

BGH-polyA reg(6868,7091)>>>

6841 gtgagaggccttgacattgtttaaacccgctgatcagcctcgcactgtgccttctagttgc 6900
CACTCTCCGGAACGTAAACAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACG

6901 cagccatctggtgtttgcccctccccgctgccttccttgaccctggaaggtgccactccc 6960
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6961 actgtcctttcctaataaaaatgaggaaattgcatcgcattgtctgagtaggtgtcattct 7020
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7021 attctgggggggtgggggtggggcaggacagcaagggggaggattgggaagacaatagcagg 7080
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7081 catgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctct 7140
GTACGACCCCTACGCCACCCGAGATACCGAAGACTCCGCCTTTCTTGGTTCGACCCCGAGA

f1 origin(7171,7477)>>>

7141 aggggggatccccacgcgccctgtagcggcgcatthaagcgcggcggggtgtgggtgggttacg 7200
TCCCCCATAGGGGTGCGCGGGACATCGCCGCGTAATTCGCGCCGCCACACCACCAATGC

7201 cgcagcgtgaccgctacacttgccagcgccttagcgcggcctcctttcgctttcttccct 7260
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7261 tcctttctcgccacgcttcgcccggctttccccgctcaagctctaaatcgggggctcccttta 7320
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7321 ggggtccgatttagtgctttacggcacctcgacccccaaaaaacttgattaggggtgatggg 7380
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7441 ttctttaatagtggaactcttggtccaaactggaacaacactcaaccctatctcggtctat 7500
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7501 tcttttgatttataagggattttgccgatttcggcctattgggttaaaaaatgagctgatt 7560
AGAAAATAAATATTCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

SV40-

Enhancer(DTS) other(7609,7680)>>>

|

SV40

prom(7609,7930)>>>

|

7561 taacaaaaatthaacgcgaattaattctgtggaatgtgtgtcagttaggggtgtggaaagt 7620

ATTGTTTTTAAATTGCGCTTAATTAAGACACCTTACACACAGTCAATCCCACACCTTTCA

7621 ccccaggctcccagcagggcagaagatgcaaagcatgcatctcaattagtcagcaacca 7680
GGGGTCCGAGGGGTTCGTCCGTCTTCATACGTTTTCGTACGTAGAGTTAATCAGTCGTTGGT

SV40-Enhancer(DTS) other(7681,7752)>>>
|

7681 ggtgtggaaagtcccaggctcccagcagggcagaagatgcaaagcatgcatctcaatt 7740
CCACACCTTTCAGGGGTCCGAGGGGTTCGTCCGTCTTCATACGTTTTCGTACGTAGAGTTAA

SV40 origin(7776,7853)>>>
|

7741 agtcagcaaccatagtcgcccctaactccgcccatactccgcccctaactccgcccagtt 7800
TCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAA

7801 ccgcccattctccgcccatactccgcccatactccgcccctaactccgcccagtt 7860
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7861 cctctgcctctgagctattccagaagtagtgaggaggcttttttggaggcctaggctttt 7920
GGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAACCTCCGGATCCGAAAA

HygroR

marker(7979,8996)>>>
|

7921 gcaaaaagctcccgggagcttgtatatccattttcggatctgatcagcacgtgatgaaaa 7980
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7981 agcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtct 8040
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8041 ccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggag 8100
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8101 ggcgtggatagtcctcgggtaaatagctgcgcgatggtttctacaaagatcgttatg 8160
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8161 tttatcggcactttgcatcggccgagctcccattccggaagtgcttgacattggggaaat 8220
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8401 aatacactacatggcgtgatttcatatgcccgtattgctgatccccatggtgatcactggc 8460
TTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCG

8461 aaactgtgatggacgacaccgtcagtgccgtccgtcgcgcagggctctcgatgagctgatgc 8520
TTTGACACTACCTGCTGTGGCAGTCACGCAGGCAGCGCTCCGAGAGCTACTCGACTACG

8521 tttggggccgaggactgccccgaagtccggcacctcgtgcacgaggatttcggctccaaca 8580
AAACCCGGCTCCTGACGGGGCTTCAGGCCGTGGAGCACGTGCGCCTAAAGCCGAGGTTGT

8581 atgtcctgacggacaatggccgcataaacagcgggtcattgactggagcggagcggatggttcg 8640
TACAGGACTGCCTGTTACCGGCGTATTGTGCGCCAGTAACTGACCTCGCTCCGCTACAAGC

8641 gggattcccaatacagaggtcgccaacatcttcttctgaggccggtgggttggttgtatgg 8700
CCCTAAGGGTTATGCTCCAGCGGTTGTAGAAGAAGACCTCCGGCACCAACCGAACATACC

8701 agcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctcc 8760
TCGTGCTCTGCGCGATGAAGCTCGCCTCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGG

8761 gggcgatatatgctccgcattgggtcttgaccaactctatcagagcttgggtgacggcaatt 8820
CCCGCATATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAA

8821 tcgatgatgcagcttgggpcgagggctgatgagcgaatcgtccgatccggagccggga 8880
AGCTACTACGTGGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCT

8881 ctgtcgggpcgtacacaaatcgcccgcagaagcgcggccgctctggaccgatggctgtgtag 8940
GACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATC

8941 aagtactcgccgatagtggaaccgacgccccagcactcgtccgagggcaaaggaatagc 9000
TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTCTGAGCAGGCTCCCGTTTCCTTATCG

9001 acgtgctacgagatctcgattccaccgccccttctatgaaaggttgggcttcggaatcg 9060
TGCACGATGCTCTAAAGCTAAGGTGGCGGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061 ttttcgggacgcccggctggatgatcctccagcgcgggatctcatgctggagttcttcg 9120
AAAAGGCCCTGCGGCCGACCTACTAGGAGGTGCGGCCCTAGAGTACGACCTCAAGAAGC

SV40-polyA-signal

reg(9160,9194)>>>

9121 cccacccaacttgtttattgagcttataatgggttacaataaagcaatagcatcacia 9180
GGGTGGGGTTGAACAAATAACGTGCAATATTACCAATGTTTATTTTCGTTATCGTAGTGTT

9181 atttcacaataaagcatttttttactgcattctagttgtggtttgtccaaactcatca 9240
TAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGTTTTGAGTAGT

9241 atgtatcttatcatgtctgtataccgctcgacctctagctagagcttggcgtaaatcatggt 9300
TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCA

lac prom(9342,9425)<<<

9301 catagctgtttcctgtgtgaaattggtatccgctcacaattccacacaacatacagagccg 9360
GTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGC

9361 gaagcataaagtgtaaagcctggggtgcctaataagtgagtgagctaaactcacattaattgct 9420
CTTCGTATTTACATTTTCGGACCCACGGATTACTCACTCGATTGAGTGTAATTAACGCA

9421 tgcgctcactgcccgtttccagtcgggaaacctgtcgtgccagctgcattaatgaatcg 9480
ACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTTCGACGTAATTACTTAGC

9481 gccaacgcgcggggagagggcgggtttgctgattgggpcgctcttccgcttcctcgctcactg 9540
CGGTTGCGCGCCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGAC

9541 actcgctgpcgctcggtcggttcggctgcccgcagcgggtatcagctcactcaaagggcggtaa 9600
TGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTTCCGCCATT

9601 tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc 9660

ATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACTCGTTTTCCGGTTCG

pUC origin(9686,10305)<<<

|

9661 aaaagggccaggaaccgtaaaaaagggccgcttgctggcggtttttccataggctccgcccc 9720
TTTTCCGGTCTTTGGCATTTCGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGG

9721 ctgacgagcatcacaaaaatcgacgctcaagtcaagggtggcgaaacccgacaggactat 9780
GACTGCTCGTAGTGTTCCTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATA

9781 aaagataccagggcgtttccccctggaagctccctcgtgctctcctgttccgaccctgc 9840
TTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACG

9841 cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct 9900
GCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901 cacgctgtaggtatctcagttcgggtgtaggtcgctcgaagctgggctgtgtgcacg 9960
GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTTCGACCCGACACACGTGC

9961 aaccccccgcttcagccccgaccgctgctccttatccggtaactatcgtcttgagtccaacc
10020
TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021 cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga
10080
GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081 ggtatgtagggcgtgctacagagttcttgaagtggtggcctaactacggctacactagaa
10140
CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141 gaacagtatctgggtatctgctcctgctgaagccagttaccttcggaaaaagagttggta
10200
CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCAT

10201 gctcttgatccggcaaaaccaccgctggtagcgggtgggttttttggttgcaagcagc
10260
CGAGAACTAGGCCGTTTGTTCCTGGTGGCGACCATCGCCACCAAAAAACAAACGTTTCGTCG

10261 agattacgctcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggctc
10320
TCTAATGCGCGTCTTTTTTCTTAGAGTTCCTTAGGAACTAGAAAAGATGCCCCAGAC

10321 acgctcagtggaacgaaaactcacgttaagggatcttgggtcatgagattatcaaaaagga
10380
TGCGAGTCACCTTGCTTTTGGAGTGAATTCCCTAAAACAGTACTCTAATAGTTTTTCT

10381 tcttcacctagatccttttaaatataaatgaagttttaaataaatcaatctaaagtatatatg
10440
AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAATTTAGTTAGATTTTCATATATAC

AmpR marker(10460,11320)<<<

|

10441 agtaaaacttgggtctgacagttaccaatgcttaatacagtgaggcacctatctcagcgatct
10500
TCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGA


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11341 attgaagcatttatcaggggttattgtctcatgagcggatacatatttgaatgtatttaga
11400
      TAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCT

11401 aaaataaaciaaataggggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg
11460
      TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGCTGC

                                                    SV40-polyA-signal
reg(11504,11538)>>>

11461 gatcgggagatcaacttgtttattgcagcttataatggttacaaataaagcaatagcatc
11520
      CTAGCCCTCTAGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTTCGTTATCGTAG

11521 acaaatttcacaaataaagcatttttttactgcattctagttgtggtttgtccaaactc
11580
      TGTTTAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG

11581 atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc
11640
      TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTCGATTGGTTTTAGTAGGG

11641 aaacttcccaccccataccctattaccactgcccaattacctgtggtttcatttactctaa
11700
      TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT

11701 acctgtgattcctctgaattattttcattttaaagaaattgtatttgtaaataatgtact
11760
      TGGACACTAAGGAGACTTAATAAAAAGTAAAATTTCTTTAACATAAAACAATTTTATACATGA

11761 acaaacttagtagt 11774
      TGTTTGAATCATCA

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Restriction analysis of pGIPZ lentiviral vector

AhdI (GACnn_n'nnGTC) [Eam1105I,AspEI,DriI,EclHKI]
 Cuts 1 time.
 Cuts at position 10533.
 Fragment sizes 10533, 1241.

AleI (CACnn'nnGTG) [OliI]
 Cuts 1 time.
 Cuts at position 1577.
 Fragment sizes 1577, 10197.

AloI (GAACnnnnnnTCCnnnnnnn_nnnnn')
 Cuts 1 time.
 Cuts at position 7423.

Fragment sizes 7423, 4351.

AloI (GGAnnnnnnGTTcnnnnnnn_nnnnn')
Cuts 1 time.
Cuts at position 7455.
Fragment sizes 7455, 4319.

AsiSI (GCG_AT'CGC) [SgfI]
Cuts 1 time.
Cuts at position 8338.
Fragment sizes 8338, 3436.

BbvCI (CC'TCA_GC)
Cuts 1 time.
Cuts at position 1424.
Fragment sizes 1424, 10350.

BlpI (GC'TnA_GC) [Bpu1102I,Bsp1720I,CelIII]
Cuts 1 time.
Cuts at position 3564.
Fragment sizes 3564, 8210.

Bpu10I (CC'TnA_GC)
Cuts 1 time.
Cuts at position 1424.
Fragment sizes 1424, 10350.

BsaBI (GATnn'nnATC) [Bse8I,BseJI,MamI]
[dam methylated]
Cuts 1 time.
Cuts at position [3853].
Fragment sizes 3853, 7921.

BsiWI (C'GTAC_G) [Pfl123II,PspLI,SunI]
Cuts 1 time.
Cuts at position 4749.
Fragment sizes 4749, 7025.

BsrGI (T'GTAC_A) [Bsp1407I,BstAUI,SspBI]
Cuts 1 time.
Cuts at position 4089.
Fragment sizes 4089, 7685.

BstEII (G'GTnAC_C) [BstPI,Eco91I,Eco065I,PspEI]
Cuts 1 time.
Cuts at position 4827.
Fragment sizes 4827, 6947.

BstZ17I (GTA'TAC) [BssNAI,Bst1107I]
Cuts 1 time.
Cuts at position 9261.
Fragment sizes 9261, 2513.

Bsu36I (CC'TnA_GG) [AxyI,Bse21I,Eco81I]

Cuts 1 time.

Cuts at position 6469.

Fragment sizes 6469, 5305.

CspCI (CAAnnnnnGTGGnnnnnnnnnn_nn')

Cuts 1 time.

Cuts at position 3141.

Fragment sizes 3141, 8633.

CspCI (CCACnnnnnTTGnnnnnnnnnn_nn')

Cuts 1 time.

Cuts at position 3106.

Fragment sizes 3106, 8668.

EcoNI (CCTnn'n_nnAGG) [BstENI,XagI]

Cuts 1 time.

Cuts at position 1170.

Fragment sizes 1170, 10604.

FspI (TGC'GCA) [Acc16I,AviII,NsbI]

Cuts 1 time.

Cuts at position 10755.

Fragment sizes 10755, 1019.

HpaI (GTT'AAC) [KspAI]

Cuts 1 time.

Cuts at position 5376.

Fragment sizes 5376, 6398.

MluI (A'CGCG_T)

Cuts 1 time.

Cuts at position 5736.

Fragment sizes 5736, 6038.

NotI (GC'GGCC_GC) [CciNI]

Cuts 1 time.

Cuts at position 4100.

Fragment sizes 4100, 7674.

NruI (TCG'CGA) [Bsp68I]

[dam methylated]

Cuts 1 time.

Cuts at position [833].

Fragment sizes 833, 10941.

PmeI (GTTT'AAAC) [MssI]

Cuts 1 time.

Cuts at position 6862.

Fragment sizes 6862, 4912.

PpuMI (rG'GwC_Cy) [PpuXI,Psp5II,PspPPI]
[dcm methylated]
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9840.

PshAI (GACnn'nnGTC) [BoxI,BstPAI]
Cuts 1 time.
Cuts at position 8001.
Fragment sizes 8001, 3773.

SanDI (GG'GwC_CC)
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9840.

SfiI (GGCCn_nnn'nGGCC)
[dcm methylated]
Cuts 1 time.
Cuts at position 2621.
Fragment sizes 2621, 9153.

SgrAI (Cr'CCGG_yG)
Cuts 1 time.
Cuts at position 2500.
Fragment sizes 2500, 9274.

SnaBI (TAC'GTA) [BstSNI,Eco105I]
Cuts 1 time.
Cuts at position 3070.
Fragment sizes 3070, 8704.

SspI (AAT'ATT)
Cuts 1 time.
Cuts at position 11337.
Fragment sizes 11337, 437.

XbaI (T'CTAG_A)
[dam methylated]
Cuts 1 time.
Cuts at position 2707.
Fragment sizes 2707, 9067.

XhoI (C'TCGA_G) [BssHI,Paer7I,Sfr274I,SlaI,StrI,TliI]
Cuts 1 time.
Cuts at position 5391.
Fragment sizes 5391, 6383.

IMPORTANT SAFETY NOTE:

Follow NIH guidelines regarding lentiviral production and transduction; follow Biosafety Level 2 (BL2) or BL2+ laboratory criteria.

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