

## **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.

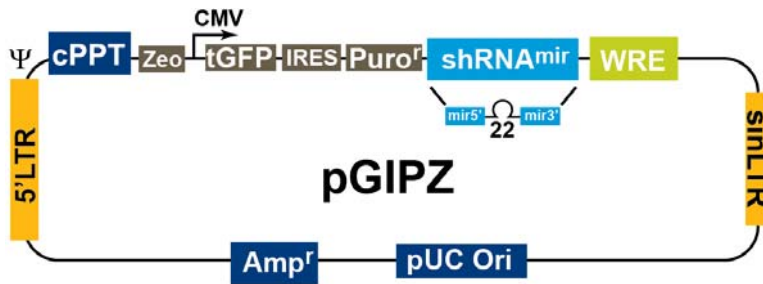


Figure 1: pGIPZ lentiviral vector

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 <sup>r</sup>	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 <sup>r</sup>	Bacterial selectable marker

Table 2: Antibiotic Resistances Conveyed by pGIPZ

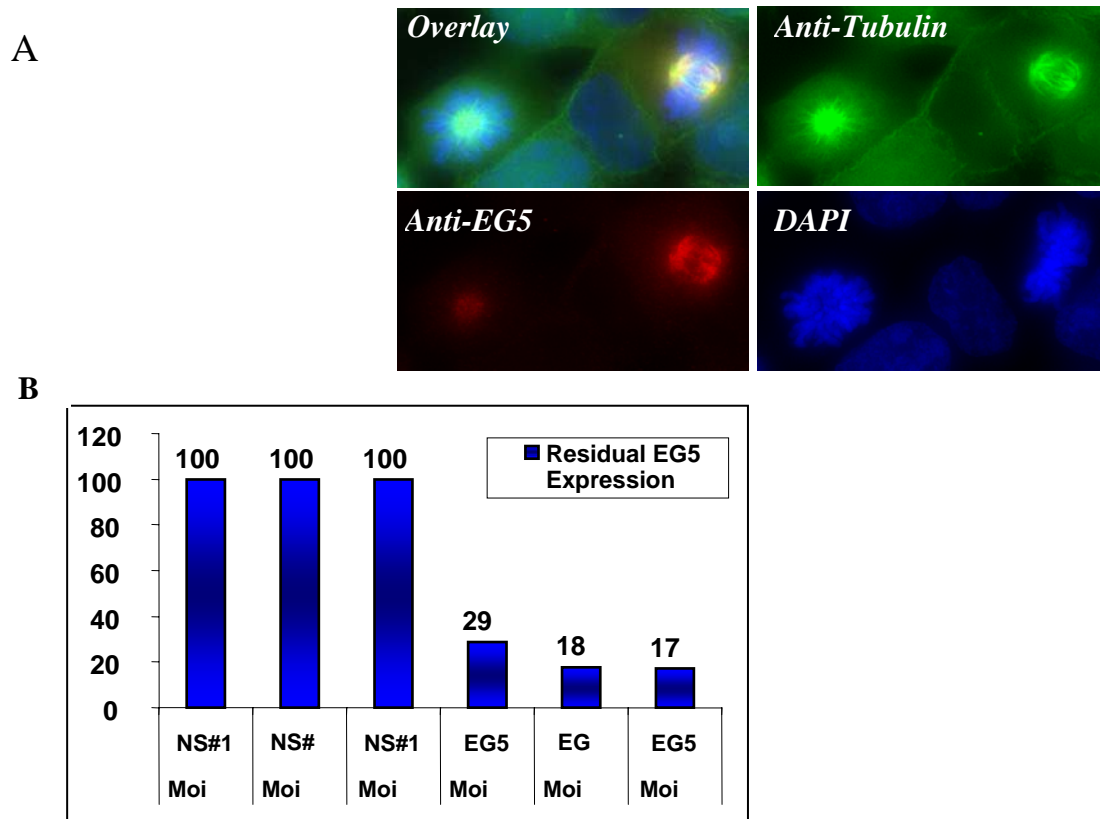
Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100µg/ml	Bacterial selection marker
Zeocin	25µg/ml	Bacterial selection marker (vector)
Puromycin	Variable	Mammalian selectable marker

### 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 $\mu$ l serum-free media and lentiviral particles containing GAPDH or EG5 shRNAir, 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

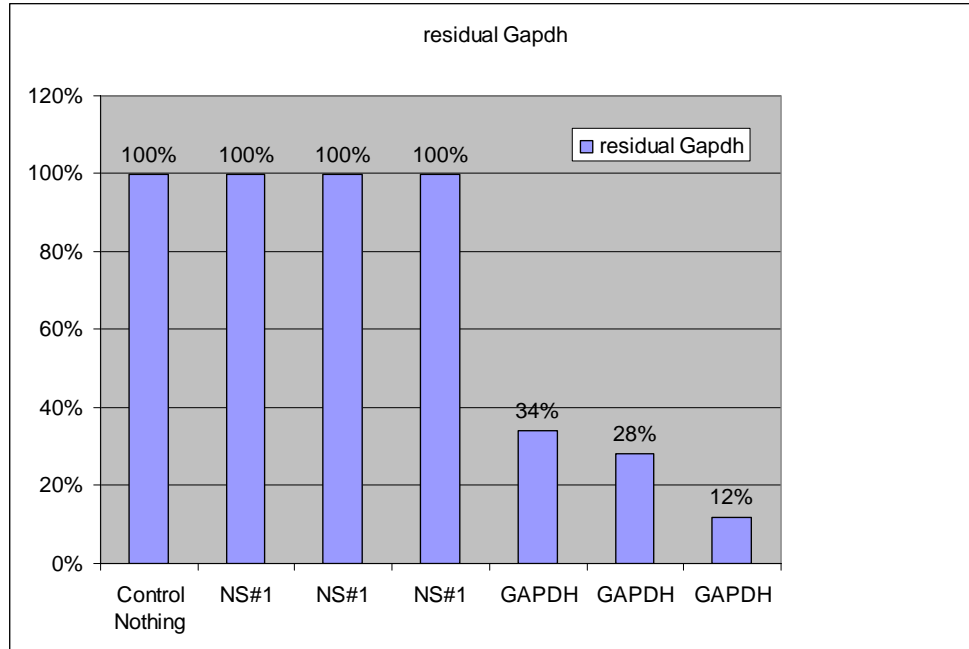
At 48 hours post-transduction, transduced cells were lysed and total RNA was extracted using the Qiagen RNAeasy Kit (cat# 74104). The RNA was converted to cDNA using the ABI- High Capacity cDNA RT Kit (cat#4368813), using 500ng total RNA in a 100 $\mu$ l reaction. A 1/100 dilution of the cDNA was used 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.



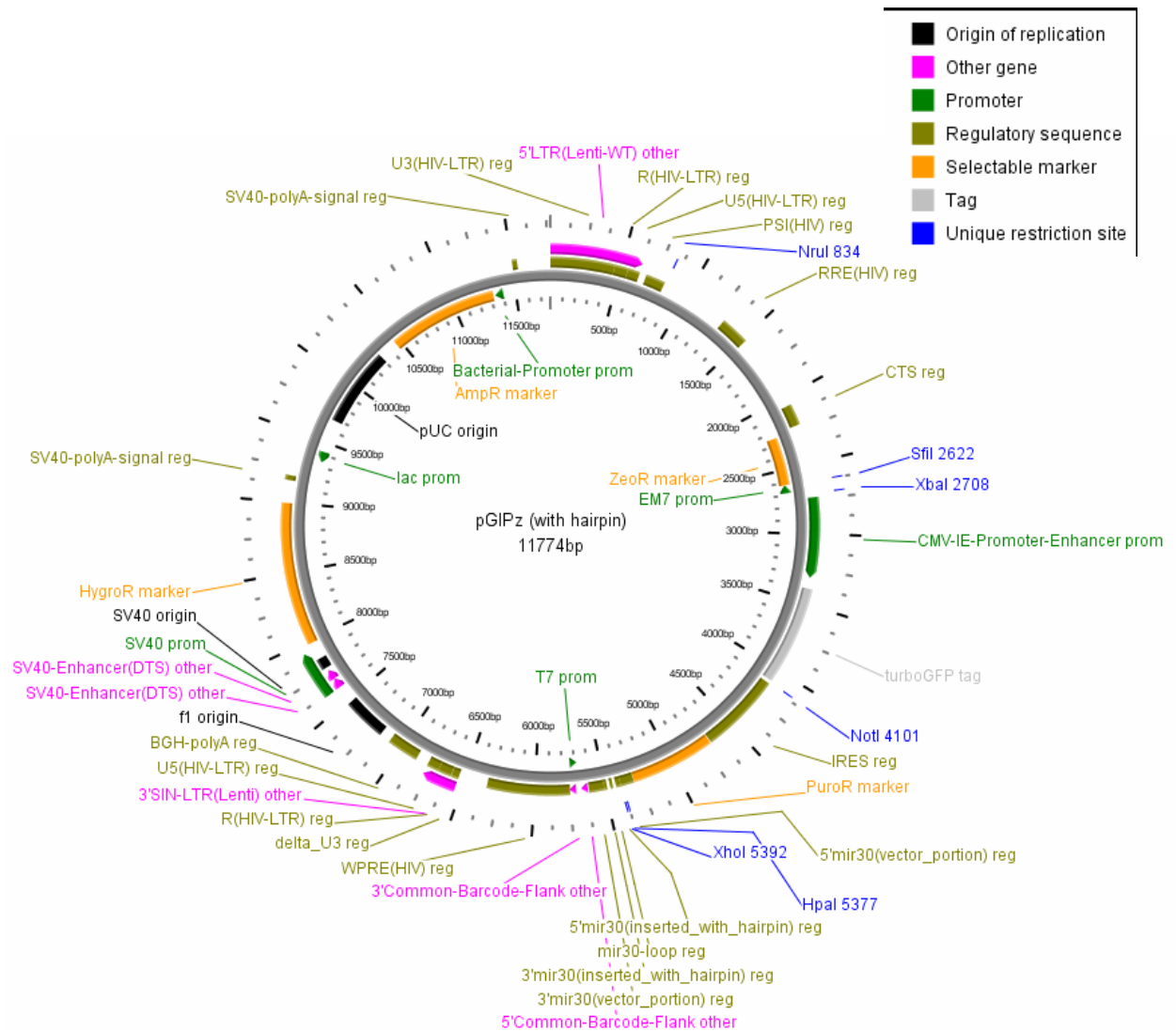
**Figure 2.** Knockdown of the EG5 (KIF11) gene allowed evaluation of phenotypic evidence of RNAi as well as its molecular manifestation

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
    ACTGGAAACCTACCACGATGTTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT
181  ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgaccgg 240
    TATTTCTCTCTTGTGGTTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC
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    TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC
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                                     R(HIV-LTR) reg(456,550)>>>
                                     |
421  cctgcatataagcagctgctttttgctgtactgggtctctctggttagaccagatctga 480
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                                     U5(HIV-LTR) reg(551,635)>>>
                                     |
541  tgagtgcttcaagtagtgtgtgcccgtctggtgtgtgactctggtaactagagatccctc 600
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601  agacccttttagtcagtggtgaaaatctctagcagtgggcggccgaacagggacttgaaag 660
    TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC
                                     PSI(HIV) reg(685,822)>>>
                                     |
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    GCTTTCCCTTTGGTCTCCTCGAGAGAGCTGCGTCTGAGCCGAACGACTTCGCGCGTGCC
721  caagagggcagggggcggcgactggtgagtacgcaaaaattttgactagcggaggctaga 780
    GTTCTCCGCTCCCCGCCGCTGACCACTCATGCGGTTTTTAAACTGATCGCCTCCGATCT
                                     NruI
                                     |
781  aggagagagatgggtgagagagcgtcagtattaagcgggggagaattagatcgcgatggg 840
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1141 aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag 1200  
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1201 tgaattatataaatataaagtagtaaaaattgaaccattaggagtagcaccaccaaggc 1260  
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RRE (HIV)

reg(1314,1518)>>>

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1321 gttccttgggagcagcaggaagcactatgggagcagcgtcaatgacgctgacggtacagggc 1380  
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1381 cagacaattattgtctgggtatagtgagcagcagacaacaatttgctgagggctattgagggc 1440  
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1441 gcaacagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaagaatcct 1500  
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1921 cccaacccccgaggggacccgacaggccccgaaggaatagaagaagaaggtggagagagaga 1980  
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1981 cagagacagatccattcgattagtgaaacggatcggcactgctgctgcgccaattctgcagac 2040  
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CTS reg(2064,2214)>>>

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2101 gggaaagaatagtagacataatagcaacagacatacaaaactaagaattacaaaaacaaa 2160  
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2161 ttacaaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220  
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ZeoR marker(2245,2619)<<<

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2281 gccggccgggtcgcgagggcgaactcccgccccacggctgctcgccgatctcggatcat 2340  
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2341 ggccggccggaggcgtcccgaagtctcgtggacacgacctccgaccactcggcgtacag 2400  
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2401 ctcgctcagggcgcgacccacacccaggccaggggtgtgtcggccaccacctggtcctg 2460  
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2461 gaccgcgctgatgaacaggggtcacgtcgtcccggaccacaccggcgaagtgcctccac 2520  
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2521 gaagtcccgggagaaacccgagccgggtcgggtccagaactcgaccgctccggcgacgtcgcg 2580  
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SfiI

EM7 prom(2620,2683)<<<

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2641 cgtattatactatgccgatatactatgccgatgattaattgtcaacacgtgctgcaggtc 2700  
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XbaI

CMV-IE-Promoter-Enhancer

prom(2738,3311)>>>

2701 cgaggttctagacgtattaccgccaatgcattagttattaatagtaataattacgggggtc 2760  
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2761 attagttcatagcccatatatggagttccgcttacataacttacggtaaatggcccgcg 2820  
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5041 cggcccaaggagcccgcgctgggttcctggccaccgctcggcgtctcgcccgaccaccagggc 5100
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5101 aagggctctgggcagcgcgctcgtgctccccggagtggaggcggccgagcgcgctggggtg 5160
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5161 cccgccttcctggagacctccgcgccccgcaacctccccttctacgagcggctcggcttc 5220
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5221 accgtcaccgcccagcgtcgaggtgcccgaaggaccgcgcacctgggtgcatgaccgcaag 5280
TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCTGGCGCGTGGACCACGTACTGGGCGTTC

5'mir30(vector_portion) reg(5296,5390)>>>
|
5281 cccgggtgcctgagtttgtttgaatgaggcttcagtactttacagaatcgttgcctgcaca 5340
GGGCCACGGACTCAAACAACTTACTCCGAAGTCATGAAATGTCTTAGCAACGGACGTGT

HpaI XhoI
| |
5'mir30(inserted_with_hairpin) reg(5391,5423)>>>
| |
5341 tcttgaaacacttgctgggattacttcttcaggttaacccaacagaaggctcgagAAGG 5400
AGAACCTTTGTGAACGACCCTAATGAAGAAGTCCAATTGGGTTGTCTTCCGAGCTCTTCC

mir30-loop
reg(5446,5464)>>>
|
5401 TATATTGCTGTTGACAGTGAGCGACCTCCACCCTCACTCTGCCATTAGTGAAGCCACAGA 5460
ATATAACGACAACCTGTCACTCGCTGGAGGTGGGAGTGAGACGGTAATCACTTCCGGTGTCT

3'mir30(vector_portion) reg(5507,5614)>>>
|
3'mir30(inserted_with_hairpin)
reg(5487,5506)>>>
| |
5461 TGTAATGGCAGAGTGAGGGTGGAGGGTGCCTACTGCCTCGgaattcaaggggctacttta 5520
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5521 ggagcaattatcttgtttactaaaactgaataccttgctatctctttgatacatttttac 5580
CCTCGTTAATAGAACAAATGATTTTGACTTATGGAACGATAGAGAACTATGTAAAAATG

5' Common-Barcode-
Flank other(5626,5646)>>>
|
5581 aaagctgaattaaaatgggtataaattaaatcacttttttcaattggaagactaatgcggc 5640
TTTCGACTTAATTTTACCATATTTAATTTAGTGAAAAAAGTTAACCTTCTGATTACGCCG

5641 cggccattactccgctcctcgtgctcttggtgcatatgtctgctggtttggttgatggtggtt 5700
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T7 prom(5710,5729)<<< WPRE(HIV)
reg(5749,6337)>>>

```



6661 ttaagcctcaataaagcttgcccttgagtgcttcaagtagtggtgcccgtctgttggtg 6720  
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6721 actctggtaactagagatccctcagacccttttagtcagtggtgaaaatctctagcagta 6780  
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6781 gtagttcatgtcatcttattattcagatatttataacttgcaaagaaatgaatatcagaga 6840  
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BGH-polyA reg(6868,7091)>>>

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6901 cagccatctgttgtttgcccctcccccgctgccttccttgaccctggaaggtgccactccc 6960  
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7021 attctgggggggtgggggtggggcaggacagcaagggggaggattgggaagacaatagcagg 7080  
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f1 origin(7171,7477)>>>

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7201 cgcagcgtgaccgctacacttgccagcgccttagcgcggcctcctttcgctttcttccct 7260  
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7321 ggggtccgatttagtgctttacggcacctcgacccccaaaaaacttgattaggggtgatgg 7380  
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AAGAAATTATCACCTGAGAACAAGGTTTGACCTTGTTGTGAGTTGGGATAGAGCCAGATA

7501 tcttttgatttataagggattttgccgatttcggcctattgggttaaaaaatgagctgatt 7560  
AGAAAATAAATATTCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

SV40-

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

|  
SV40

prom(7609,7930)>>>

7561 taacaaaaatttaacgcgaattaattctgtggaatgtgtgtcagttaggggtgtggaaagt 7620



8581 atgtcctgacggacaatggccgcataaacagcgggtcattgactggagcggagcggatggttcg 8640  
TACAGGACTGCCTGTTACCGGCGTATTGTGCGCCAGTAACTGACCTCGCTCCGCTACAAGC

8641 gggattcccaatacagaggtcgccaacatcttcttctgaggccggtgggttggttgtatgg 8700  
CCCTAAGGGTTATGCTCCAGCGGTTGTAGAAGAAGACCTCCGGCACCAACCGAACATACC

8701 agcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctcc 8760  
TCGTGCTCTGCGCGATGAAGCTCGCCTCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGG

8761 gggcgtatatgctccgcattgggtcttgaccaactctatcagagcttgggtgacggcaatt 8820  
CCCGCATATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAA

8821 tcgatgatgcagcttgggpgcaggggtcgatgagcgaatcgtccgatccggagccggga 8880  
AGCTACTACGTGGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCT

8881 ctgtcgggpgtacacaaatcgcccgcagaagcgcggccgctctggaccgatggctgtgtag 8940  
GACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATC

8941 aagtactcgccgatagtggaaccgacgccccagcactcgtccgaggggcaaaggaatagc 9000  
TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTGCTGAGCAGGCTCCCGTTTCCTTATCG

9001 acgtgctacgagatctcgattccaccgccccttctatgaaaggttgggcttcggaatcg 9060  
TGCACGATGCTCTAAAGCTAAGGTGGCGGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061 ttttcgggacgcccggctggatgatcctccagcgcggggatctcatgctggagttcttcg 9120  
AAAAGGCCCTGCGGCCGACCTACTAGGAGGTGCGGCCCTAGAGTACGACCTCAAGAAGC

SV40-polyA-signal

reg(9160,9194)>>>

9121 cccacccaacttgtttattgagcttataatgggttacaataaagcaatagcatcacia 9180  
GGGTGGGGTTGAACAAATAACGTGCAATATTACCAATGTTTATTTTCGTTATCGTAGTGTT

9181 atttcacaataaagcatttttttactgcattctagttgtggtttgtccaaactcatca 9240  
TAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGTTTTGAGTAGT

9241 atgtatcttatcatgtctgtataccgctcgacctctagctagagcttggcgtaatcatggt 9300  
TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCA

lac prom(9342,9425)<<<

9301 catagctgtttcctgtgtgaaattggtatccgctcacaattccacacaacatacagagccg 9360  
GTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGC

9361 gaagcataaagtgtaaagcctggggtgcctaataagtgagtgagctaaactcacattaattgct 9420  
CTTCGTATTTACATTTTCGGACCCACGGATTACTCACTCGATTGAGTGTAATTAACGCA

9421 tgcgctcactgcccgtttccagtcgggaaacctgtcgtgccagctgcattaatgaatcg 9480  
ACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTTCGACGTAATTACTTAGC

9481 gccaacgcgcggggagagggcgggtttgctgattgggpgctcttccgcttcctcgctcactg 9540  
CGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGAC

9541 actcgctgagctcggtcggttcggctgccccgagcgggtatcagctcactcaaagggcggtaa 9600  
TGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTTCCGCCATT

9601 tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc 9660

ATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACTCGTTTTCCGGTTCG

pUC origin(9686,10305)<<<

|

9661 aaaagggccaggaaccgtaaaaaagggcgcggttgctggcggtttttccataggctccgcccc 9720  
TTTTCCGGTCTTTGGCATTTCGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGG

9721 ctgacgagcatcacaaaaatcgacgctcaagtcaaggtggcgaaacccgacaggactat 9780  
GACTGCTCGTAGTGTTCCTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATA

9781 aaagataccagggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgc 9840  
TTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACG

9841 cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct 9900  
GCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901 cacgctgtaggtatctcagttcgggtgtaggtcgctcagctccaagctgggctgtgtgcacg 9960  
GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTTCGACCCGACACACGTGC

9961 aaccccccggttcagccccgaccgctgcgccttatccggtaactatcgtcttgagtccaacc  
10020  
TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021 cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga  
10080  
GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081 ggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaa  
10140  
CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141 gaacagtatctgggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggta  
10200  
CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCAT

10201 gctcttgatccggcaaaaccaccgctggtagcgggtgggttttttggtttgcaagcagc  
10260  
CGAGAACTAGGCCGTTTGTTCGGTGGCGACCATCGCCACCAAAAAACAAACGTTTCGTCG

10261 agattacgcgagaaaaaaggatctcaagaagatcctttgatcttttctacggggctcg  
10320  
TCTAATGCGCGTCTTTTTTCTTAGAGTTCCTTAGGAACTAGAAAAGATGCCCCAGAC

10321 acgctcagtggaacgaaaactcacgttaagggatcttgggtcatgagattatcaaaaagga  
10380  
TGCGAGTCACCTTGCTTTTGTAGTGAATTCCCTAAAACAGTACTCTAATAGTTTTTCT

10381 tcttcacctagatccttttaaatataaatgaagttttaaataaatcaatctaaagtatatatg  
10440  
AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAATTTAGTTAGATTTTCATATATAC

AmpR marker(10460,11320)<<<

|

10441 agtaaaacttgggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatct  
10500  
TCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGA

10501 gtctatcttcggttcatccatagttgcctgactccccgtcgtgtagataactacgatacggg  
10560 CAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCC  
10561 agggcttaccatctggccccagtgctgcaatgataccgcgagaccacgctcaccggctc  
10620 TCCCGAATGGTAGACCGGGGTCACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAG  
10621 cagatcttatcagcaataaaccagccagccggaagggccgagcgcagaagtggctcctgcaa  
10680 GTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGCTCGCGTCTTACCAGGACGTT  
10681 ctttatccgcctccatccagctctattaattgctgcccgggaagctagagtaagtagttcgc  
10740 GAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCCCTTCGATCTCATTTCATCAAGCG  
10741 cagttaatagtttgcgcaacggttggccattgctacaggcatcgtggtgtcacgctcgt  
10800 GTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACACAGTGCAGCA  
10801 cgtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatccc  
10860 GCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGG  
10861 ccatggttggtgcaaaaaagcgggttagctccttcggctcctccgatcgttgtcagaagtaagt  
10920 GGTACAACACGTTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCA  
10921 tggccgcagtggttatcactcatgggttatggcagcactgcataattctcttactgtcatgc  
10980 ACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACG  
10981 catccgtaagatgcttttctgtgactgggtgagtactcaaccaagtcattctgagaatagt  
11040 GTAGGCATTCTACGAAAAGACACTGACCACTCATGAGTTGGTTTCAGTAAGACTCTTATCA  
11041 gtatgcggcgaccgagttgctcctgcccggcgtcaatacgggataataccgcgccacata  
11100 CATACGCCGCTGGCTCAACGAGAACGGGCCGAGTTATGCCCTATTATGGCGCGGTGTAT  
11101 gcagaactttaaaagtgctcatcattggaaaacggttcttcggggcgaaaactctcaagga  
11160 CGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGGAGAGTTCCT  
11161 tcttaccgctggttgagatccagttcagatgtaaccactcgtgcacccaactgatcttcag  
11220 AGAATGGCGACAACCTTAGGTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTC  
11221 catcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaa  
11280 GTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTTGTCTTCCGTTTTACGGCGTT  
11281 aaaaggaataagggcgacacggaaatggtgaatactcatactcttcctttttcaatatt  
11340 TTTTCCCTTATTCCCGCTGTGCCTTTACAACCTTATGAGTATGAGAAGGAAAAAGTTATAA

Bacterial-Promoter prom(11362,11400)<<<

```

11341 attgaagcatttatcaggggttattgtctcatgagcggatacatatttgaatgtatttaga
11400
      TAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCT

11401 aaaataaacaatataggggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg
11460
      TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGCTGC

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

11461 gatcgggagatcaacttgtttattgcagcttataatggttacaaataaagcaatagcatc
11520
      CTAGCCCTCTAGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTTCGTTATCGTAG

11521 acaaatttcacaaataaagcatttttttctactgcattctagttgtggtttgtccaaactc
11580
      TGTTTAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG

11581 atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc
11640
      TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTCGATTGGTTTTAGTAGGG

11641 aaacttcccaccccataccctattaccactgcccaattacctgtggtttcatttactctaa
11700
      TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT

11701 acctgtgattcctctgaattattttcattttaagaaattgtatttgtaaataatgtact
11760
      TGGACACTAAGGAGACTTAATAAAAAGTAAAATTTCTTTAACATAAAACAATTTTATACATGA

11761 acaaacttagtagt 11774
      TGTTTGAATCATCA

```

**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,AviIII,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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