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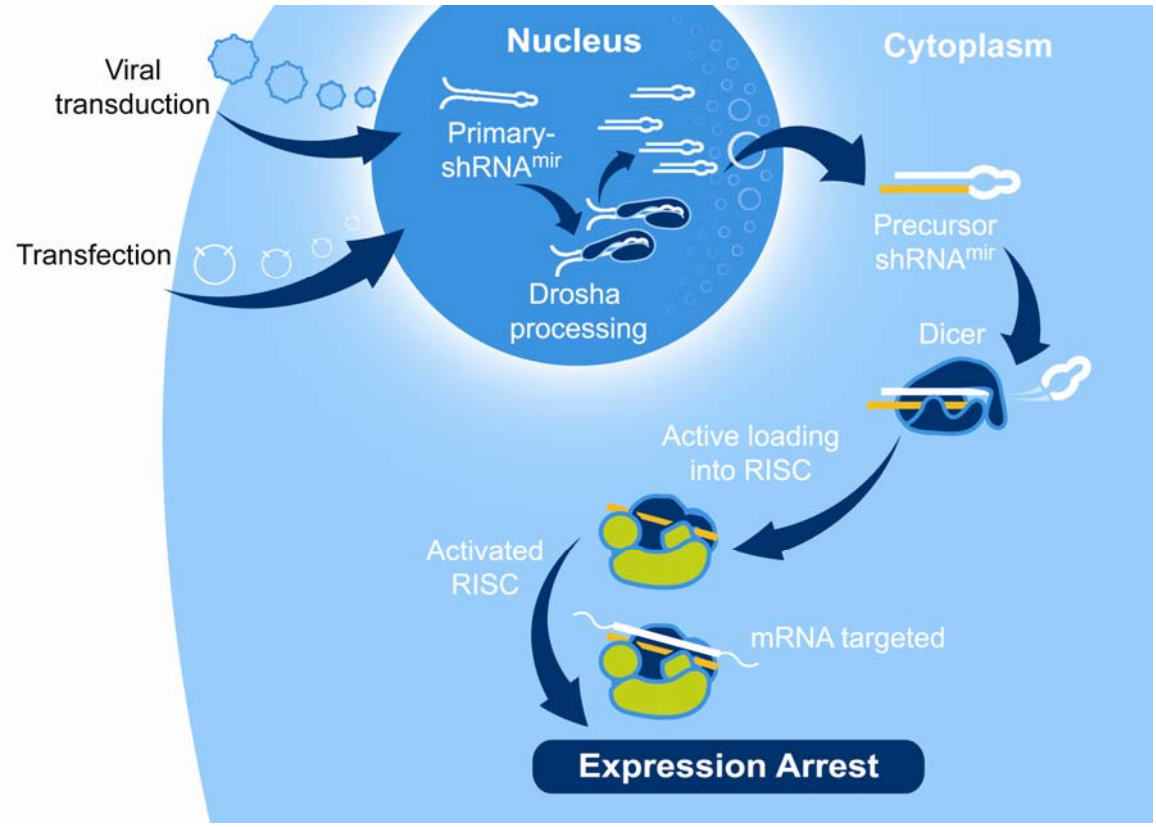
# Expression Arrest™ GIPZ lentiviral shRNAmir library



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**Expression Arrest™ Human GIPZ lentiviral shRNAmir library**
**RHS4430, RHS4477**

The human GIPZ lentiviral shRNAmir library was developed by Open Biosystems in collaboration with Dr. Greg Hannon (CSHL) and Dr. Steve Elledge (Harvard). This library combines the design advantages of microRNA-adapted shRNA (shRNAmir) with the pGIPZ lentiviral vector to create a powerful RNAi trigger capable of producing RNAi in most cell types including primary and non-dividing cells.

shRNAmir triggers have been designed to mimic a natural microRNA primary transcript and each target sequence has been selected based on thermodynamic criteria for optimal small RNA performance. Validation of this design is detailed in Silva *et al* (2005) showing a substantial increase in knockdown efficiency.

**Unique features of the GIPZ lentiviral shRNAmir library include:**

- shRNAmir constructs targeting the entire human genome already cloned into the pGIPZ lentiviral vector
- Efficient low copy knockdown - Important for pooled screens
- TurboGFP (tGFP) and shRNAmir are part of a bicistronic transcript allowing the visual marking of shRNAmir expressing cells
- Effective transduction of primary and non-dividing cell lines e.g. neurons
- Unique 60nt molecular barcode facilitate pooled screens

**Shipping and Storage**
The Expression Arrest Human GIPZ shRNAmir lentiviral library is provided in 96-well microtiter plates containing frozen stock cultures of *E. coli* (Prime+) in LB-Lennox (low salt) broth with 8% glycerol, 100µg/ml carbenicillin and 25µg/ml zeocin.

Individual constructs are shipped as bacterial cultures of *E. coli* (prime+) in LB-Lennox (low salt) broth with 8% glycerol and carbenicillin (100ug/ml) and zeocin (25ug/ml). Individual constructs are shipped on wet ice. Open Biosystems checks all cultures for growth prior to shipment.

The GIPZ human lentiviral shRNAmir library and individual constructs should be stored at -80$^{o}$C.

## shRNAmir design

- Replaced mature microRNA sequence in human microRNA 30 (mir-30) with gene specific duplexes
- Adding mir-30 loop and context sequences adds endogenous processing by Drosha which increases subsequent Dicer recognition and specificity
- Dicer processing promotes active loading into the RISC complex
- Rules-based design includes destabilizing the 5'end of the antisense strand for strand specific incorporation into RISC

***Increased Drosha/Dicer processing=More siRNA=Greater knockdown***

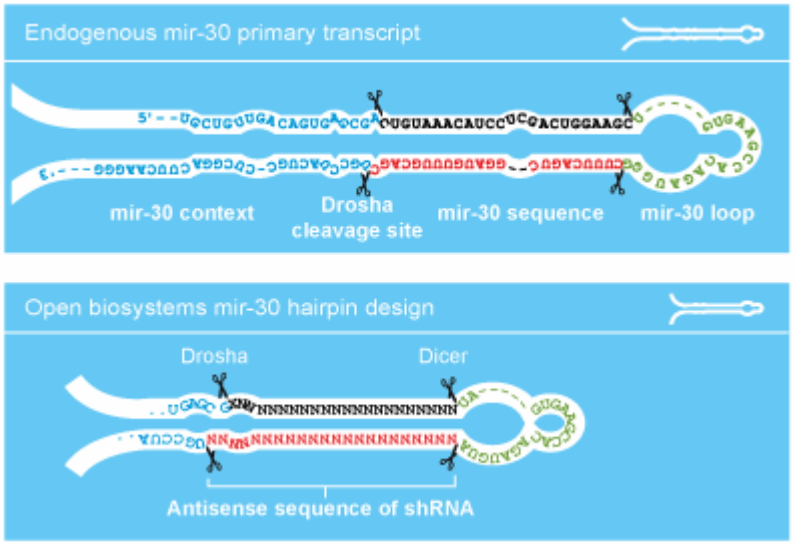


**Figure 1**: **Expression Arrest shRNAmir are expressed as mir-30 primary transcripts**

Use of the miR-30 design allowed the use of **'rules-based' designs** for target sequence selection. One such rule is the destabilizing of the 5' end of the antisense strand that results in strand specific incorporation of miRNAs into RISC. The proprietary design algorithm targets coding regions and the untranslated region (UTR) with the additional requirement that they contain greater than 3 mismatches to any other sequence in the human or mouse genomes.

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

- Ability to perform transfections or transductions using the replication incompetent lentivirus
- 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

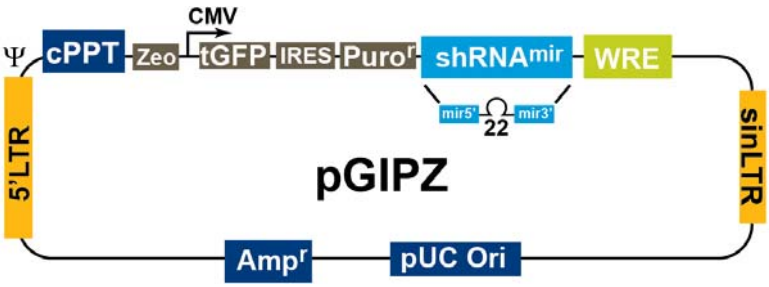


**Figure 2**: **pGIPZ lentiviral vector**

**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 |
| tGFP | Marker to track shRNAmir expression |
| IRES-Puro | Mammalian selectable marker |
| AMPr | Ampicillin bacterial selectable marker. |
| 5'LTR | 5' long terminal repeat |
| pUC ori | High copy replication and maintenance of plasmid in *E.coli* |
| SIN-LTR | 3' Self inactivating long terminal repeat |
| RRE | Rev response element |
| ZEOr | 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 |

## Culturing protocols and maintenance of pGIPZ

It is well known that viral vectors have a tendency to recombine producing background recombinants. Recombination occurs at the long terminal repeat regions (LTR's). The LTR recombination, which results in loss of most of the plasmid, can confer a growth advantage on the cells. It is therefore critical to maintain careful growth conditions when culturing viral vectors in *E.coli* in order to reduce the number and abundance of background recombinants.
The GIPZ lentiviral shRNAmir library has passed through internal QC processes to ensure high quality and low recombination.

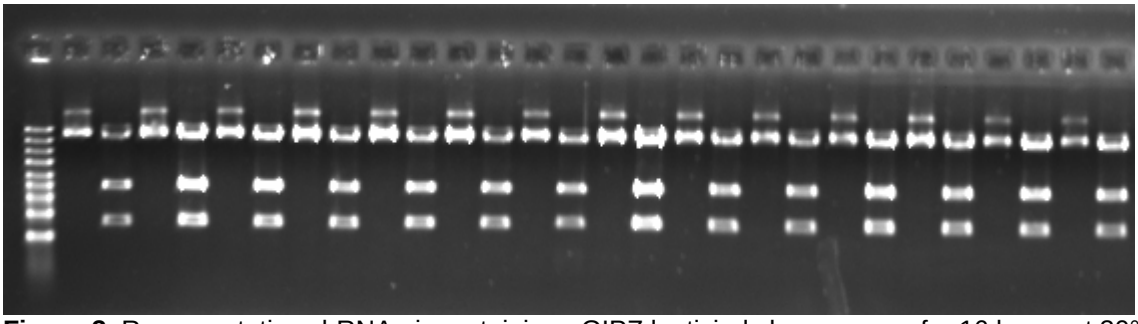

**Figure 3**. Representative shRNAmir containing pGIPZ lentiviral clones grown for 16 hours at 30° C and the plasmid isolated and normalized to a standard concentration. Clones were then digested with *Sac*II and run out on a gel. The expected band sizes are (bp)= **1259, 2502, 7927.** No recombinant products are visible. 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb)

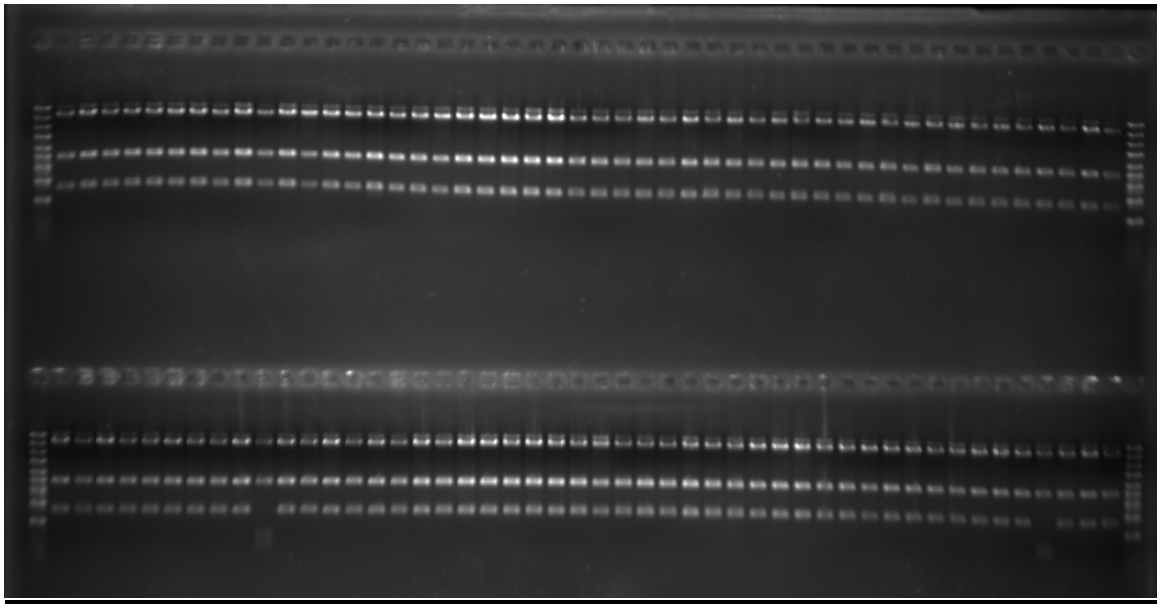

Figure 4. Gel image of a single plate from the GIPZ library cultured for 10 successive generations in an attempt to determine the tendency of the pGIPZ vector to recombine. Each generation was thawed, replicated and incubated O/N for 16 hours at 30° C then frozen, thawed and replicated. This process was repeated for 10 growth cycles. After the 10th growth cycle, plasmid was isolated and normalized to a standard concentration. Clones were then digested with *Sac*II and run on a gel. Expected band sizes (bp) = **1259, 2502, 7927.** 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb) The pGIPZ vector appears stable without showing any recombination.

**Culture conditions for individual plasmid preparations**

Most plasmid mini-prep kits recommend a culture volume of 1–10ml for good yield.
For shRNAmir constructs, 5ml of culture can be used for one plasmid mini-prep generally producing 5–10μg of plasmid DNA.

1. Upon receiving your glycerol stock(s) containing the shRNAmir of interest store at –80°C until ready to begin.
2. To prepare plasmid DNA first thaw your glycerol stock culture and pulse vortex to resuspend any *E. coli* that may have settled to the bottom of the tube.
3. Take a 10μl inoculum from the glycerol stock into 3-5ml of LB (low salt) with 100μg/ml carbenicillin and 25ug/ml zeocin. Incubate at 37°C for 16 hours with vigorous shaking. Return the glycerol stock(s) to -80°C. If a larger culture volume is desired, use the 3-5ml overnight culture as a starter inoculum. Incubate at 37°C for 16 hrs with vigorous shaking.
4. Pellet the 3-5ml culture and begin preparation of plasmid DNA.
5. Run 3-5μl of the plasmid DNA on a 1% agarose gel. pGIPZ with shRNAmir is 11744bp.

*Note: Due to the tendency of all viral vectors to recombine we recommend keeping the incubation times as short as possible and avoid subculturing. Return to your original glycerol stock or the colony glycerol stock for each plasmid preparation.*

**Restriction Digests of pGIPZ**

The following is a sample protocol for restriction enzyme digestion using *Kpn*I, *Sac*II, *Sal*I, *Xho*I *and/or Not*I for diagnostic quality control of pGIPZ lentiviral vectors.

1. Using filtered pipette tips and sterile conditions add the following components, in the order stated, to a sterile PCR thin-wall tube.

| | |
|---|---|
| Sterile, nuclease-free water | X μl |
| Restriction enzyme 10X buffer | 1μl |
| BSA (10X, 10mg/ml) if required | 1μl |
| DNA sample 80 -240ng, in water or TE buffer | X μl |
| Restriction enzyme 20U | 0.25μl |
| **Final volume** | **10μl** |

2. Mix gently by pipetting.
3. Incubate in a thermalcycler at 37°C for 2 hours to digest
4. Load the gel with 10μl of each of the digested samples (*Kpn*I, *Sac*II, *Sal*I, *Xho*I and/or *Not*I) on a 1% agarose gel. Run uncut sample alongside the digested samples.

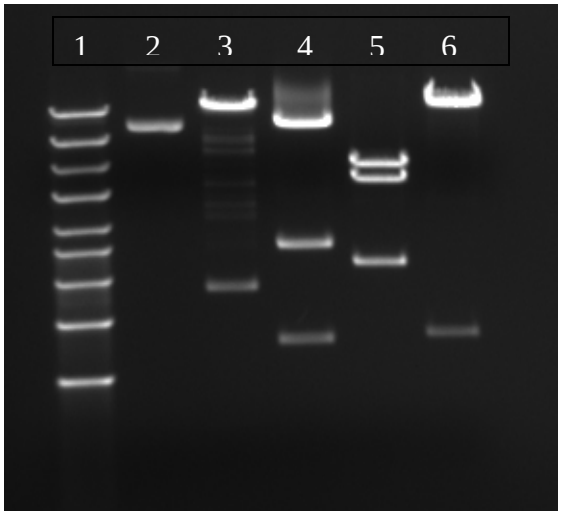


**Figure 5**: Restriction digests with pGIPZ. Lane 1– 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb). Lane2 - Uncut pGIPZ vector. Lane 3 - *Kpn*I digested pGIPZ produces 2 bands at 1750bp and 9860bp. Lane4- *Sac*II digest produces 3 bands at 1178bp, 2502bp and 7930bp. Lane 5 -*Sal*I produces 3 bands at 2188bp, 4298bp and 5124bp. Lane 6 – *Xho*I *Not*I double digest produces 2 bands at 1210bp and 10400bp.

## Culture conditions for 96-well plasmid preparation

Inoculate 96-well bio-block containing 1ml per well of the above media with 1µl of the culture. Incubate at 37°C with shaking (~170-200 RPM). We have observed that incubation times from 16 hours produces good plasmid yield. For plasmid preparation, follow the kit protocols recommended by the manufacturer.

*Note: The cells can be grown at 37°C for purposes of template preparation or sequencing. For archive replication, grow all pGIPZ clones at 30°C in LB-Lennox (low salt) media plus 25ug/ml zeocin and 100ug/ml carbenicillin in order to provide maximum stability of the clones.*

## Materials Required

LB-Lennox Broth (low salt) – VWR item# EM1.00547.0500
Glycerol – VWR item# EM-4760
Carbenicillin or Ampicillin – VWR item# EM-2200 or 80030-956
Zeocin – Invivogen item# ant-zn-5p
96-well microplates – VWR item# 62407-174
Aluminum seals – VWR item# 73520-056
Disposable replicators – Genetix item# X5054

## $CaPO_4$ Transfection Protocol for pGIPZ Lentiviral packaging

(100-mm dish format)

1. Approximately 24 hours before transfection, seed 6.0 X $10^6$ 293T cells in 14-ml of complete media (Dulbecco's modified Eagle's medium (DMEM), 10% FBS, 2 mM L-glutamine, 1X Pen-Strep).

2. Incubate at 37°C, 5% $CO_2$ overnight. Transfection should begin when cells are approximately 90% confluent.

3. The following describes the preparation of DNA-$CaPO_4$ mixture and the protocol for performing 1 transfection (one 100-mm dish). Transfection reactions are carried out in a 5-ml polystyrene round-bottom tube (Falcon catalog # 352058)

DNA Preparation

DNA to be co-transfected, add volume to 945 μl with sterile water:

1. Transgene (gene transfer vector): 21μg
2. pCMV-Gag-Pol (2nd Generation eg. psPAX2): 21μg
3. pCMV-VSV-G-poly A (e.g pMD2.G) 10.5μg

Note: The number of transfection reactions is scalable. For example, if transfecting numerous 100-mm dishes to generate larger volumes or higher titers of the same vector stock, a master mix of the DNA-water stock is made and aliquoted into 50-ml **polystyrene** tubes. A maximum of seven 100-mm dishes can be transfected from one 50-ml tube. For seven transfections, pipette 6615 μl of the DNA-water mix maintaining the same ratio of each of the vector plasmids as well as DNA to water.

4. The following describes the $CaPO_4$ precipitation reaction in both one and seven 100-mm dish formats.

One 100-mm dish:

In one 5-ml snap cap polystyrene tube mix:

a. DNA plus sterile water to final volume of 945 μl.
b. Add 105 μl of 2.5 M $CaCl_2$.
c. While vortexing tube, add dropwise 1050 μl of 2X HBSS (2100 μl total volume). Make sure vortexer is set so that the contents mix thoroughly without spilling over.

For seven 100-mm dishes:

In one 50-ml polystyrene tube mix:

a. DNA plus sterile water to final volume of 6615 μl.
b. Add 735 μl of 2.5 M $CaCl_2$.
c. While vortexing tube, add dropwise 7350 μl of 2X HBSS (14,700 μl total volume). Make sure vortexer is set so that the contents mix thoroughly without spilling over.

5. Incubate at room temperature for 3 minutes. A chalky white precipitate should be visible in the tube. If no precipitate is noticeable, allow the incubation to continue at room temperature until it is visible.

6. Following incubation, vortex contents of the tube a few seconds, and pipette 2100 μl of the transfection mixture dropwise into one well. Do not add the transfection mixture to only one area of the well but instead spread the drops over the entire surface of well.

7. Incubate at 37°C, 5% $CO_2$ for 12-16 hours.

8. Remove media from each plate and slowly pipette 14 ml of DMEM, **5% FBS**, 2 mM L-glutamine, 1X Pen-Strep) to each well. **DO NOT WASH** cells. 5% FBS is used to decrease the amount of serum proteins pelleted with the Vector stock during ultracentrifugation.

9. Incubate at 37°C, 5% $CO_2$ for an additional 48 hours.

10. Harvest virus-containing supernatant. Pellet cells/debris by low-speed centrifugation (1600 x g for 10 min.

11. Aliquot virus and store at -80°C.

12. Virus can be concentrated by ultracentrifugation (SW28, 23,000rpm, 1.5h @ 4°C).

**Reagents:**

**2.5 M $CaCl_2$**
(For 100 ml):
36.75 g $CaCl_2$ (Sigma, Cat. No. C-7902)
Add sterile $dH_2O$ to 100 ml

Filter-sterilize through 0.22 μm filter flask (Millipore)

**2X HBSS** (Hepes Buffered Saline Solution)

50 mM Hepes (pH 7.1)
280 mM NaCl
1.5 mM Sodium Phosphate

**The final pH should be 7.1**

(For 1 liter):

| | |
|---|---|
| 11.915 g | Hepes (Sigma, Cat. No. H-3375) |
| 16.363 g | NaCl (Sigma, Cat. No. S-3014) |
| 0.090 g | $NaH_2PO_4$ (Sigma, Cat. No. S-3139) |
| 0.107 g | $Na_2HPO_4$ (Sigma, Cat. No. S-3264) |

Add sterile $dH_2O$ to 990 ml
pH to 7.1 by dropwise adding 10 N NaOH

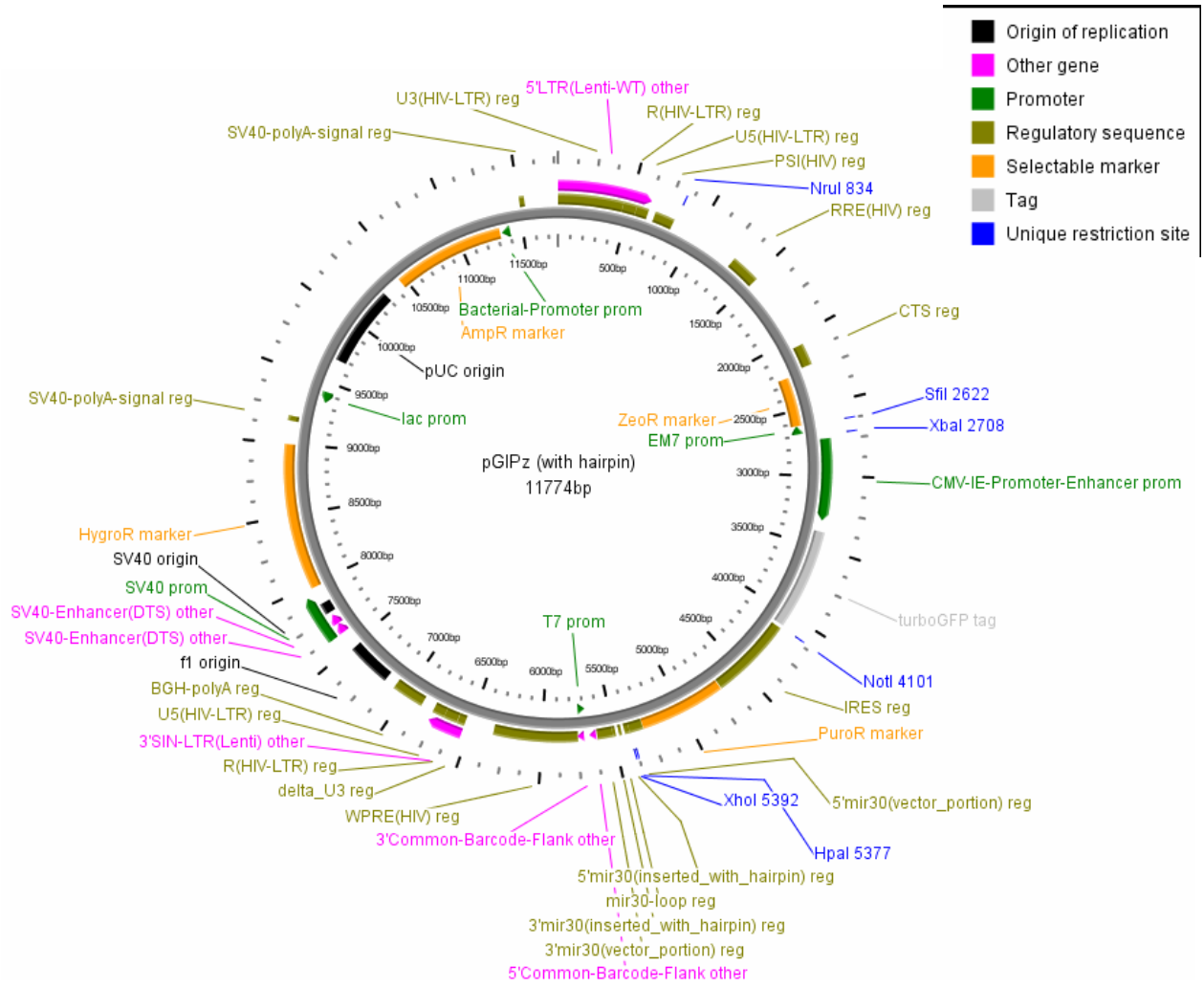


**Figure 6: Detailed Vector Map of pGIPZ lentiviral vector**

## Sequence of pGIPZ lentiviral vector (11774bp)

```
5'LTR(Lenti-WT) other(1,635)>>>
      |
      U3(HIV-LTR) reg(1,455)>>>
      |
1     tggaagggctaattcactcccaaagaagacaagatatccttgatctgtggatctaccaca 60
      ACCTTCCCGATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT

61    cacaaggctacttccctgattagcagaactacacaccagggccaggggtcagatatccac 120
      GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCCAGTCTATAGGTG

121   tgacctttggatggtgctacaagctagtaccagttgagccagataaggtagaagaggcca 180
      ACTGGAAACCTACCACGATGTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT

181   ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgacccgg 240
      TATTTCCTCTCTTGTGGTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC
```

```
241   agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccgag 300
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301   agctgcatccggagtacttcaagaactgctgatatcgagcttgctacaagggactttccg 360
      TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC

361   ctggggactttccagggaggcgtggcctgggcgggactggggagtggcgagccctcagat 420
      GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA

                                        R(HIV-LTR) reg(456,550)>>>
                                        |
421   cctgcatataagcagctgctttttgcctgtactgggtctctctggttagaccagatctga 480
      GGACGTATATTCGTCGACGAAAAACGGACATGACCCAGAGAGACCAATCTGGTCTAGACT

481   gcctgggagctctctggctaactagggaacccactgcttaagcctcaataaagcttgcct 540
      CGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTCGAACGGA

                U5(HIV-LTR) reg(551,635)>>>
                |
541   tgagtgcttcaagtagtgtgtgcccgtctgttgtgtgactctggtaactagagatccctc 600
      ACTCACGAAGTTCATCACACACGGGCAGACAACACACTGAGACCATTGATCTCTAGGGAG

601   agacccttttagtcagtgtggaaaatctctagcagtggcgcccgaacagggacttgaaag 660
      TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC

                              PSI(HIV) reg(685,822)>>>
                              |
661   cgaaagggaaaccagaggagctctctcgacgcaggactcggcttgctgaagcgcgcacgg 720
      GCTTTCCCTTTGGTCTCCTCGAGAGAGCTGCGTCCTGAGCCGAACGACTTCGCGCGTGCC

721   caagaggcgaggggcggcgactggtgagtacgccaaaaattttgactagcggaggctaga 780
      GTTCTCCGCTCCCCGCCGCTGACCACTCATGCGGTTTTTAAAACTGATCGCCTCCGATCT

                                                              NruI
                                                              |
781   aggagagagatgggtgcgagagcgtcagtattaagcgggggagaattagatcgcgatggg 840
      TCCTCTCTCTACCCACGCTCTCGCAGTCATAATTCGCCCCCTCTTAATCTAGCGCTACCC

841   aaaaaattcggttaaggccaggggggaaagaaaaaatataaattaaaacatatagtatggg 900
      TTTTTTAAGCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATACCC

901   caagcagggagctagaacgattcgcagttaatcctggcctgttagaaacatcagaaggct 960
      GTTCGTCCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTTCCGA

961   gtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagat 1020
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1021  cattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagaca 1080
      GTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTTCTGT

1081  ccaaggaagctttagacaagatagaggaagagcaaaacaaaagtaagaccaccgcacagc 1140
      GGTTCCTTCGAAATCTGTTCTATCTCCTTCTCGTTTTGTTTTCATTCTGGTGGCGTGTCG

1141  aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag 1200
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```

```
1201  tgaattatataaatataaagtagtaaaaattgaaccattaggagtagcacccaccaaggc 1260
      ACTTAATATATTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTTCCG

                                                                RRE(HIV)
reg(1314,1518)>>>
                                                                |
1261  aaagagaagagtggtgcagagagaaaaaagagcagtgggaataggagctttgttccttgg 1320
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1321  gttcttgggagcagcaggaagcactatgggcgcagcgtcaatgacgctgacggtacaggc 1380
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1381  cagacaattattgtctggtatagtgcagcagcagaacaatttgctgagggctattgaggc 1440
      GTCTGTTAATAACAGACCATATCACGTCGTCGTCTTGTTAAACGACTCCCGATAACTCCG

1441  gcaacagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaagaatcct 1500
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1501  ggctgtggaaagatacctaaaggatcaacagctcctggggatttggggttgctctggaaa 1560
      CCGACACCTTTCTATGGATTTCCTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTT

1561  actcatttgcaccactgctgtgccttggaatgctagttggagtaataaatctctggaaca 1620
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1621  gatttggaatcacacgacctggatggagtgggacagagaaattaacaattacacaagctt 1680
      CTAAACCTTAGTGTGCTGGACCTACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGAA

1681  aatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattatt 1740
      TTATGTGAGGAATTAACTTCTTAGCGTTTTGGTCGTTCTTTTCTTACTTGTTCTTAATAA

1741  ggaattagataaatgggcaagtttgtggaattggtttaacataacaaattggctgtggta 1800
      CCTTAATCTATTTACCCGTTCAAACACCTTAACCAAATTGTATTGTTTAACCGACACCAT

1801  tataaaattattcataatgatagtaggaggcttggtaggtttaagaatagtttttgctgt 1860
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1861  actttctatagtgaatagagttaggcagggatattcaccattatcgtttcagacccacct 1920
      TGAAAGATATCACTTATCTCAATCCGTCCCTATAAGTGGTAATAGCAAAGTCTGGGTGGA

1921  cccaaccccgaggggacccgacaggcccgaaggaatagaagaagaaggtggagagagaga 1980
      GGGTTGGGGCTCCCCTGGGCTGTCCGGGCTTCCTTATCTTCTTCTTCCACCTCTCTCTCT

1981  cagagacagatccattcgattagtgaacggatcggcactgcgtgcgccaattctgcagac 2040
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2101  gggaaagaatagtagacataatagcaacagacatacaaactaaagaattacaaaaacaaa 2160
      CCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTAATGTTTTTGTTT

2161  ttacaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220
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```

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                              ZeoR marker(2245,2619)<<<
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2281  gccggccgggtcgcgcagggcgaactcccgcccccacggctgctcgccgatctcggtcat 2340
      CGGCCGGCCCAGCGCGTCCCGCTTGAGGGCGGGGGTGCCGACGAGCGGCTAGAGCCAGTA

2341  ggccggcccggaggcgtcccggaagttcgtggacacgacctccgaccactcggcgtacag 2400
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2401  ctcgtccaggccgcgcacccacacccaggccagggtgttgtccggcaccacctggtcctg 2460
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2461  gaccgcgctgatgaacagggtcacgtcgtcccggaccacaccggcgaagtcgtcctccac 2520
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2521  gaagtcccgggagaacccgagccggtcggtccagaactcgaccgctccggcgacgtcgcg 2580
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                                                  SfiI
                                                  |
                                             EM7 prom(2620,2683)<<<
                                             | |
2581  cgcggtgagcaccggaacggcactggtcaacttggccatggtggccctcctatagtgagt 2640
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2641  cgtattatactatgccgatatactatgccgatgattaattgtcaacacgtgctgcaggtc 2700
      GCATAATATGATACGGCTATATGATACGGCTACTAATTAACAGTTGTGCACGACGTCCAG

            XbaI                              CMV-IE-Promoter-Enhancer
prom(2738,3311)>>>
            |                                 |
2701  cgaggttctagacgtattaccgccatgcattagttattaatagtaatcaattacggggtc 2760
      GCTCCAAGATCTGCATAATGGCGGTACGTAATCAATAATTATCATTAGTTAATGCCCCAG

2761  attagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcc 2820
      TAATCAAGTATCGGGTATATACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGG

2821  tggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagt 2880
      ACCGACTGGCGGGTTGCTGGGGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATCA

2881  aacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgccca 2940
      TTGCGGTTATCCCTGAAAGGTAACTGCAGTTACCCACCTCATAAATGCCATTTGACGGGT

2941  cttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg 3000
      GAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGGGGGATAACTGCAGTTACTGCC

3001  taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggca 3060
      ATTTACCGGGCGGACCGTAATACGGGTCATGTACTGGAATACCCTGAAAGGATGAACCGT

3061  gtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaa 3120
      CATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTT

3121  tgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaa 3180
```

```
      ACCCGCACCTATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTT

3181  tgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgc 3240
      ACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTGTTGAGGCG

3241  cccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcg 3300
      GGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGAGC

3301  tttagtgaaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaag 3360
      AAATCACTTGGCAGTCTAGCGGACCTCTGCGGTAGGTGCGACAAAACTGGAGGTATCTTC

                                turboGFP tag(3390,4088)>>>
                                |
3361  acaccgactctactagaggatctgccaccatggagagcgacgagagcggcctgcccgcca 3420
      TGTGGCTGAGATGATCTCCTAGACGGTGGTACCTCTCGCTGCTCTCGCCGGACGGGCGGT

3421  tggagatcgagtgccgcatcaccggcaccctgaacggcgtggagttcgagctggtgggcg 3480
      ACCTCTAGCTCACGGCGTAGTGGCCGTGGGACTTGCCGCACCTCAAGCTCGACCACCCGC

3481  gcggagagggcacccccgagcagggccgcatgaccaacaagatgaagagcaccaaaggcg 3540
      CGCCTCTCCCGTGGGGGCTCGTCCCGGCGTACTGGTTGTTCTACTTCTCGTGGTTTCCGC

3541  ccctgaccttcagcccctacctgctgagccacgtgatgggctacggcttctaccacttcg 3600
      GGGACTGGAAGTCGGGGATGGACGACTCGGTGCACTACCCGATGCCGAAGATGGTGAAGC

3601  gcacctaccccagcggctacgagaacccccttcctgcacgccatcaacaacggcggctaca 3660
      CGTGGATGGGGTCGCCGATGCTCTTGGGGAAGGACGTGCGGTAGTTGTTGCCGCCGATGT

3661  ccaacacccgcatcgagaagtacgaggacggcggcgtgctgcacgtgagcttcagctacc 3720
      GGTTGTGGGCGTAGCTCTTCATGCTCCTGCCGCCGCACGACGTGCACTCGAAGTCGATGG

3721  gctacgaggccggccgcgtgatcggcgacttcaaggtgatgggcaccggcttccccgagg 3780
      CGATGCTCCGGCCGGCGCACTAGCCGCTGAAGTTCCACTACCCGTGGCCGAAGGGGCTCC

3781  acagcgtgatcttcaccgacaagatcatccgcagcaacgccaccgtggagcacctgcacc 3840
      TGTCGCACTAGAAGTGGCTGTTCTAGTAGGCGTCGTTGCGGTGGCACCTCGTGGACGTGG

3841  ccatgggcgataacgatctggatggcagcttcacccgcaccttcagcctgcgcgacggcg 3900
      GGTACCCGCTATTGCTAGACCTACCGTCGAAGTGGGCGTGGAAGTCGGACGCGCTGCCGC

3901  gctactacagctccgtggtggacagccacatgcacttcaagagcgccatccaccccagca 3960
      CGATGATGTCGAGGCACCACCTGTCGGTGTACGTGAAGTTCTCGCGGTAGGTGGGGTCGT

3961  tcctgcagaacgggggccccatgttcgccttccgccgcgtggaggaggatcacagcaaca 4020
      AGGACGTCTTGCCCCCGGGGTACAAGCGGAAGGCGGCGCACCTCCTCCTAGTGTCGTTGT

4021  ccgagctgggcatcgtggagtaccagcacgccttcaagaccccggatgcagatgccggtg 4080
      GGCTCGACCCGTAGCACCTCATGGTCGTGCGGAAGTTCTGGGGCCTACGTCTACGGCCAC

                        NotI          IRES reg(4114,4689)>>>
                        |             |
4081  aagaataatgtacaagtagcggccgcaaattccgcccctctccctcccccccccctaacg 4140
      TTCTTATTACATGTTCATCGCCGGCGTTTAAGGCGGGGAGAGGGAGGGGGGGGGGATTGC

4141  ttactggccgaagccgcttggaataaggccggtgtgcgtttgtctatatgttattttcca 4200
      AATGACCGGCTTCGGCGAACCTTATTCCGGCCACACGCAAACAGATATACAATAAAAGGT
```

```
4201  ccatattgccgtcttttggcaatgtgagggcccggaaacctggccctgtcttcttgacga 4260
      GGTATAACGGCAGAAAACCGTTACACTCCCGGGCCTTTGGACCGGGACAGAAGAACTGCT

4261  gcattcctaggggtctttcccctctcgccaaaggaatgcaaggtctgttgaatgtcgtga 4320
      CGTAAGGATCCCCAGAAAGGGGAGAGCGGTTTCCTTACGTTCCAGACAACTTACAGCACT

4321  aggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgacccctttgca 4380
      TCCTTCGTCAAGGAGACCTTCGAAGAACTTCTGTTTGTTGCAGACATCGCTGGGAAACGT

4381  ggcagcggaacccccacctggcgacaggtgcctctgcggccaaaagccacgtgtataag 4440
      CCGTCGCCTTGGGGGGTGGACCGCTGTCCACGGAGACGCCGGTTTTCGGTGCACATATTC

4441  atacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtggaaa 4500
      TATGTGGACGTTTCCGCCGTGTTGGGGTCACGGTGCAACACTCAACCTATCAACACCTTT

4501  gagtcaaatggctctcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtac 4560
      CTCAGTTTACCGAGAGGAGTTCGCATAAGTTGTTCCCCGACTTCCTACGGGTCTTCCATG

4561  cccattgtatgggatctgatctggggcctcggtgcacatgctttacatgtgtttagtcga 4620
      GGGTAACATACCCTAGACTAGACCCCGGAGCCACGTGTACGAAATGTACACAAATCAGCT

4621  ggttaaaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaacac 4680
      CCAATTTTTTTGCAGATCCGGGGGGCTTGGTGCCCCTGCACCAAAAGGAAACTTTTTGTG

                    PuroR marker(4696,5292)>>>
                    |
4681  gataataccatggccaccgagtacaagcccacggtgcgcctcgccacccgcgacgacgtc 4740
      CTATTATGGTACCGGTGGCTCATGTTCGGGTGCCACGCGGAGCGGTGGGCGCTGCTGCAG

4741  ccccgggccgtacgcaccctcgccgccgcgttcgccgactaccccgccacgcgccacacc 4800
      GGGGCCCGGCATGCGTGGGAGCGGCGGCGCAAGCGGCTGATGGGGCGGTGCGCGGTGTGG

4801  gtcgacccggaccgccacatcgagcgggtcaccgagctgcaagaactcttcctcacgcgc 4860
      CAGCTGGGCCTGGCGGTGTAGCTCGCCCAGTGGCTCGACGTTCTTGAGAAGGAGTGCGCG

4861  gtcgggctcgacatcggcaaggtgtgggtcgcggacgacggcgccgcggtggcggtctgg 4920
      CAGCCCGAGCTGTAGCCGTTCCACACCCAGCGCCTGCTGCCGCGGCGCCACCGCCAGACC

4921  accacgccggagagcgtcgaagcgggggcggtgttcgccgagatcggctcgcgcatggcc 4980
      TGGTGCGGCCTCTCGCAGCTTCGCCCCCGCCACAAGCGGCTCTAGCCGAGCGCGTACCGG

4981  gagttgagcggttcccggctggccgcgcagcaacagatggaaggcctcctggcgccgcac 5040
      CTCAACTCGCCAAGGGCCGACCGGCGCGTCGTTGTCTACCTTCCGGAGGACCGCGGCGTG

5041  cggcccaaggagcccgcgtggttcctggccaccgtcggcgtctcgcccgaccaccagggc 5100
      GCCGGGTTCCTCGGGCGCACCAAGGACCGGTGGCAGCCGCAGAGCGGGCTGGTGGTCCCG

5101  aagggtctgggcagcgccgtcgtgctccccggagtggaggcggccgagcgcgctggggtg 5160
      TTCCCAGACCCGTCGCGGCAGCACGAGGGGCCTCACCTCCGCCGGCTCGCGCGACCCCAC

5161  cccgccttcctggagacctccgcgccccgcaacctccccttctacgagcggctcggcttc 5220
      GGGCGGAAGGACCTCTGGAGGCGCGGGGCGTTGGAGGGGAAGATGCTCGCCGAGCCGAAG

5221  accgtcaccgccgacgtcgaggtgcccgaaggaccgcgcacctggtgcatgacccgcaag 5280
      TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCCTGGCGCGTGGACCACGTACTGGGCGTTC
```

```
                    5'mir30(vector_portion) reg(5296,5390)>>>
                    |
5281  cccggtgcctgagtttgtttgaatgaggcttcagtactttacagaatcgttgcctgcaca 5340
      GGGCCACGGACTCAAACAAACTTACTCCGAAGTCATGAAATGTCTTAGCAACGGACGTGT

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

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

                                                     3'mir30(vector_portion)
reg(5507,5614)>>>
                                                     |
                                  3'mir30(inserted_with_hairpin)
reg(5487,5506)>>>
                                  |                  |
5461  TGTAATGGCAGAGTGAGGGTGGAGGGTGCCTACTGCCTCGgaattcaaggggctacttta 5520
      ACATTACCGTCTCACTCCCACCTCCCACGGATGACGGAGCCTTAAGTTCCCCGATGAAAT

5521  ggagcaattatcttgtttactaaaactgaataccttgctatctctttgatacatttttac 5580
      CCTCGTTAATAGAACAAATGATTTTGACTTATGGAACGATAGAGAAACTATGTAAAAATG

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

5641  cggccattactccgtctcgtgtcttgttgcatatgtctgctggtttgtttgatgttgttt 5700
      GCCGGTAATGAGGCAGAGCACAGAACAACGTATACAGACGACCAAACAAACTACAACAAA

               T7 prom(5710,5729)<<<                  WPRE(HIV)
reg(5749,6337)>>>
               |                                      |
            3'Common-Barcode-Flank other(5707,5729)>>>
            |  |                                      |
5701  gcgggcgggccctatagtgagtcgtattacctaggacgcgtctggaacaatcaacctctg 5760
      CGCCCGCCCGGGATATCACTCAGCATAATGGATCCTGCGCAGACCTTGTTAGTTGGAGAC

5761  gattacaaaatttgtgaaagattgactggtattcttaactatgttgctccttttacgcta 5820
      CTAATGTTTTAAACACTTTCTAACTGACCATAAGAATTGATACAACGAGGAAAATGCGAT

5821  tgtggatacgctgctttaatgcctttgtatcatgctattgcttcccgtatggctttcatt 5880
      ACACCTATGCGACGAAATTACGGAAACATAGTACGATAACGAAGGGCATACCGAAAGTAA

5881  ttctcctccttgtataaatcctggttgctgtctctttatgaggagttgtggcccgttgtc 5940
```

```
      AAGAGGAGGAACATATTTAGGACCAACGACAGAGAAATACTCCTCAACACCGGGCAACAG

5941  aggcaacgtggcgtggtgtgcactgtgtttgctgacgcaacccccactggttggggcatt 6000
      TCCGTTGCACCGCACCACACGTGACACAAACGACTGCGTTGGGGGTGACCAACCCCGTAA

6001  gccaccacctgtcagctcctttccgggactttcgctttccccctccctattgccacggcg 6060
      CGGTGGTGGACAGTCGAGGAAAGGCCCTGAAAGCGAAAGGGGGAGGGATAACGGTGCCGC

6061  gaactcatcgccgcctgccttgcccgctgctggacaggggctcggctgttgggcactgac 6120
      CTTGAGTAGCGGCGGACGGAACGGGCGACGACCTGTCCCCGAGCCGACAACCCGTGACTG

6121  aattccgtggtgttgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgcc 6180
      TTAAGGCACCACAACAGCCCCTTCGACTGCAGGAAAGGTACCGACGAGCGGACACAACGG

6181  acctggattctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggac 6240
      TGGACCTAAGACGCGCCCTGCAGGAAGACGATGCAGGGAAGCCGGGAGTTAGGTCGCCTG

6241  cttccttcccgcggcctgctgccggctctgcggcctcttccgcgtcttcgccttcgccct 6300
      GAAGGAAGGGCGCCGGACGACGGCCGAGACGCCGGAGAAGGCGCAGAAGCGGAAGCGGGA

6301  cagacgagtcggatctccctttgggccgcctccccgcctggaattaattctgcagtcgag 6360
      GTCTGCTCAGCCTAGAGGGAAACCCGGCGGAGGGGCGGACCTTAATTAAGACGTCAGCTC

6361  acctagaaaaacatggagcaatcacaagtagcaatacagcagctaccaatgctgattgtg 6420
      TGGATCTTTTTGTACCTCGTTAGTGTTCATCGTTATGTCGTCGATGGTTACGACTAACAC

6421  cctggctagaagcacaagaggaggaggaggtgggttttccagtcacacctcaggtacctt 6480
      GGACCGATCTTCGTGTTCTCCTCCTCCTCCACCCAAAAGGTCAGTGTGGAGTCCATGGAA

6481  taagaccaatgacttacaaggcagctgtagatcttagccacttttaaaagaaaagaggg 6540
      ATTCTGGTTACTGAATGTTCCGTCGACATCTAGAATCGGTGAAAAATTTTCTTTTCTCCC

         3'SIN-LTR(Lenti) other(6544,6779)>>>
         |
         delta_U3 reg(6544,6596)>>>                              R(HIV-LTR)
reg(6599,6693)>>>
         |                                                       |
6541  gactggaagggctaattcactcccaacgaagacaagatctgcttttgcttgtactgggt 6600
      CTGACCTTCCCGATTAAGTGAGGGTTGCTTCTGTTCTAGACGAAAAACGAACATGACCCA

6601  ctctctggttagaccagatctgagcctgggagctctctggctaactagggaacccactgc 6660
      GAGAGACCAATCTGGTCTAGACTCGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACG

                                      U5(HIV-LTR) reg(6694,6778)>>>
                                      |
6661  ttaagcctcaataaagcttgccttgagtgcttcaagtagtgtgtgcccgtctgttgtgtg 6720
      AATTCGGAGTTATTTCGAACGGAACTCACGAAGTTCATCACACACGGGCAGACAACACAC

6721  actctggtaactagagatccctcagacccttttagtcagtgtggaaaatctctagcagta 6780
      TGAGACCATTGATCTCTAGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCAT

6781  gtagttcatgtcatcttattattcagtatttataacttgcaaagaaatgaatatcagaga 6840
      CATCAAGTACAGTAGAATAATAAGTCATAAATATTGAACGTTTCTTTACTTATAGTCTCT

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

```
6841  gtgagaggccttgacattgtttaaacccgctgatcagcctcgactgtgccttctagttgc 6900
      CACTCTCCGGAACTGTAACAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACG

6901  cagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactccc 6960
      GTCGGTAGACAACAAACGGGGAGGGGGCACGGAAGGAACTGGGACCTTCCACGGTGAGGG

6961  actgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattct 7020
      TGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGA

7021  attctgggggtggggtggggcaggacagcaaggggaggattgggaagacaatagcagg 7080
      TAAGACCCCCCACCCCACCCCGTCCTGTCGTTCCCCCTCCTAACCCTTCTGTTATCGTCC

7081  catgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctct 7140
      GTACGACCCCTACGCCACCCGAGATACCGAAGACTCCGCCTTTCTTGGTCGACCCCGAGA

                                    f1 origin(7171,7477)>>>
                                    |
7141  agggggtatccccacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacg 7200
      TCCCCCATAGGGGTGCGCGGGACATCGCCGCGTAATTCGCGCCGCCCACACCACCAATGC

7201  cgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttccct 7260
      GCGTCGCACTGGCGATGTGAACGGTCGCGGGATCGCGGGCGAGGAAAGCGAAAGAAGGGA

7261  tcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctcccttta 7320
      AGGAAAGAGCGGTGCAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGCCCCCGAGGGAAAT

7321  gggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggt 7380
      CCCAAGGCTAAATCACGAAATGCCGTGGAGCTGGGGTTTTTTGAACTAATCCCACTACCA

7381  tcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacg 7440
      AGTGCATCACCCGGTAGCGGGACTATCTGCCAAAAAGCGGGAAACTGCAACCTCAGGTGC

7441  ttctttaatagtggactcttgttccaaactggaacaacactcaaccctatctcggtctat 7500
      AAGAAATTATCACCTGAGAACAAGGTTTGACCTTGTTGTGAGTTGGGATAGAGCCAGATA

7501  tcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatt 7560
      AGAAAACTAAATATTCCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

                                                      SV40-Enhancer(DTS)
other(7609,7680)>>>
                                                      |
                                                      SV40 prom(7609,7930)>>>
                                                      |
7561  taacaaaaatttaacgcgaattaattctgtggaatgtgtgtcagttagggtgtggaaagt 7620
      ATTGTTTTTAAATTGCGCTTAATTAAGACACCTTACACACAGTCAATCCCACACCTTTCA

7621  ccccaggctccccagcaggcagaagtatgcaaagcatgcatctcaattagtcagcaacca 7680
      GGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGT

      SV40-Enhancer(DTS) other(7681,7752)>>>
      |
7681  ggtgtggaaagtccccaggctccccagcaggcagaagtatgcaaagcatgcatctcaatt 7740
      CCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAA

                                          SV40 origin(7776,7853)>>>
                                          |
```

```
7741  agtcagcaaccatagtcccgcccctaactccgcccatcccgcccctaactccgcccagtt 7800
      TCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAA

7801  ccgcccattctccgccccatggctgactaatttttttatttatgcagaggccgaggccg 7860
      GGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGC

7861  cctctgcctctgagctattccagaagtagtgaggaggctttttggaggcctaggctttt 7920
      GGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAA

                                                                HygroR
marker(7979,8996)>>>
                                                                |
7921  gcaaaaagctcccgggagcttgtatatccattttcggatctgatcagcacgtgatgaaaa 7980
      CGTTTTTCGAGGGCCCTCGAACATATAGGTAAAAGCCTAGACTAGTCGTGCACTACTTTT

7981  agcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtct 8040
      TCGGACTTGAGTGGCGCTGCAGACAGCTCTTCAAAGACTAGCTTTTCAAGCTGTCGCAGA

8041  ccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggag 8100
      GGCTGGACTACGTCGAGAGCCTCCCGCTTCTTAGAGCACGAAAGTCGAAGCTACATCCTC

8101  ggcgtggatatgtcctgcgggtaaatagctgcgccgatggtttctacaaagatcgttatg 8160
      CCGCACCTATACAGGACGCCCATTTATCGACGCGGCTACCAAAGATGTTTCTAGCAATAC

8161  tttatcggcactttgcatcggccgcgctcccgattccggaagtgcttgacattggggaat 8220
      AAATAGCCGTGAAACGTAGCCGGCGCGAGGGCTAAGGCCTTCACGAACTGTAACCCCTTA

8221  tcagcgagagcctgacctattgcatctcccgccgtgcacagggtgtcacgttgcaagacc 8280
      AGTCGCTCTCGGACTGGATAACGTAGAGGGCGGCACGTGTCCCACAGTGCAACGTTCTGG

8281  tgcctgaaaccgaactgcccgctgttctgcagccggtcgcggaggccatggatgcgatcg 8340
      ACGGACTTTGGCTTGACGGGCGACAAGACGTCGGCCAGCGCCTCCGGTACCTACGCTAGC

8341  ctgcggccgatcttagccagacgagcgggttcggcccattcggaccgcaaggaatcggtc 8400
      GACGCCGGCTAGAATCGGTCTGCTCGCCCAAGCCGGGTAAGCCTGGCGTTCCTTAGCCAG

8401  aatacactacatggcgtgatttcatatgcgcgattgctgatccccatgtgtatcactggc 8460
      TTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCG

8461  aaactgtgatggacgacaccgtcagtgcgtccgtcgcgcaggctctcgatgagctgatgc 8520
      TTTGACACTACCTGCTGTGGCAGTCACGCAGGCAGCGCGTCCGAGAGCTACTCGACTACG

8521  tttgggccgaggactgccccgaagtccggcacctcgtgcacgcggatttcggctccaaca 8580
      AAACCCGGCTCCTGACGGGGCTTCAGGCCGTGGAGCACGTGCGCCTAAAGCCGAGGTTGT

8581  atgtcctgacggacaatggccgcataacagcggtcattgactggagcgaggcgatgttcg 8640
      TACAGGACTGCCTGTTACCGGCGTATTGTCGCCAGTAACTGACCTCGCTCCGCTACAAGC

8641  gggattcccaatacgaggtcgccaacatcttcttctggaggccgtggttggcttgtatgg 8700
      CCCTAAGGGTTATGCTCCAGCGGTTGTAGAAGAAGACCTCCGGCACCAACCGAACATACC

8701  agcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctcc 8760
      TCGTCGTCTGCGCGATGAAGCTCGCCTCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGG

8761  gggcgtatatgctccgcattggtcttgaccaactctatcagagcttggttgacggcaatt 8820
      CCCGCATATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAA
```

```
8821  tcgatgatgcagcttgggcgcagggtcgatgcgacgcaatcgtccgatccggagccggga 8880
      AGCTACTACGTCGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCT

8881  ctgtcgggcgtacacaaatcgcccgcagaagcgcggccgtctggaccgatggctgtgtag 8940
      GACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATC

8941  aagtactcgccgatagtggaaaccgacgccccagcactcgtccgagggcaaaggaatagc 9000
      TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTCGTGAGCAGGCTCCCGTTTCCTTATCG

9001  acgtgctacgagatttcgattccaccgccgccttctatgaaaggttgggcttcggaatcg 9060
      TGCACGATGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061  ttttccgggacgccggctggatgatcctccagcgcggggatctcatgctggagttcttcg 9120
      AAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGC

                                                SV40-polyA-signal
reg(9160,9194)>>>
                                                |
9121  cccaccccaacttgtttattgcagcttataatggttacaaataaagcaatagcatcacaa 9180
      GGGTGGGGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTT

9181  atttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactcatca 9240
      TAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGT

9241  atgtatcttatcatgtctgtataccgtcgacctctagctagagcttggcgtaatcatggt 9300
      TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCA

                                                  lac prom(9342,9425)<<<
                                                  |
9301  catagctgtttcctgtgtgaaattgttatccgctcacaattccacacaacatacgagccg 9360
      GTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGC

9361  gaagcataaagtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcgt 9420
      CTTCGTATTTCACATTTCGGACCCCACGGATTACTCACTCGATTGAGTGTAATTAACGCA

9421  tgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcg 9480
      ACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTCGACGTAATTACTTAGC

9481  gccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactg 9540
      CGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGAC

9541  actcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaa 9600
      TGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTCCGCCATT

9601  tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc 9660
      ATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCG

                                pUC origin(9686,10305)<<<
                                |
9661  aaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccc 9720
      TTTTCCGGTCCTTGGCATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGG

9721  ctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactat 9780
      GACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATA
```

```
9781  aaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgc 9840
      TTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACG

9841  cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct 9900
      GCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901  cacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacg 9960
      GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTCGACCCGACACACGTGC

9961  aacccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacc 10020
      TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021 cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga 10080
      GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081 ggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaa 10140
      CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141 gaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggta 10200
      CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCAT

10201 gctcttgatccggcaaacaaaccaccgctggtagcggtggttttttgtttgcaagcagc 10260
      CGAGAACTAGGCCGTTTGTTTGGTGGCGACCATCGCCACCAAAAAAACAAACGTTCGTCG

10261 agattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctg 10320
      TCTAATGCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGAC

10321 acgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaagga 10380
      TGCGAGTCACCTTGCTTTTGAGTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCT

10381 tcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatatatg 10440
      AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATATAC

                          AmpR marker(10460,11320)<<<
                          |
10441 agtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatct 10500
      TCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGA

10501 gtctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacgatacggg 10560
      CAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCCC

10561 agggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctc 10620
      TCCCGAATGGTAGACCGGGGTCACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAG

10621 cagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaa 10680
      GTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGCTCGCGTCTTCACCAGGACGTT

10681 ctttatccgcctccatccagtctattaattgttgccgggaagctagagtaagtagttcgc 10740
      GAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCCCTTCGATCTCATTCATCAAGCG

10741 cagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgt 10800
      GTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACCACAGTGCGAGCA

10801 cgtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatccc 10860
      GCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGG
```

```
10861 ccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagt 10920
      GGTACAACACGTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCA

10921 tggccgcagtgttatcactcatggttatggcagcactgcataattctcttactgtcatgc 10980
      ACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACG

10981 catccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatagt 11040
      GTAGGCATTCTACGAAAAGACACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCA

11041 gtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacata 11100
      CATACGCCGCTGGCTCAACGAGAACGGGCCGCAGTTATGCCCTATTATGGCGCGGTGTAT

11101 gcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaagga 11160
      CGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCT

11161 tcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcag 11220
      AGAATGGCGACAACTCTAGGTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTC

11221 catcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaa 11280
      GTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTGTCCTTCCGTTTTACGGCGTT

11281 aaaagggaataagggcgacacggaaatgttgaatactcatactcttccttttttcaatatt 11340
      TTTTCCCTTATTCCCGCTGTGCCTTTACAACTTATGAGTATGAGAAGGAAAAAGTTATAA

                            Bacterial-Promoter prom(11362,11400)<<<
                            |
11341 attgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttaga 11400
      TAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCT

11401 aaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg 11460
      TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGCTGC

                                                    SV40-polyA-signal
reg(11504,11538)>>>
                                                    |
11461 gatcgggagatcaacttgtttattgcagcttataatggttacaaataaagcaatagcatc 11520
      CTAGCCCTCTAGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAG

11521 acaaatttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactc 11580
      TGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG

11581 atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc 11640
      TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTCGATTGGTTTTAGTAGGG

11641 aaacttcccaccccataccctattaccactgccaattacctgtggtttcatttactctaa 11700
      TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT

11701 acctgtgattcctctgaattattttcattttaaagaaattgtatttgttaaatatgtact 11760
      TGGACACTAAGGAGACTTAATAAAAGTAAAATTTCTTTAACATAAACAATTTATACATGA

11761 acaaacttagtagt 11774
      TGTTTGAATCATCA
```

**Restriction analysis of pGIPZ lentiviral vector**

*********************************************************

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

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

***Alo*I** **(GAACnnnnnnTCCnnnnnnn_nnnnn')**
**Cuts 1 time.**
**Cuts at position 7423.**
**Fragment sizes 7423, 4351.**

***Alo*I** **(GGAnnnnnnGTTCnnnnnnn_nnnnn')**
**Cuts 1 time.**
**Cuts at position 7455.**
**Fragment sizes 7455, 4319.**

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

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

***Blp*I** **(GC'TnA_GC) [Bpu1102I,Bsp1720I,CelII]**
**Cuts 1 time.**
**Cuts at position 3564.**
**Fragment sizes 3564, 8210.**

***Bpu*10I** **(CC'TnA_GC)**
**Cuts 1 time.**
**Cuts at position 1424.**
**Fragment sizes 1424, 10350.**

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

***Bsi*WI** **(C'GTAC_G) [Pfl23II,PspLI,SunI]**

**Cuts 1 time.**
**Cuts at position 4749.**
**Fragment sizes 4749, 7025.**

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

***Bst*EII** **(G'GTnAC_C) [BstPI,Eco91I,EcoO65I,PspEI]**
**Cuts 1 time.**
**Cuts at position 4827.**
**Fragment sizes 4827, 6947.**

***Bst*Z17I** **(GTA'TAC) [BssNAI,Bst1107I]**
**Cuts 1 time.**
**Cuts at position 9261.**
**Fragment sizes 9261, 2513.**

***Bsu*36I** **(CC'TnA_GG) [AxyI,Bse21I,Eco81I]**
**Cuts 1 time.**
**Cuts at position 6469.**
**Fragment sizes 6469, 5305.**

***Csp*CI** **(CAAnnnnnGTGGnnnnnnnnnn_nn')**
**Cuts 1 time.**
**Cuts at position 3141.**
**Fragment sizes 3141, 8633.**

***Csp*CI** **(CCACnnnnnTTGnnnnnnnnnnn_nn')**
**Cuts 1 time.**
**Cuts at position 3106.**
**Fragment sizes 3106, 8668.**

***Eco*NI** **(CCTnn'n_nnAGG) [BstENI,XagI]**
**Cuts 1 time.**
**Cuts at position 1170.**
**Fragment sizes 1170, 10604.**

***Fsp*I** **(TGC'GCA) [Acc16I,AviII,NsbI]**
**Cuts 1 time.**
**Cuts at position 10755.**
**Fragment sizes 10755, 1019.**

***Hpa*I** **(GTT'AAC) [KspAI]**
**Cuts 1 time.**
**Cuts at position 5376.**
**Fragment sizes 5376, 6398.**

***Mlu*I** **(A'CGCG_T)**
**Cuts 1 time.**

**Cuts at position 5736.**
**Fragment sizes 5736, 6038.**

***Not*I** **(GC'GGCC_GC) [CciNI]**
**Cuts 1 time.**
**Cuts at position 4100.**
**Fragment sizes 4100, 7674.**

***Nru*I** **(TCG'CGA) [Bsp68I]**
**[dam methylated]**
**Cuts 1 time.**
**Cuts at position [833].**
**Fragment sizes 833, 10941.**

***Pme*I** **(GTTT'AAAC) [MssI]**
**Cuts 1 time.**
**Cuts at position 6862.**
**Fragment sizes 6862, 4912.**

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

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

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

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

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

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

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

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

***Xho*I** **(C'TCGA_G) [BssHI,PaeR7I,Sfr274I,SlaI,StrI,TliI]**
**Cuts 1 time.**
**Cuts at position 5391.**
**Fragment sizes 5391, 6383.**

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