

pGIPZ non-silencing shRNAmir lentiviral control vector RHS 4346

The pGIPZ non-silencing shRNAmir lentiviral vector (Figure 1) is a negative control for any RNAi experiment performed using the pGIPZ shRNAmir-containing lentiviral vectors. The non-silencing shRNAmir sequence is expressed under the control of the CMV promoter and contains no homology to known mammalian genes.

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
- tGFP (turboGFP) 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

The pGIPZ lentiviral non-silencing shRNAmir vector is shipped as a bacterial culture of *E. coli* (prime+) in LB-Lennox (low salt) broth with 8% glycerol, carbenicillin (100ug/ml) and zeocin (25ug/ml). Individual vials are shipped on wet ice. Open Biosystems checks all cultures for growth prior to shipment. Store at -80°C

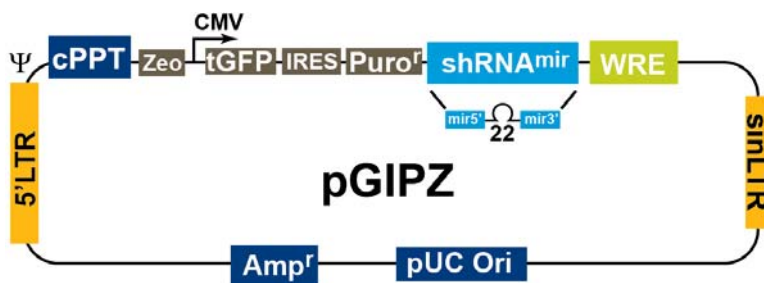


Figure1: pGIPZ lentiviral vector

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
AMPr	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
pBR322 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
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

Validation of the non-silencing shRNAmir

High purity, transfection ready plasmid DNA was prepared and resuspended in Tris buffer (pH 8.5). Following plasmid DNA preparation, the non-silencing shRNAmir was used in a transient transfection with Arrest-In™ transfection reagent to verify it as a negative control; i.e. that it will not knockdown endogenous genes, and thus serve to control for transfection and transduction related processes. Hek293T cells were plated in a 24-well plate at a density of 5×10^4 cells per well one day prior to transfection. The following day non-silencing shRNAmir constructs were transfected into the cells using Arrest-In™ reagent. At 48 hours post-transfection total RNA was extracted, converted into cDNA and quantitative PCR was performed on an ABI 3700 to establish that the baseline values of GAPDH, EG5 and PP1B did not change after exposure to non-silencing shRNAmir (Figure 2).

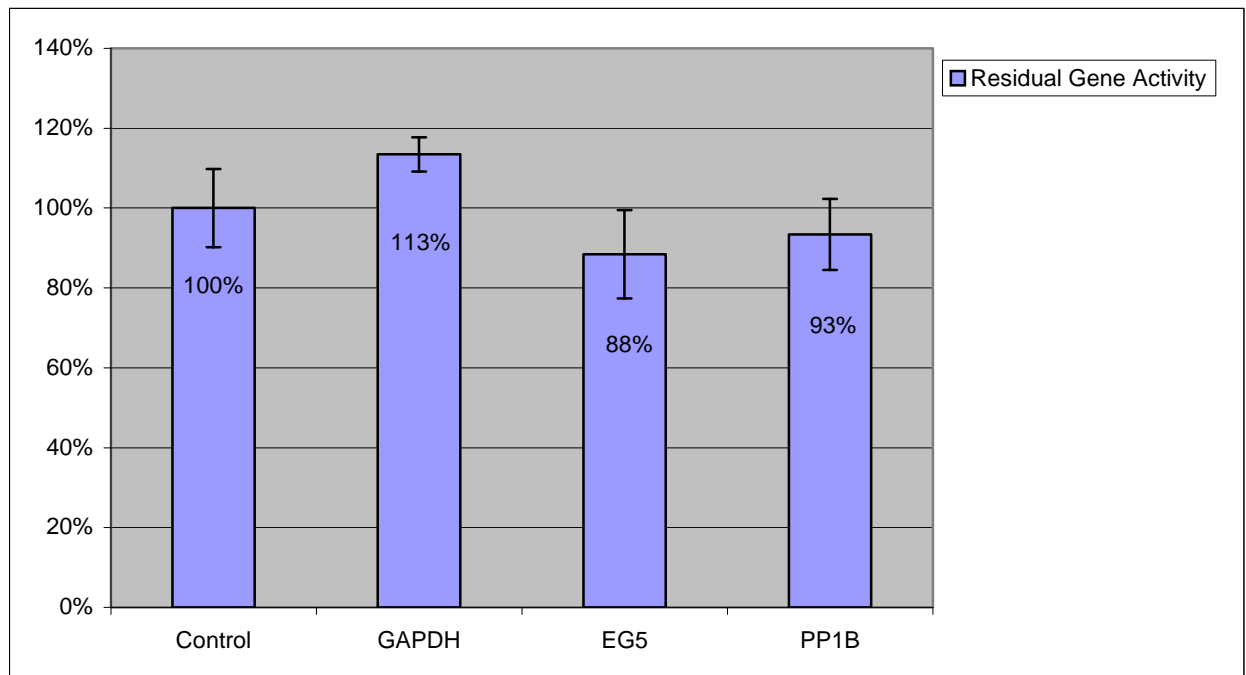


Figure 2: Non-silencing lentiviral shRNAmir control does not knockdown endogenous genes. The above data represents the baseline amount of GAPDH, EG5 or PP1B mRNA set at 100% in the control. The relative amounts of each of these mRNAs are then represented after treatment with non-silencing shRNAmir. Thus the non-silencing shRNAmir has no significant effect on endogenous gene expression.

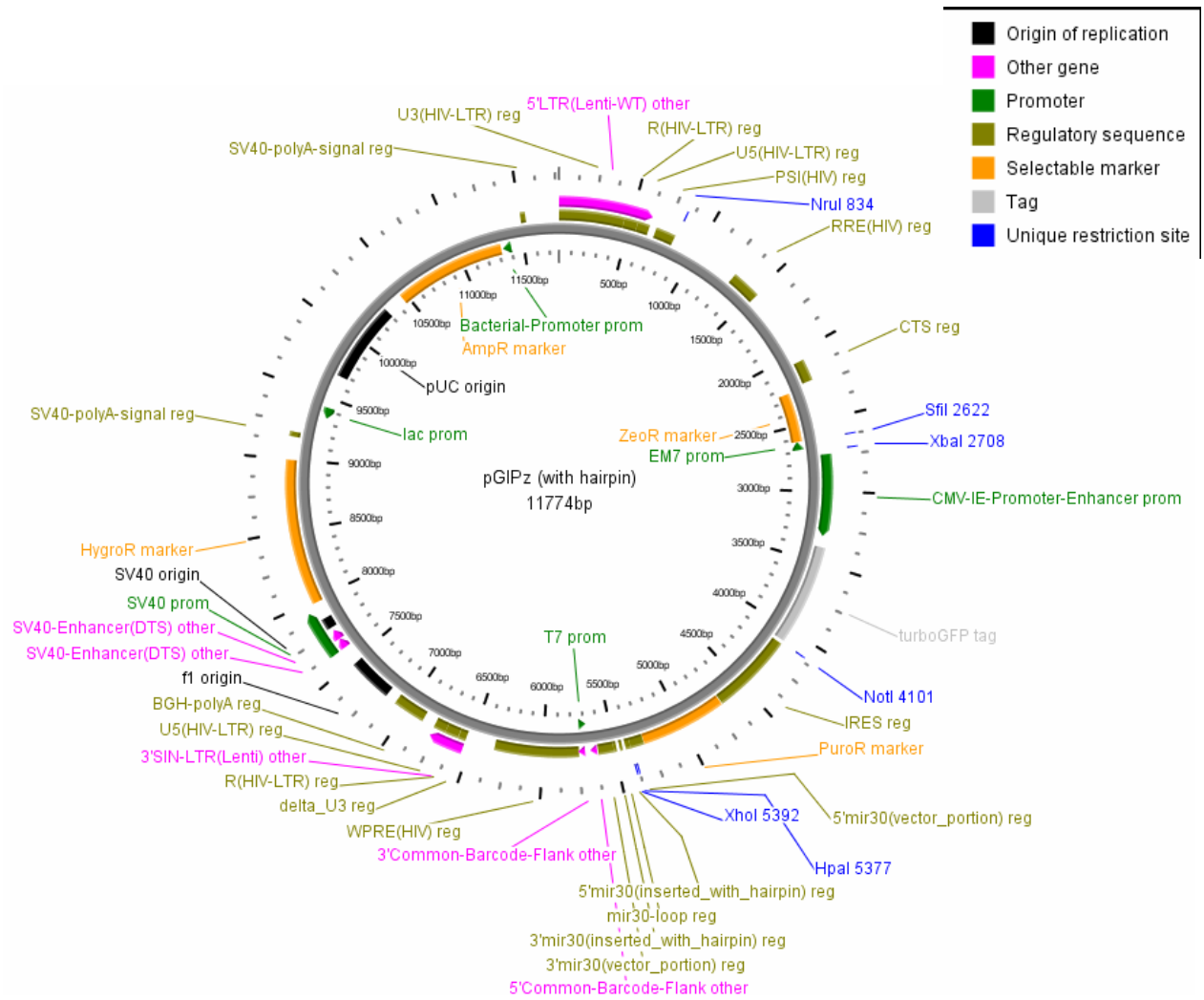


Figure 3: Detailed Vector Map of pGIPZ. The non-silencing shRNAmir sequence was designed using unique microRNA-based design rules to mimic those used in the shRNAmir Lentiviral Library, and subsequently cloned into the *XhoI* and *MluI* restriction sites.

Sequence of pGIPZ lentiviral vector (11774bp)

```

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

```

ACCTTCCCGATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT

61 cacaaggctacttcctgattagcagaactacacaccagggccaggggtcagatatccac 120
GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCAGTCTATAGGTG

121 tgacctttggatgggtgctacaagctagtaccagttgagccagataaggtagaagaggcca 180
ACTGGAAACCTACCACGATGTTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT

181 ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgaccggg 240
TATTTCTCTCTTGTGGTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC

241 agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccgag 300
TCTCTCTTCACAATCTCACCTCCAAACTGTCGGCGGATCGTAAAGTAGTGCACCGGGCTC

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TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC

361 ctggggactttccagggagggcgtggcctggggcgggactggggagtggcgagccctcagat 420
GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA

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

421 cctgcatataagcagctgctttttgcctgtactgggtctctctggttagaccagatctga 480
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481 gcctgggagctctctggctaactagggaaaccactgcttaagcctcaataaagcttgct 540
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U5(HIV-LTR) reg(551,635)>>>
|

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601 agacccttttagtcagtgtggaaaatctctagcagtggcgcccgaacagggacttgaaag 660
TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC

PSI(HIV) reg(685,822)>>>
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721 caagagggcagggggcggcgactgggtgagtacgcaaaaattttgactagcggaggctaga 780
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NruI
|

781 aggagagagatgggtgcgagagcgtcagtattaagcgggggagaattagatcgcgatggg 840
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RRE (HIV)

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1321 gttccttgggagcagcaggaagcactatgggpcgagcgtcaatgacgctgacggtacaggc 1380
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1381 cagacaattattgtctggtatagtgagcagcagacaacaatttgctgagggctattgaggc 1440
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1561 actcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctggaaca 1620
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1981 cagagacagatccattcgattagtgaaacggatcggcactgctgctgccaattctgcagac 2040
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2101 gggaaagaatagtagacataatagcaacagacatacaaaactaaagaattacaaaaacaaa 2160
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2161 ttacaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220
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ZeoR marker (2245, 2619) <<<
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SfiI
 |
 EM7 prom(2620, 2683) <<<
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2641 cgtattatactatgccgatatactatgccgatgattaattgtcaacacgtgctgcaggtc 2700
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XbaI
 |
 prom(2738, 3311) >>>

CMV-IE-Promoter-Enhancer
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2761 attagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcg 2820
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NotI

IRES reg(4114,4689)>>>

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4141 ttactggccgaagccgcttggaataagggcgggtgtgctgttatatgttattttcca 4200
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4321 aggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgacccttgca 4380
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4621 ggttaaaaaaacgtctagggccccccgaaccacggggacgtggttttcctttgaaaaacac 4680
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PuroR marker (4696, 5292) >>>

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5221  accgtcaccgcccgcgacgtcgaggtgcccgaaggaccgcgcacctggtgcatgacccgcaag 5280
      TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCTGGCGCGTGGACCACGTACTIONGGGCGTTC

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

                                          XhoI
                                          |
          HpaI
          |
5'mir30(inserted_with_hairpin) reg(5391,5423)>>>
          |
5341  tcttgaaacacttgctgggattacttcttcaggttaacccaacagaaggctcgagAAGG 5400
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                                          mir30-loop
reg(5446,5464)>>>
          |
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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
      CCTCGTTAATAGAACAAATGATTTTGACTIONTATGGAACGATAGAGAACTATGTAAAAATG

          5' Common-Barcode-
Flank other(5626,5646)>>>
          |
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5641  cggccattactccgtctcgtgtcttggtgcatatgtctgctggtttgttgatggtgttt 5700
      GCCGGTAATGAGGCAGAGCACAGAACAACGTATACAGACGACCAAACAACTACAACAAA

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

5881 ttctcctccttgtataaatcctgggtgctgtctctttatgaggagtgtgtggcccgttgtc 5940
AAGAGGAGGAACATATTTAGGACCAACGACAGAGAAATACTCCTCAACACCGGGCAACAG

5941 aggcaacgtggcggtggtgtgactgtgtttgctgacgcaacccccactggttggggcatt 6000
TCCGTTGCACCGCACCACACGTGACACAAACGACTGCGTTGGGGGTGACCAACCCCGTAA

6001 gccaccacctgtcagctcctttccgggactttcgtttccccctccctattgccacggcg 6060
CGGTGGTGGACAGTCGAGGAAAGGCCCTGAAAGCGAAAGGGGGAGGGATAACGGTGCCGC

6061 gaactcatcgccgcctgccttgcccgtgctggacaggggctcggctggtgggactgac 6120
CTTGAGTAGCGGCGGACGGAACGGGCGACGACCTGTCCCCGAGCCGACAACCCGTGACTG

6121 aattccgtgggtggtgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgcc 6180
TTAAGGCACCACAACAGCCCCTTCGACTGCAGGAAAGGTACCGACGAGCGGACACAACGG

6181 acctggattctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggac 6240
TGGACCTAAGACGCGCCCTGCAGGAAGACGATGCAGGGAAGCCGGGAGTTAGGTGCGCTG

6241 cttccttcccgcggcctgctgcccgtctgcccctcttccgcgtcttcgccttcgcct 6300
GAAGGAAGGGCGCCGGACGACGGCCGAGACGCCGGAGAAGGCGCAGAAGCGGAAGCGGGA

6301 cagacgagtcggatctccctttgggcccctccccgcctggaattaattctgcagtcgag 6360
GTCTGCTCAGCCTAGAGGGAACCCGGCGGAGGGGCGGACCTTAATTAAGACGTCAGCTC

6361 acctagaaaaacatggagcaatcacaagtagcaatacagcagctaccaatgctgattgtg 6420
TGGATCTTTTTGTACCTCGTTAGTGTTCATCGTTATGTCTGTCGATGGTTACGACTAACAC

6421 cctggctagaagcacaagaggaggagggtgggttttccagtcacacctcaggtacctt 6480
GGACCGATCTTCGTGTTCTCCTCCTCCTCCACCCAAAAGGTCAGTGTGGAGTCCATGGAA

6481 taagaccaatgacttacaaggcagctgtagatcttagccactttttaaaagaaaagaggg 6540
ATTCTGGTTACTGAATGTTCCGTCGACATCTAGAATCGGTGAAAAATTTTCTTTTCTCCC

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

6541 gactggaagggctaattcactcccaacgaagacaagatctgctttttgcttgtactgggt 6600
CTGACCTTCCCATTAAAGTGAGGGTTGCTTCTGTTCTAGACGAAAAACGAACATGACCCA

6601 ctctctgggttagaccagatctgagcctgggagctctctgggtaactagggaaacctgc 6660
GAGAGACCAATCTGGTCTAGACTCGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACG

U5(HIV-LTR) reg(6694,6778)>>>
|

6661 ttaagcctcaataaagcttgccttgagtgttcaagtagtgtgtgcccgtctgttgtgtg 6720
AATTCCGAGTTATTTTCGAACGGAACCTCACGAAGTTCATCACACACGGGCAGACAACACAC

6721 actctggtaactagagatccctcagacccttttagtcagtggtggaaaatctctagcagta 6780
TGAGACCATTGATCTCTAGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCAT

6781 gtagttcatgtcatcttattattcagtatattataacttgcaaagaaatgaatatcagaga 6840
CATCAAGTACAGTAGAATAATAAGTCATAAATATTGAACGTTTCTTTACTTATAGTCTCT

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

6841 gtgagaggccttgacattgtttaaacccgctgatcagcctcgactgtgccttctagttgc 6900
 CACTCTCCGGAAGTGTAAACAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACG

6901 cagccatctgtttgtttgccccctcccccgctgccttccttgaccctggaaggtgccactccc 6960
 GTCGGTAGACAACAAACGGGGAGGGGCGACGGAAGGAAGTGGGACCTTCCACGGTGAGGG

6961 actgtcctttcctaataaaaatgaggaaattgcatcgcattgtctgagtaggtgtcattct 7020
 TGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGA

7021 attctgggggggtgggggtggggcaggacagcaagggggaggattgggaagacaatagcagg 7080
 TAAGACCCCCACCCACCCCGTCCTGTCGTTCCCCCTCCTAACCTTCTGTTATCGTCC

7081 catgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctct 7140
 GTACGACCCCTACGCCACCCGAGATACCGAAGACTCCGCCTTTCTTGGTCGACCCCGAGA

f1 origin(7171,7477)>>>

7141 aggggggatccccacgcgcctgtagcggcgcatataagcgcggcggtgtgggtggttacg 7200
 TCCCCATAGGGGTGCGCGGGACATCGCCGCGTAATTCGCGCCGCCACACCACCAATGC

7201 cgcagcgtgaccgctacacttgccagcgccttagcgcgccgctcctttcgctttcttccct 7260
 GCGTCGCACTGGCGATGTGAACGGTTCGCGGGATCGCGGGCGAGGAAAGCGAAAGAAGGGA

7261 tcctttctcgccacgttcgcccggctttccccgtcaagctctaaatcgggggctcccttta 7320
 AGGAAAGAGCGGTGCAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGCCCCGAGGGAAAT

7321 ggggtccgatttagtgctttacggcacctcgacccccaaaaaacttgattaggggtgatgg 7380
 CCCAAGGCTAAATCACGAAATGCCGTGGAGCTGGGGTTTTTTGAACTAATCCCACTACCA

7381 tcacgtagtgggccatcgccctgatagacgggtttttcgccctttgacggttgagtcacg 7440
 AGTGCATCACCCGGTAGCGGGACTATCTGCCAAAAGCGGGAAACTGCAACCTCAGGTGC

7441 ttctttaatagtggaactcttggtccaaactggaacaacactcaaccctatctcggtctat 7500
 AAGAAATTATCACCTGAGAACAAGGTTTGACCTTGTTGTGAGTTGGGATAGAGCCAGATA

7501 tcctttgatattataagggatthttgcccgatthtcggcctattgggttaaaaaatgagctgatt 7560
 AGAAAATAAATATTCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

SV40-

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

|
SV40

prom(7609,7930)>>>

7561 taacaaaaatthaacgcgaattaattctgtggaatgtgtgtcagttaggggtgtggaaagt 7620
 ATTGTTTTTAAATTGCGCTTAATTAAGACACCTTACACACAGTCAATCCCACACCTTTCA

7621 ccccaggctccccagcagggcagaagtatgcaaagcatgcatctcaattagtcagcaacca 7680
 GGGGTCCGAGGGGTTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGT

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

7681 ggtgtggaaagtccccaggctccccagcagggcagaagtatgcaaagcatgcatctcaatt 7740
 CCACACCTTTCAGGGGTCCGAGGGGTTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAA

SV40 origin(7776,7853)>>>

7741 agtcagcaacccatagctcccgccttaactccgcccattcccgccttaactccgcccagtt 7800
TCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAA
7801 ccgcccattctccgccccatggctgactaattttttttatattatgcagaggccgaggccg 7860
GGCGGGTAAGAGGCGGGGTACCGACTGATTAATAAATAAATACGTCTCCGGCTCCGGC
7861 cctctgcctctgagctattccagaagtagtgaggaggcttttttggaggcctaggctttt 7920
GGAGACGGGACTCGATAAGGTCTTCATCACTCCTCCGAAAAACCTCCGGATCCGAAAA

HygroR

marker(7979,8996)>>>

7921 gcaaaaagctcccgggagcttgtatatccattttcggatctgatcagcacgtgatgaaaa 7980
CGTTTTTCGAGGGCCCTCGAACATATAGGTAAAAGCCTAGACTAGTCGTGCACTACTTTT
7981 agcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtct 8040
TCGGACTTGAGTGGCGCTGCAGACAGCTCTTCAAAGACTAGCTTTTCAAGCTGTGCAGAG
8041 ccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggag 8100
GGCTGGACTACGTCGAGAGCCTCCCGCTTCTTAGAGCACGAAAGTCGAAGCTACATCCTC
8101 ggcgtggatatgtcctgcgggtaaatagctgcgcccgatggtttctacaaagatcgttatg 8160
CCGCACCTATACAGGACGCCATTTATCGACGCGGCTACCAAAGATGTTTCTAGCAATAC
8161 tttatcggcactttgcatcggccgcgctcccgattccggaagtgccttgacattggggaat 8220
AAATAGCCGTGAAACGTAGCCGGCGCGAGGGCTAAGGCCTTCACGAACTGTAACCCCTTA
8221 tcagcgagagcctgacctattgcatctcccgcctgacacaggggtgacagcttgcaagacc 8280
AGTCGCTCTCGGACTGGATAACGTAGAGGGCGGCACGTGTCCACAGTGCAACGTTCTGG
8281 tgacctgaaaccgaactgcccgtgttctgcagccggctcgcggaggccatggatgcatcg 8340
ACGGACTTTGGCTTGACGGGCGACAAGACGTGCGCCAGCGCCTCCGGTACCTACGCTAGC
8341 ctgcggccgatcttagccagacgagcgggttcggcccattcggaccgcaaggaatcggtc 8400
GACGCCGGCTAGAATCGGTCTGCTCGCCCAAGCCGGGTAAGCCTGGCGTTCTTACCCAG
8401 aatacactacatggcgtgatttcatatgcgctgattgctgatccccatgtgatcactggc 8460
TTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCG
8461 aaactgtgatggacgacaccgtcagtgctcgtcgcgcagcctctcgatgagctgatgc 8520
TTTGACTACTGCTGTGGCAGTCACGCAGGCAGCGCTCCGAGAGCTACTCGACTACG
8521 tttgggcccaggactgccccgaagtccggcacctcgtgcacgcggatttcggctccaaca 8580
AAACCCGGCTCCTGACGGGGCTTCAGGCCGTGGAGCACGTGCGCCTAAAGCCGAGGTTGT
8581 atgtcctgacggacaatggccgcataaacagcggctcattgactggagcagggcgatggtcg 8640
TACAGGACTGCCTGTTACCGGCGTATTGTGCGCCAGTAAGTACCTCGCTCCGCTACAAGC
8641 gggattcccacaatacagaggtcgccaacatcttctcttgaggccgctgggttgctgatgg 8700
CCCTAAGGGTTATGCTCCAGCGGTTGTAGAAGAAGACCTCCGGCACCAACCGAACATAAC
8701 agcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgaggctcc 8760
TCGTGCTCTGCGCGATGAAGCTCGCCTCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGG

8761 gggcgtatatgctccgcattgggtcttgaccaactctatcagagcttggttgacggcaatt 8820
 CCCGCATATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAA

8821 tcgatgatgcagcttgggscgagggctcgatgacgacgcaatcgtccgatccggagccggga 8880
 AGCTACTACGTGGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCT

8881 ctgtcgggscgtacacaaatcgcccgcagaagcgcggccgctctggaccgatggctgtgtag 8940
 GACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATC

8941 aagtactcgccgatagtggaaccgacgccccagcactcgtccgagggcaaaggaatagc 9000
 TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTCTGAGCAGGCTCCCGTTTTCTTATCG

9001 acgtgctacgagatttcgattccaccgccccttctatgaaaggtgggcttcggaatcg 9060
 TGCACGATGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061 ttttcgggacgcccggctggatgatcctccagcgcggggatctcatgctggagttcttcg 9120
 AAAAGGCCCTGCGGCCGACCTACTAGGAGGTGCGCCCCCTAGAGTACGACCTCAAGAAGC

SV40-polyA-signal

reg(9160,9194)>>>

9121 cccacccaacttgtttattgagcttataatgggttacaataaagcaatagcatcacia 9180
 GGGTGGGGTTGAACAAATAACGTGCAATATTACCAATGTTTATTTTCGTTATCGTAGTGT

9181 atttcacaataaagcatttttttactgcatctagttgtggtttgtccaaactcatca 9240
 TAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGT

9241 atgtatcttatcatgtctgtataaccgtcgacctctagctagagcttggcgtaatcatggt 9300
 TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCCGATTAGTACCA

lac prom(9342,9425)<<<

9301 catagctgtttcctgtgtgaaattggtatccgctcacaattccacacaacatacagagccg 9360
 GTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTAAAGGTGTGTTGTATGCTCGGC

9361 gaagcataaagtgtaaagcctgggggtgcctaagttagtgagctaactcacattaattgct 9420
 CTTTCGTATTTACATTTTCGACCCACGGATTACTCACTCGATTGAGTGTAAATTAACGCA

9421 tgcgctcactgcccgtttccagtcgggaaacctgtcgtgcccagctgcattaatgaatcg 9480
 ACGCGAGTGACGGGCGAAAGGTGAGCCCTTTGGACAGCACGGTCGACGTAATTAAGTAC

9481 gccaacgcgccccggagagggcgttttgcgtattgggscgctcttccgcttcctcgctcactg 9540
 CGGTTGCGCGCCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGAC

9541 actcgctgcgctcggctcgttcggctgccccgagcgggtatcagctcactcaaagggcggtaa 9600
 TGAGCGACGCGAGCCAGCAAGCCGACGCGCTCGCCATAGTCGAGTGAGTTTCCGCCATT

9601 tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc 9660
 ATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACTACTCGTTTTCCGGTGC

pUC origin(9686,10305)<<<

9661 aaaaggccaggaaccgtaaaaaggccgctgtgctggcgtttttccataggctccgcccc 9720
 TTTTCCGGTCTTTGGCATTTCGCGGCAACGACCGCAAAAAGGTATCCGAGGCGGGGG

9721 ctgacgagcatcacaataatcgacgctcaagttagaggtggcgaaacccgacaggactat 9780
 GACTGCTCGTAGTGTTCCTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATA

9781 aaagataccagggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgc 9840
 TTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACG

9841 cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct 9900
 CGGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901 cacgctgtaggtatctcagttcgggtgtaggtcgttcgctccaagctgggctgtgtgcacg 9960
 GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTTCGACCCGACACACGTGC

9961 aaccccccgttcagccccgaccgctgcgccttatccggtaactatcgtcttgagtccaacc
 10020 TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021 cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga
 10080 GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081 ggtatgtaggcggtgctacagagttcttgaagtggggcctaactacggctacactagaa
 10140 CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141 gaacagtatgttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggta
 10200 CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTTCTCAACCAT

10201 gctcttgatccggcaaaacaaaccaccgctggtagcgggtgggtttttttgtttgcaagcagc
 10260 CGAGAACTAGGCCGTTTTGTTTGGTGGCGACCATCGCCACCAAAAAACAAACGTTTCGTGC

10261 agattacgcgcagaaaaaaggatctcaagaagatcctttgatcttttctacggggctg
 10320 TCTAATGCGCGTCTTTTTTTTCTAGAGTTCTTCTAGGAACTAGAAAAGATGCCCCAGAC

10321 acgctcagtggaacgaaaactcacgttaagggatgttggtcatgagattatcaaaaagga
 10380 TGCAGTACCTTGCTTTTGTAGTGAATTCCCTAAAACAGTACTCTAATAGTTTTTCTCT

10381 tcttcacctagatccttttaaattaaaaatgaagttttaaataatctaaagtatatatg
 10440 AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAATTTAGTTAGATTTTCATATATAC

AmpR marker(10460,11320)<<<
 |

10441 agtaaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatct
 10500 TCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGA

10501 gtctatctcggtcatccatagttgcctgactccccgctcgtgtagataactacgatacggg
 10560 CAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCCC

10561 agggcttaccatctggccccagtgctgcaatgataccgcgagaccacgctcaccggctc
 10620 TCCCGAATGGTAGACCGGGTACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAG

10621 cagatattatcagcaataaaccagccagccggaagggccgagcgcagaagtggctcctgcaa
10680 GTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGCTCGCGTCTTACCAGGACGTT

10681 ctttatccgcctccatccagctctattaattggtgcccgggaagctagagtaagtagttcgc
10740 GAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCCCTTCGATCTCATTTCATCAAGCG

10741 cagttaatagtttgcgcaacggttggtgccattgctacaggcatcgtgggtgtcacgctcgt
10800 GTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACACAGTGCAGCA

10801 cgtttggatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatccc
10860 GCAAACCATAACGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGG

10861 ccatggttgcaaaaaagcgggttagctccttcggtcctccgatcgttgtcagaagtaagt
10920 GGTACAACACGTTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCA

10921 tggccgcagtggtatcactcatggttatggcagcactgcataattctcttactgtcatgc
10980 ACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACG

10981 catccgtaagatgcttttctgtgactggtagtactcaaccaagtcattctgagaatagt
11040 GTAGGCATTCTACGAAAAGACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCA

11041 gtatgcccgcaccgagttgctcctgcccggcgtcaatacgggataataccgcgccacata
11100 CATAACGCGCTGGCTCAACGAGAACGGGCCGAGTTATGCCCTATTATGGCGCGGTGTAT

11101 gcagaactttaaaagtgctcatcattggaaaacggttcttcggggcgaaaactctcaagga
11160 CGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGGAGAGTTCCT

11161 tcttaccgctggtgagatccagttcgatgtaaccactcgtgcacccaactgatcttcag
11220 AGAATGGCGACAACCTTAGGTCAAGCTACATTGGGTGAGCACGTGGGTGACTAGAAAGTC

11221 catcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaa
11280 GTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTTGTCCTTCCGTTTTACGGCGTT

11281 aaaaggaataagggcgacacggaaatggtgaataactcactcttctttttcaatatt
11340 TTTTCCCTTATTCCCGCTGTGCCTTTACAACCTTATGAGTATGAGAAGGAAAAAGTTATAA

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

11341 attgaagcatttatcaggggttattgtctcatgagcggatacatatttgaatgtatttaga
11400 TAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCT

11401 aaaataaaciaaataggggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg
11460 TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTTCACGGTGGACTGCAGCTGC

SV40-polyA-signal

reg(11504,11538)>>>

```
11461 gatcgggagatcaacttggtttattgcagcttataatggttacaaataaagcaatagcatc
11520 CTAGCCCTCTAGTTGAACAAATAACGTGCAATATTACCAATGTTTATTTTCGTTATCGTAG
11521 acaaatttcacaaataaagcatttttttactgcattctagttgtgggtttgtccaaactc
11580 TGTTTAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG
11581 atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc
11640 TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTTCGATTGGTTTTAGTAGGG
11641 aaacttcccaccccataccctattaccactgcccaattacctgtgggtttcatttactctaa
11700 TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT
11701 acctgtgattcctctgaattattttcattttaaagaaattgtatttgtaaatatgtact
11760 TGGACACTAAGGAGACTTAATAAAAAGTAAAATTTCTTTAACATAAACAATTTATACATGA
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 (CAAnnnnnGTGGnnnnnnnnnnn_nn')
Cuts 1 time.
Cuts at position 3141.
Fragment sizes 3141, 8633.

CspCI (CCACnnnnnTTGnnnnnnnnnnn_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.

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