Expression Arrest™ GIPZ lentiviral shRNAmir library
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°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 Drosophila 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 Drosophila/Dicer processing = More siRNA = Greater knockdown*

![Diagram of shRNAmir design](image)

**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

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

Table 2: Antibiotic Resistances Conveyed by pGIPZ

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (carbenicillin)</td>
<td>100µg/ml</td>
<td>Bacterial selection marker</td>
</tr>
<tr>
<td>Zeocin</td>
<td>25µg/ml</td>
<td>Bacterial selection marker (vector)</td>
</tr>
<tr>
<td>Puromycin</td>
<td>variable</td>
<td>Mammalian selectable marker</td>
</tr>
</tbody>
</table>
**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](image). 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 SacII 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](image). 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 SacII 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 25µg/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**


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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile, nuclease-free water</td>
<td>X µl</td>
</tr>
<tr>
<td>Restriction enzyme 10X buffer</td>
<td>1 µl</td>
</tr>
<tr>
<td>BSA (10X, 10mg/ml) if required</td>
<td>1 µl</td>
</tr>
<tr>
<td>DNA sample 80 -240ng, in water or TE buffer</td>
<td>X µl</td>
</tr>
<tr>
<td>Restriction enzyme 20U</td>
<td>0.25µl</td>
</tr>
<tr>
<td><strong>Final volume</strong></td>
<td><strong>10µl</strong></td>
</tr>
</tbody>
</table>

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). Lane 2 - Uncut pGIPZ vector. Lane 3 - KpnI digested pGIPZ produces 2 bands at 1750bp and 9860bp. Lane 4 - SacII digest produces 3 bands at 1178bp, 2502bp and 7930bp. Lane 5 - SalI produces 3 bands at 2188bp, 4298bp and 5124bp. Lane 6 – XhoI NotI 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 25µg/ml zeocin and 100µg/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
CaPO₄ Transfection Protocol for pGIPZ Lentiviral packaging

(100-mm dish format)

1. Approximately 24 hours before transfection, seed 6.0 X 10⁶ 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% CO₂ overnight. Transfection should begin when cells are approximately 90% confluent.

3. The following describes the preparation of DNA-CaPO₄ 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): 21 µg
2. pCMV-Gag-Pol (2nd Generation eg. psPAX2): 21 µg
3. pCMV-VSV-G-poly A (e.g pMD2.G) 10.5 µg

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 CaPO₄ 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 CaCl₂.
   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 CaCl₂.
   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% CO₂ 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 oC, 5% CO₂ 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 CaCl₂**  
(For 100 ml):  
36.75 g CaCl₂ (Sigma, Cat. No. C-7902)  
Add sterile dH₂O 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 Heps (Sigma, Cat. No. H-3375)  
16.363 g NaCl (Sigma, Cat. No. S-3014)  
0.090 g NaH₂PO₄ (Sigma, Cat. No. S-3139)  
0.107 g Na₂HPO₄ (Sigma, Cat. No. S-3264)  
Add sterile dH₂O 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 tggagggctaatctcatcccaagagacagagatctcttgatctgtgcatctaccaca 60
    ACCTTCCGAATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT
61   cacaagggctacttccctgattagcagaactacaccgagcgctgcatgggatggatgacccgg 120
    GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGGTCCCGGTCCCCAGTCTATAGGTG
121  tggacctttggatggtgctacaagctagtaccagttgagccagataaggtagaagaggcca 180
    ACTGGAAACCTACCACGATGTTCGATCATGGTCAACTCGGTCTATTCCATCTTCCGGT
181  ataaaaagaggaacaccagcagctgtgtaacccctcttgagctgctgctgtgatgacccgg 240
    TATTTCTTCTCTTGTGATCGACACAGTACCTACTGCGGAC

Phone: 1-888-412-2225                  FAX: 1-256-704-4849               info@openbiosystems.com
V010304                                  For Research Use Only
241  agagagaagtgttagagtgagtttgacagccgcttagcatttcatcacgtggcccgag  
     TCTCTTTCCAATACTCACCTCCAAAATCTGTGGCCGAGATGTAAAGTAGTGTGACCGGGCTC

301  agctgcacccgagacttttcaagaaactgtgtggtgctggtctgatcagccgaagtctcgc  
     TCGACGTCAGCCCTCATGAAGTTCATCTGACTCGAGATGGTCTCCCTGAAAGGCC

361  ctggggacattttccagagagcctgctgtgcctggatctgagatctgaataagggactttc  
     GACCCCCTCGAGACCACTCGACCCGGAACCCGCCTCAGCAGCCGGCTCAGGGAGTCTAC

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

481  gcctgggactttccagggaggcgtggcctgggcgggactggggagctggcagccctcagat  
     CGGACCCCTCGAGACCACTCGACCCGGAACCCGCCTCAGCAGCCGGCTCAGGGAGTCTAC

541  tggtagtgcttctcgccagtctgtgctgactgtgactcctgtagagatcagctcctgcttg  
     ACTCAGAACATTCATCAGAAGCAGACCCGAGAACACACATCGAGACCATTTATCTGCAAGGAG

601  agaccccccttgcttgcaactagagacccagctaatataagttcagctggtaggataaatgg  
     TCTGGAAAATCAGTCACACCTTTTAGAGATCGTCAAGGCTCCGGGCTTGGTGCGTCTAC

US(HIV-LTR) reg(551,635)>>>
     |
661  cggaggggacagtctctcccgtctgagccagactcgcgtctgctgagcgcagcagcaggg  
     GCTTTCCCTTTGTTGCTCCTCCGAGAGATCGGCCTGGCGAGCCGAACCCGCCTCAGCAGG

721  caagagggcgagggcgctgttggtgactgatagctgcatgccgaaagagagatcctggcgggg  
     GTTCTCCGCTCCCCGGCGGCTGACCACCTGTCCGCTGGGCTTGGTTTTAAAAACTGATGCTCCGGGATCTC

PSI(HIV) reg(685,822)>>>
     |
781  agggagagatgggtgcgagagcgtcagtattaagcgggggagaattagttcgcgatgggg  
     TCCTCTCCTCGCCAGCTTCGAGGTCAATTCGCCCCCTCCTTACTTGCGGTCTACCC

841  aaaaaattcgttgaaggccaggggggaagaaaaattatataaatataaattaaatatgtgatgg  
     TTTTTAAGGCCAAATTCCGGCTGCCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
V010304

1201  tgaattatatatatatagaataaaaattgaaaccattaggagtagcaccacccaccaaggc 1260
ACTTAAATATATTATATTATTTTCACTATTTTTTAACTTGGATACTCTCCTGTTGGTGGTCCCG

RRE(HIV)

reg(1314,1518)>>>
|
1261  aaagagaaagagtgtgctgacagagaaaaagagcagtggagctttgctcttgqg 1320
TTTCTCTTCACCACGTCTCTCTTTTTTTTCTGCTACAACCTTTATCTCGAAAAACAGAAAAC

1321  gtctctgggagccagacagacagctcattgctacagcagctgtgacagtcagcagc 1380
CAAGAACCTCTGCTGCTCTTGTGATACCCCGCTGCGACTTTACTGCGACTCGCTGCCCTCG

1381  cagacaattttgctggtgataggcagcagcagcagcagcagcagcagcagcagcagc 1440
GTCCTGTTAAACAGACACATATCAGCCTGCTCTTTTTATGAAAGACTCCCCGATACCTCG

1441  gcaacagcatctgtgctgcacggtggcatcaagcgctccagcaagaatctct 1500
CGTTGCTGAAGACACAGTGTGTCGAGGACCTCTCAAGAAGACACTCGCTGCTGTTCCCTTG

1501  ggcgtcgcagaaagataccttaaaaagagtaacaagactctctggggattttgctctggqaa 1560
CCGACACCTTTCTATTGGATTTTCTAGTTGTCGAGGACCCCTAAACCCCAACGAGACTTT

1561  actcatttcgacacactctgtctgcttcggtaatgcttttgctctggtaaaca 1620
TGAGTAACACTTGCGACACCGACCTGATACCTGACTATTATTGAGACACTTG

1621  gatttggaatcacaacagcctggtgtagttggaagtaataaatttcctggaaca 1680
CTAAAACCTTAGTGCTGGACCTAAGCTACCTCCGTCGCTTCCGTGGATACCCCGCGTCTC

1681  aatatacctccaatattggagacagacaaaccagacagaaaaagaaagaacgacgatatt 1740
TTATGTGAGGAAAATACTTCTTTGCAGTTTTTGCTGCTTTTTCTTTCTTTGCTCTTTAAA

1741  ggattagataaatcggaatagttttttggtgaatttttttaacataaaaaatggtcttggt 1800
CCTTAATCTATTTACCCCGTGTCACACAAACAAATGTTTTTTTTAAAAAGACACAG

1801  tataaaattttcataaatagatcagagctctggtctttgagtttaaagatagttttttttctgt 1860
ATATATATAAGTTTACACTTATCTGCTTCCCGAACCACATCCCAATTCTTTATAAAAACACG

1861  actttctctattgagatagttggtccagggatcattcctagtctctcttcgattatcctgq 1920
TTGAGAGATACACCTACACTACCTCCCGTCTTTAAATGAGCAGACTGGGTGGTGAG

1921  cccacccgccggacagccacggacacggccaggaagaagagacaggtgagagagqaga 1980
GGGGGTGGCTCCCTGTGGGCTGCTGGGCTTCCTTCCTTTCTCTTCTCTCTCTCTCT

1981  cagagacagatccatctctttgattagcagaacggtagctgcagcctcactctgctcgagac 2040
GTCCTGCTCTGATGTAAGTCCTACCTTGGCTGAGGACCGCCGTTAGACGCCTGCT

CTS reg(2064,2214)>>>
|
2041  aaatggcaggtattttctcatcaacatattttttaaaagaaaaaaagggggagtttggtgcag 2100
TTTACCCTACATAGTAGGTGTTTTTTTTTTTTTTTCCCCCTAACCACCCCATGTCAGCT

2101  gggagagataatgatgagaataatagcareacatcataaactaanagatattacaacaaaaa 2160
CCCTTTCTATCTAGTGTATATTACGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTT

2161  ttacaaaaatttttctgggtttatttacagggagacagacagatcctgctttgttgtag 2220
AATGTAAAAAGTTTTTTTTAAGCCCAAAAAATGTGCTTCTGCTGACTTTAGCTAAAAACACATC
ZeoR marker (2245, 2619) <<<

```
2221  tacccgggccgcgtcttagtccggaatcagtcctgtccctgaggccacgaagtcagcagtt 2280
  ATGGCCGGCGCAGATCAGCCTTAGTCAAGGACCGGAGCGGGGTGTGCTTACGTGCGTCAA
2281  gcgcgcgggggtgctcgccgagggcaactccgcccccccacggtcgtcggatctgtcat 2340
  CGGCCGCCACCGCGCTCCGGCTTAGTGCTGGGTTGGTGGCGCAGCCCGCTAGAACGCTGTTG
2341  gcgcgcgcggggggtcgcgcagggcgaactcccgcccccacggtcgtcggatctgtcat 2400
  CGGCCGCCACCGCGCTCCGGCTTAGTGCTGGGTTGGTGGCGCAGCCCGCTAGAACGCTGTTG
2401  ctctgctacgacgcacccgaccacacaccaggccacaggggtgtgtctgtccggaccactg 2460
  GAGCAGGTCTCAGGGCGTGGTGGTGGCAGAATCAACGCTGCTGGAAGACGGGTGGAGAAC
2461  gaccgcgtgatgacaggggtcggtcgtctccggaccacacccgacaggggtgtgtctgtc 2520
  CTGGGCCGACTACCTGTCCCGATGAGCAGCGGACTGGGCTTCAACGAGAATGGAGAGT
2521  gaaatcggcgggaacgacccggtggtcgtcgtcagtcgagtcgctcggcgtcagtg 2580
  CTTCAGGGGCCCTTGGGTGGGTGGGGTGCCGACGAGGCTAGAGCCAGTA
SfiI
  |  
EM7 prom (2620, 2683) <<<

```

```
2581  cgcggtgagcaccggacagggcaactgtgctggctcctctactaaggtgtgtgtgtgtgt 2640
  GCGCCACTCAGGGGGGCTGGGCTGGGCTGACGTAATTGATTAGATGATGATGATGATGATG
2641  cgtattatatactcggtagcataatggccctctcgattagttcattataaggtgtgtgt 2700
  GCCATAATATGATACGGCTATATGATACGGCTACTAATTAACAGTTGTGCACGACGCCAG
2701  cgaatcggcgggttcgggttctagacgtattacccgtgctgtaatgctgtaatgctgta 2760
  GCTCCAAGATCTGCAATGCGCTGGGCTGAGATATCAACTTCTGTACGCGGAGGATTTAGG
2761  attaggtcatgatatgggctcgtgacgtaataatggtggaggtgaggtgaggtgaggt 2820
  TAATCAAGTTCTCCGCTTAGTAAAGGCCTGCTATGACGCTGTGCTGCTGCTGCTGCTGCTGCT
2821  tggctgacggggcggcgtctgaccggtaataatgctgtaatgctgtaatgctgta 2880
  ACCGACTGGGGGGTTGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGG
2881  aacggtatatagggacttttctgataatctgctgtactgtactgtactgtactgtact 2940
  TTTCGCTGATAATCCGGAGTTATGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
2941  cttgggactatcacaatgtataatgtataatgtataatgtataatgtataatgtataa 3000
  GAAGGTGCTCATGTGTTTGCATGGGGCTATTGAGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGG
3001  tataattgcccgttcggttcggttcggttcggttcggttcggttcggttcggttcggttc 3060
  ATTTACGGGCGCGGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
3061  gtaatcataatgtgatgatgatgatgatgatgatgatgatgatgatgatgatgatgat 3120
  CATGTAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
3121  tgggggctgtgatccagctgggattttcaagttcctccccacattgccgtc 3180

XbaI
  |  
CMV-IE-Promoter-Enhancer
prom (2738, 3311) >>>

```
2700  cgcggtgagcaccggacagggcaactgtgctggctcctctactaaggtgtgtgtgtgtgt 2760
  GCGCCACTCAGGGGGGCTGGGCTGGGCTGACGTAATTGATTAGATGATGATGATGATGATG
2760  attaggtcatgatatgggctcgtgacgtaataatggtggaggtgaggtgaggtgaggt 2820
  TAATCAAGTTCTCCGCTTAGTAAAGGCCTGCTATGACGCTGTGCTGCTGCTGCTGCTGCTGCTGCT
2820  tggctgacggggcggcgtctgaccggtaataatgctgtaatgctgtaatgctgta 2880
  ACCGACTGGGGGGTTGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGG
2880  aacggtatatagggacttttctgataatctgctgtactgtactgtactgtactgtact 2940
  TTTCGCTGATAATCCGGAGTTATGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
2940  cttgggactatcacaatgtataatgtataatgtataatgtataatgtataatgtataa 3000
  GAAGGTGCTCATGTGTTTGCATGGGGCTATTGAGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGG
3000  tataattgcccgttcggttcggttcggttcggttcggttcggttcggttcggttcggttc 3060
  ATTTACGGGCGCGGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
3060  gtaatcataatgtgatgatgatgatgatgatgatgatgatgatgatgatgatgatgat 3120
  CATGTAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
3120  tgggggctgtgatccagctgggattttcaagttcctccccacattgccgtc 3180
```
4201 ccatattgcgttttggcaatgtgagggccggaacctggccctgtcttcttgacga 4260
GGTATAACGGCAAGAAACCGTTAACTCCCGGGCCTTTGGACCAGGACAGAACCTGCT
4261 gcattctagggtcctttcccccttcgccaaggaataaagtcatttgttaaagttcga 4320
CGTAAGGATCCCCAGAAAGGGGAGAGCGGTTCCTACAGATCCAGACACTTACACGAC
4321 aggaaagctgctttcctgctaaaatcctaaacgataaggtgtggaatgtgtgtatcga 4380
TCTTTCTGCAAGGAGACAGCTTTGTTGGTACACAGATCGCTGGGAAACGT
4381 ggccacggaaccccccaacctggcagactggccgaccacacgtgtataag 4440
CCGTCGCCCTGGGGGTGAGCCGGCTGTCCACGGAGACGCCGGTTTTCGGTGCACATATTC
4441 atacacctgcaaaagggcgcacaacccacagctgccacgttgtgtagttgtgtgaaaa 4500
TATGTGGAGGTTGTCGCCGCAGTAAGGATCCCGAGAAGTTGGAAGTGGTCGGTGACATTT
4501 gattaaagttgctttcctcaacaggtatcaacaagggggtgaaggtgccaggaagtac 4560
CTCAGTTATCCGGAGAGAGTTGCAAGTAATTGTTGGTCCGGGGTCTACGGGCTCGGAG
4561 cccattgtatgggatctgatctggggcctcggtgcacatgctttacatgtttagtcga 4620
GGGTAACATACTGCCAGACTAGACCCCGGAGCCACGTGTACGAAATGTACACAAATCAGC
4621 gtttaaaaaagctctagccccccqaaacccccagggagcaggttgttttcttttgaaaaacac 4680
CCAAATTATTTTGCGACAGGAAAGGAAGGAGAGTTTTTG

PuroR marker (4696, 5292) >>>

| 4681 gataataccatgqccacccagagttacagcctgcctcccacccgcacgcagcgctc 4740
CTATTATGTAACCGGTGACTCATGTTCCCGGGTTCCACACGCAGGACGGGTGGGCTCTGCTGAG
4741 cccgggacctacgcacccctcgcgcgccgacgtgctacccgcacccgggacaccc 4800
GGGGCCGCGCATGCGGCGAGCGGTGGGCGCTGCTGAGCGGTGTGG
4801 gtgcgaccgggcacgcaccccgccagcagctcgtacaagctagctctccagcgcgc 4860
CAGCTGGGCTCTGGCCGAGTAGTTGCCTCCCGCGCGCGGAGGAAGGAGTGGGTG
4861 gtgcggctcagcactcgcaagaaggtgtgctgctgccagacggcgccgacgtgctcgc 4920
CAGCCCGAGACTGTGATTACCGCTTCACCAACCAACCGCTGTCGCCCGCGCACCAGGCAG
4921 accacgccccgagcagctgcaagcgggggctgtctgttccgagatacgctgccgctctgccag 4980
TGTTGCCGGCTCTGGCAGATCCTCGGCGGCCTGGGCCAGCAGGAGCGCAGAGGAGG
4981 gatgttcgacgggttccgcctggtccagcagcagaagagctcctgctgctgcgcggc 5040
CTCAATCGCGCAAGGCGCTGTCGCTTGCTACCTTCCCGAGAAGGCGCGGCG
5041 cggcacaagacccggcctgtgcctctggccaccccagaccacccgacgcagggc 5100
GCCGGCTTCTCGGGCGCAGAAGGAGCTGGTGCACAGCCAGAGGCGGTGGTGGTCCCG
5101 aaggtctgacggaagcaggaggaggttaggtgctgggagaggggctgtctggggtg 5160
TTCCCAAGCCCCGTCCGCGCCGACAGCGGGGCTACCCCTCCGGCGGCGCCAGCACCAC
5161 cccgcctctggagacctcccacccgacccctctctctctgagcgcggctctgccttc 5220
GGGCAGGACGCTTCGTGAGGCGCGCGGCGTGGAGGGGAGATGGCTCCGGAGAGCAAG
5221 acctgtcaacaggccgacgcagctgaggtgccccgaaggagacggccacactgtctagctgac 5280
TGGCAGTGGCGGCGTCAACTCCGCGTGAACCACTGACTTGGGGCGGTTC

Phone: 1-888-412-2225 FAX: 1-256-704-4849 info@openbiosystems.com

V010304 For Research Use Only
5'mir30 (vector portion) reg(5296,5390)>>>  
5281  cccgtgcctgagttgttgaatgagccttcagttcactttacagaacetgtggcaca 5340  
GGCCACGGACCTCAAACAAACTTTACTCCGAAGT CATGAAATGTCTTAGCAACGGAGCTGT  

XhoI  
HpaI  

5'mir30 (inserted with hairpin) reg(5391,5423)>>>  
5341  tctttgaaaaccttgctgtaggattacttcgaggtaacccaacagaaggctggagAAAG 5400  
AGAACCTTTGTGAAAGCACCCTAATGAAGAAGTGCTCAAATGGGTTGTCTCTCGAGCTCTTCC  

mir30-loop  

3'mir30 (vector portion) reg(5464,5464)>>>  
5401  TATATTGCTGGACGACCTCAAACACTTACTCCGAGAATTATAGGAAAGCCACAGA 5460  
ATATAACGACACTTGCAATCGCTGGAGGTTGGGAGTGACGGTAATCACTCTCCGAGCTCTTCC  

3'mir30 (inserted with hairpin) reg(5507,5514)>>>  
5461  TGTAATGGCAGAGTGAGGGTGGAGGGTGCCTACTGCCTCGgaattcaaggggctactttta 5520  
ACATTACACGTCTCTGACCACTCCACCCGATGACGGAGCCCTTAAGTCTCCCCGATGAAAT  

5'Common-Barcode-Flank other(5626,5646)>>>  
5581  aaagctgaattaaaatggtataaattaaatcacttttttcaattggagaagactaatgcgc 5640  
TTTCGACTTAATTTTACCATATTTTATATTTTAGGAAAAAGTGAACCTCTGTATTACGGCG  

3'Common-Barcode-Flank other(5707,5729)>>>  
5641  cgggccattacctccgctctgtgtctgttcacctgtctggtctgtttttagttgtggctttt 5700  
GCCGCTACTTACGAGCTGAGGCGAGGACCAAATCTACAGACGACAAACAAATCTACAAAA  

T7 prom(5710,5729)<<<  
WPRE (HIV)  

reg(5749,6337)>>>  
5701  gcgggccggcctattgatgtcgtttaccaaggggactcttctcgggaataaacatcaacccctgc 5760  
CGCCCGCCCGGGGATATCATCACCAGCATATGGATCCTGCGCAGACCTTGGTATTTAGTGGGAGAC  

5761  gattacaataatttggtaaggattgtgattcttttatactctgtgtgcttttcttcagcta 5820  
CTAAATGCCATCATCATCTACTCCCATGACCTGAGGCAAAAGTGAACCTCTGTATTACGGCG  

5821  tgtggatacgtcttcactgtctccttcctgtcttccttttagggagtgtgtgcctgtttttcatt 5880  
ACACCTATGCGACGAAATTACGGAAACATAGTACGATAACGAAGGGCATACCGAAAGTAA  

5881  ttctcttctctgtataaatttttggttgtctcctttttagggagtgtgtggtcctggccggtgtc 5940
7741  agtcagcaaccatatgtcgcgccctcaactccgcccatactccgcccctactccggcccagtt
   TCGAGCTGAGTTATACGGACGGGGATGTAGGGGGAGGGGGAGGTTGAGGGCGGGTTCA
7801  ccgcccatctctcgcgcccatggctgactaatatatatatatgacggcaggccccgc
   GGCAGTTAAGGAGCCGGGATCGACTGATAAAAAATAAATACGTCTCCGGGTCGCC
7861  cctctgcttcctgacgtactccagagtaagtctcgggttcgactttttggggccttcgct
   GGAGACGGAGACTCGATAAGGTCTTCTTACAACCTTCCCAGAAACACTCCGGATACCG

marker(7979,8996)>>>

7921  gcaaaaagctccgccccagctgtatatcccttgcgagctgactgtagatgtgtaaaa
gttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
8821  tcgatgatgcagttggccagggatctgcatgctgacgccgagccggga 8880
AGCTACTACGTGGAACCGCGTCCCGAATGCGCTTCTAGGCTAGGCTAGCTTCGCG
8881  cttgcagggtcaacaatcfcgccgagcccctgctgacgccgagccggga 8940
GACAGCCCGCATGATTGGAACCTGCTCAGGCCTGCAGCAGAATGGTCTGCTTTTCT
8941  aagtactccgcatagttgggctcaggtgtcggaggtcaggtgtgtag 9000
TTTATTCAGGCTTTCTCATATTTCTATTCTTTTCTTATTATAAGTGAGCTGAGT
9001  acgtgtctcagagttctctatctccctccggcccttcfctagctggctgaatctg 9060
TGCACTACGTCTCTAGCGAGAATGGTCTCAGGCCTGCTATGATAGCTGCAGCAGAGT
9061  ttttcctcgagccggctgctatgtcatctccagccgcggctcaggtctgctgcgctg 9120
AAAAAGCCCTGCGCCGCAGACCTACTAGGAGGTCGCGGCCCCCTAGAGTACATTAGC
9121  cccaccccaactgtttattgcagcttataatggttacaaataaagcaatgaaccaaa 9180
GGGTGGGGTGAAACAATAAAGCTCGAATATTATTAGTACATTTTCTGTTATCGTGT
9181  atttcacaaataaagcatttttttctcactcagactgttattgtattattccaaactcatca 9240
TAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACCAAAACAGGGTATGAGT
9241  atgtatatcattcatctgtcatattcgcagctcatttagctagctggctgatattgt 9300
TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGC
9301  cacagctgtttctgtgtaattttttatccctccagttataaagcaatgaacccaggg 9360
GTATCGAAGAAGGAAAAACTCATTTTCTGTTATCGTGTAGCAGCTGACGTCG
9361  gcagaatcctgctgggcaaccccctgctgcttgctctaaacccgacaggactat 9420
CGGTTGGGTCGCTCCGCCTCCGCCCTCCGGCACTGAGCTGACGTCG
9421  tggctcactgcgggtcctttctcactgctcaaccaaaccaatcaggtgcttcaggg 9480
ACCGAGATCGGCCGCCAGAATACTGGAGCAGACGTCG
9481  gccacggccggagggcaggtggctgtatattgggccccctctctccctccctccctc 9540
CTCTTGGGGGAACCGCGAAGATCGACGTCG
9541  acctgtctgcctggtctgtgaacccgagccggcagctgctgcctgctgcctgctgcctg 9600
TGACGCAAGCGGCGGCGGCAAGGCGGCGGCTCGCCATGCTGACGTCG
9601  tacggttatccacagaatcaggggataaagccgagaaacactgtgtgagctaaagggcagc 9660
ATGCCAATAGGTGTCTTAGTCCCCTATTGCTCCTTGTACACTCGTTTTTCGGCTC
9661  aaaaaggccagacagccgtaaaaagagggcaggtgtctgtgctcttaaagccgctgcggggc 9720
TTTCTCGCTCTGGCCATTTTTCCTGTCGTTCGAGGTGGCGCGGCAGCGGGCCGCTC
9721  cggacagcatacacaacagcagcggctgctgcactgctgaagctgctgctgctgcctgctg 9780
GACTGCTGAGCTTTGAGGCTGAGGGTACACTCGTTTTTCGGCTCCTCGTAC

SV40-polyA-signal
reg(9160,9194)>>>
| reg(9160,9194)>>>
]

lac prom(9342,9425)<<<<
|

pUC origin(9686,10305)<<<<
|

For Research Use Only

Phone: 1-888-412-2225                  FAX: 1-256-704-4849               info@openbiosystems.com

V010304
Restriction analysis of pGIPZ lentiviral vector

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

**AhdI**  
(GACnn_n'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**  
(GAACnnnnnnTCnnnnnnn_nnnnn')  
Cuts 1 time.  
Cuts at position 7423.  
Fragment sizes 7423, 4351.

**AloI**  
(GGAnnnnnnGTTTnnnnnnn_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.

**BpiI**  
(GC'TnA_GC)  [Bpu1102I,Bsp1720I,CelII]  
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,EcoO65I,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**
(CAAnnnnnGTGGnnnnnnn_n')
Cuts 1 time.
Cuts at position 3141.
Fragment sizes 3141, 8633.

**CspCI**
(CCACnnnnnTTGnnnnnnnnn_n')
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.

\textbf{NotI} \quad \text{(GC'GGCC_GC)} \quad \text{[CciNI]}
Cuts 1 time.
Cuts at position 4100.
Fragment sizes 4100, 7674.

\textbf{NruI} \quad \text{(TCG'CGA)} \quad \text{[Bsp68I]}
[dam methylated]
Cuts 1 time.
Cuts at position [833].
Fragment sizes 833, 10941.

\textbf{PmeI} \quad \text{(GTTT'AAAC)} \quad \text{[MssI]}
Cuts 1 time.
Cuts at position 6862.
Fragment sizes 6862, 4912.

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

\textbf{PshAI} \quad \text{(GACnn'nnGTC)} \quad \text{[BoxI,BstPAI]}
Cuts 1 time.
Cuts at position 8001.
Fragment sizes 8001, 3773.

\textbf{SanDI} \quad \text{(GG'GwC_CC)}
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9840.

\textbf{SfII} \quad \text{(GGCCn_nnn'nGGCC)}
[dcm methylated]
Cuts 1 time.
Cuts at position 2621.
Fragment sizes 2621, 9153.

\textbf{SgrAI} \quad \text{(Cr'CCGG_yG)}
Cuts 1 time.
Cuts at position 2500.
Fragment sizes 2500, 9274.

\textbf{SnaBI} \quad \text{(TAC'GTA)} \quad \text{[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.
References


Limited Use License:

This product is covered by several patent applications owned by Cold Spring Harbor Laboratory. The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) under these patent rights to perform the RNAi knockdown methods using the RNAi-inducing vectors claimed in those patent applications for research purposes solely in conjunction with this product. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include nor carry any right or license to use, develop, or otherwise exploit this product commercially, and no rights are conveyed to the buyer to use the product or components of the product for any other purposes, including without limitation, provision of services to a third party, generation of commercial databases, or clinical diagnostics or therapeutics.

In addition, any commercial organization that purchases or desires to purchase RNAi clones are outside the above research license and should contact Open Biosystems for a research use license.

This product is sold pursuant to a license from CSHL, and CSHL reserves all other rights under these patent rights. For information on purchasing a license to the patent rights for uses other than in conjunction with this product or to use this product for purposes other than research, please contact the CSHL Office of Technology Transfer at, 516-367-8312.