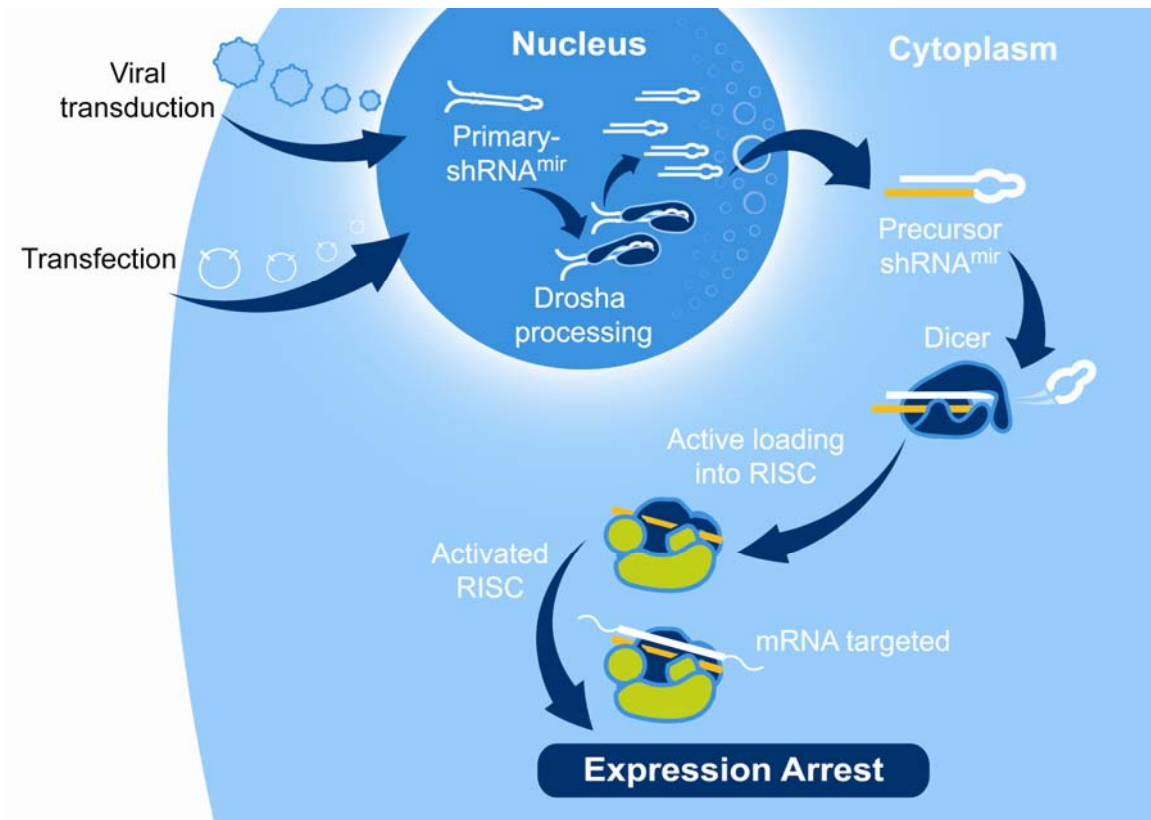


Expression Arrest™ Human lentiviral shRNAmir library RHS4186, RHS4129, RHS3991



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Expression Arrest human microRNA-adapted shRNA (shRNAmir) lentiviral library from Open Biosystems is a whole genome shRNAmir resource for mammalian RNAi. This library was developed in collaboration with Dr. Greg Hannon (CSHL) and Dr. Steve Elledge (Harvard).

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 lentiviral shRNAmir library include:

- shRNAmir constructs covering the entire human genome already cloned into the pCMV-GIN-ZEO lentiviral vector
- Efficient single copy knockdown- Important for pooled screens
- GFP 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

Library Deliverable

The Expression Arrest Human shRNAmir lentiviral library is provided in 96-well microtiter plates containing frozen stock cultures of *E. coli* (DH10 β) in LB broth with 8% glycerol, carbenicillin (100 μ g/ml) and Zeocin (25 μ g/ml). Open Biosystems checks all cultures for growth prior to shipment.

Library storage

The Expression Arrest Human lentiviral shRNAmir library should be stored at -80°C.

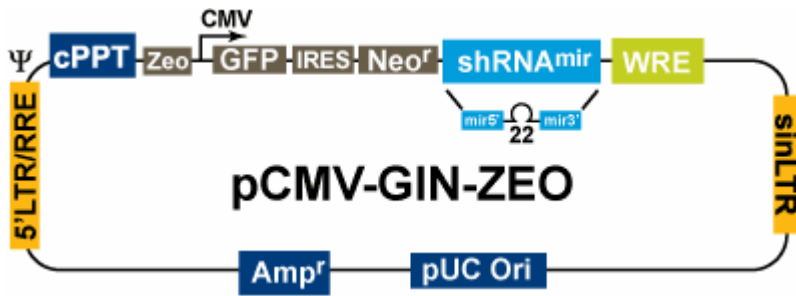


Figure 2: pCMV-GIN-ZEO lentiviral vector

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-NEO	Mammalian selectable marker
AMPr	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in <i>E.coli</i>
SIN-LTR	3' Self inactivating long terminal repeat
RRE	Rev response element
ZEO	Bacterial selectable marker

Table 1: Features of the pCMV-GIN-ZEO Vector

Antibiotic Resistance

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

Table 2: Antibiotic Resistances Conveyed by pCMV-GIN-ZEO

Culturing protocols and maintenance of pCMV-GIN-ZEO

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 lentiviral shRNAmir library has passed through internal QC processes to ensure high quality and low recombination. Open Biosystems does not recommend replication of the entire library. Backup copies of the lentiviral shRNAmir library may be purchased through Open Biosystems to ensure the same high quality and low level of recombination.

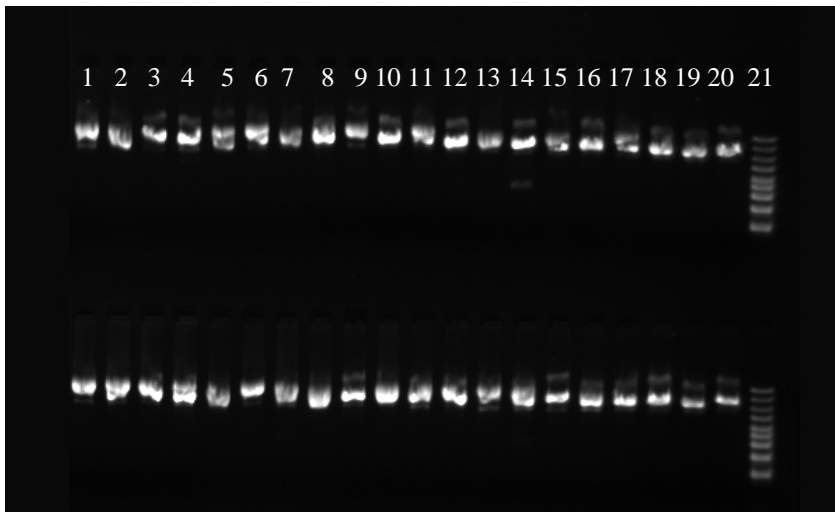


Figure 3. Representative shRNAmir lentiviral clones grown for 20 hrs and plasmid prepped. Uncut plasmids run at ~9kb and show minimal recombination products (lane 14).

Culture conditions for individual plasmid preparations

Most plasmid mini-prep kits recommend a culture volume of 1–10 ml for good yield. For shRNAmir constructs, 5ml of culture can be used for one mini-prep generally producing 5–10 μ g 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 10 μ l inoculum from the glycerol stock into 3-5ml of LB with carbenicillin (100 μ g/ml) and Zeocin (25 μ g/ml). Incubate at 37°C for 12 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 12 hrs with vigorous shaking.

Note: Long term growth in culture is not recommended in order to avoid high levels of recombination

4. Pellet the 3-5ml culture and begin preparation of plasmid DNA. We recommend

preparing Ultra-pure DNA to ensure both high-purity and low endotoxin levels (Qiagen Catalog #12123) as required for transfection into eukaryotic cells.

If you wish to continue at a later time cell pellets can be kept frozen at -20°C overnight.

5. Run 3-5 μl of the plasmid DNA on a 1% agarose gel. The uncut pCMV-GIN-ZEO shRNAmir constructs run at about 9kb
6. Prepare an 8% glycerol stock culture using the 1ml of culture you removed prior to plasmid preparation. This culture can be used for future plasmid preparations but it is still recommended you streak isolate and work from a fresh colony. Store at -80°C .

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 pCMV-GIN-ZEO

The following is a sample protocol for single restriction enzyme digestion using NotI or PvuI or XhoI for diagnostic quality control of pCMV-GIN-ZEO 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	15.8 μl
Restriction enzyme NotI 10X buffer	2 μl
BSA (10X, 10mg/ml)	0.2 μl
DNA sample 400ng, in water or TE buffer	1 μl
<u>Restriction enzyme NotI, 20U</u>	<u>1μl</u>
Final volume	20μl

2. Mix gently by pipetting.
3. Incubate in a thermalcycler at 37°C for 2.5 hours to digest then at 70°C for 20 minutes to kill the enzyme.
4. Add 4 μl of 6X Loading Dye (or another appropriate DNA loading buffer), and proceed to gel analysis.
5. Load the gel with 20 μl of each of the digested samples (NotI, PvuI or XhoI single digests) on a 1% agarose gel. Also run 1 μl (200ng) of the uncut sample combined with 16 μl of water and 3 μl of 6x dye alongside the digested samples.
6. The NotI digest will cut twice and produce two bands of 1393 and 8100bp. The PvuI digest should produce 2 bands: 1952bp and 7546bp. The XhoI digest will linearize the vector since it cuts once at position 6127.

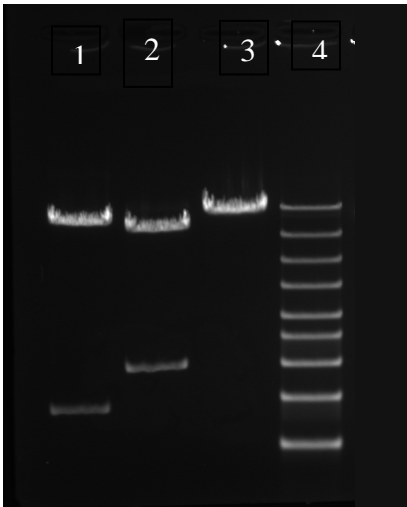


Figure 4: Restriction digests with pCMV-GIN-ZEO. Lane 1–NotI digested pCMV-GIN-ZEO produces 2 bands at 1393bp and 8100bp. Lane2- PvuII digest produces two bands at 1952bp and 7546bp. Lane 3-XhoI cuts once and produces linearized vector at ~9500bp. Lane 4 is a 10kb marker (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb).

Culture conditions for 96-well plasmid preparation

Inoculate 96-well bio-block containing 1 ml 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 12-14 hours produces good plasmid yield. For plasmid preparation, follow the kit protocols recommended by the manufacturer.

Materials Required

LB-Lennox Broth (low salt) – VWR item# EM1.00547.0500

Glycerol – VWR item# EM-4760

Ampicillin – VWR item# EM-2200

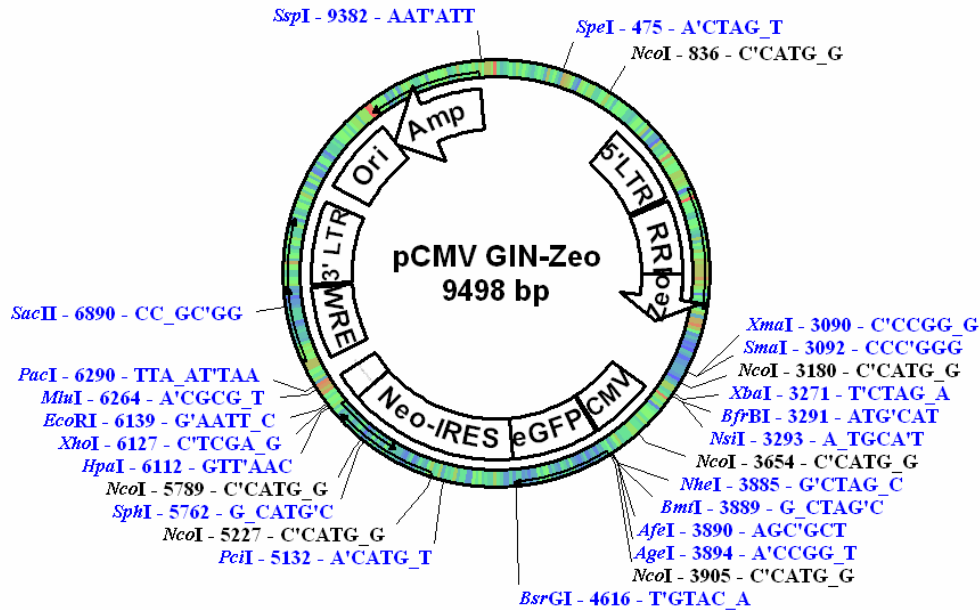
Zeocin – Invivogen item# ant-zn-5p

96-well microplates – VWR item# 62407-174

Aluminum seals – VWR item# 73520-056

Disposable replicators – Scinomix item# SCI-4010-OS

Figure 5: Detailed Vector Map of pCMV-GIN-ZEO



Sequence of pCMV-GIN-ZEO

```

1  GTCGACGGAT CGGGAGATCT CCCGATCCCC TATGGTGCAC TCTCAGTACA
   CAGCTGCCTA GCCCTCTAGA GGGCTAGGGG ATACCACGTG AGAGTCATGT

51  ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATCTGCTCC CTGCTTGTGT
   TAGACGAGAC TACGGCGTAT CAATTCGGTC ATAGACGAGG GACGAACACA

101  GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC
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151  AAGGCTTGAC CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT
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201  GCGCTGCTTC GCGATGTACG GGCCAGATAT ACGCGCGCGT TGACATTGAT
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251  TATTGACTAG TTATTAATAG TAATCAATTA CGGGGTCATT AGTTCATAGC
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5151 CGCCGTGTTT CCGCTGTCTAG CGCAGGGGCG CCCGGTTCTT TTTGTCAAGA
GCGGCACAAG GCCGACAGTC GCGTCCCCGC GGGCCAAGAA AAACAGTTCT
5201 CCGACCTGTC CCGTGCCCTG AATGAACTGC AGGACGAGGC AGCGCGGCTA
GGCTGGACAG GCCACGGGAC TTACTTGACG TCCTGCTCCG TCGCGCCGAT
5251 TCGTGGCTGG CCACGACGGG CGTTCCTTGC GCAGCTGTGC TCGACGTTGT
AGCACCGACC GGTGCTGCCC GCAAGGAACG CGTCGACACG AGCTGCAACA
5301 CACTGAAGCG GGAAGGGACT GGCTGCTATT GGGCGAAGTG CCGGGGCAGG
GTGACTTCGC CCTTCCCTGA CCGACGATAA CCCGCTTCAC GGCCCCGTCC
5351 ATCTCCTGTC ATCTCACCTT GCTCCTGCCG AGAAAGTATC CATCATGGCT
TAGAGGACAG TAGAGTGGAA CGAGGACGGC TCTTTCATAG GTAGTACCGA
5401 GATGCAATGC GCGGGCTGCA TACGCTTGAT CCGGCTACCT GCCCATTCTA
CTACGTTACG CCGCCGACGT ATGCGAACTA GGCCGATGGA CGGGTAAGCT
5451 CCACCAAGCG AAACATCGCA TCGAGCGAGC ACGTACTCGG ATGGAAGCCG
GGTGGTTTCGC TTTGTAGCGT AGCTCGCTCG TGCATGAGCC TACCTTCGGC
5501 GTCTTGTCGA TCAGGATGAT CTGGACGAAG AGCATCAGGG GCTCGCGCCA
CAGAACAGCT AGTCCTACTA GACCTGCTTC TCGTAGTCCC CGAGCGCGGT
5551 GCCGAACTGT TCGCCAGGCT CAAGGCGCGC ATGCCCGACG GCGAGGATCT
CGGCTTGACA AGCGGTCCGA GTTCCGCGCG TACGGGCTGC CGCTCCTAGA
5601 CGTCGTGACC CATGGCGATG CCTGCTTGCC GAATATCATG GTGGAAAATG
GCAGCACTGG GTACCGCTAC GGACGAACGG CTTATAGTAC CACCTTTTAC
5651 GCCGCTTTTT TGGATTTCATC GACTGTGGCC GGCTGGGTGT GCGCGACCGC
CGGCGAAAAG ACCTAAGTAG CTGACACCGG CCGACCCACA CCGCTGGCG
5701 TATCAGGACA TAGCGTTGGC TACCCGTGAT ATTGCTGAAG AGCTTGGCGG
ATAGTCCTGT ATCGCAACCG ATGGGCACTA TAACGACTTC TCGAACCGCC
5751 CGAATGGGCT GACCGCTTCC TCGTGCTTTA CCGTATCGCC GCTCCCGATT
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5801 CGCAGCGCAT CGCCTTCTAT CGCCTTCTTG ACGAGTTCTT CTGAGCGGCC
GCGTCGCGTA GCGGAAGATA GCGGAAGAAC TGCTCAAGAA GACTCGCCGG
5851 GCTGTTTTGAA TGAGGCTTCA GTACTTTACA GAATCGTTGC CTGCACATCT
CGACAAACTT ACTCCGAAGT CATGAAATGT CTTAGCAACG GACGTGTAGA
5901 TGGAAACACT TGCTGGGATT ACTTCTTCAG GTTAACCCAA CAGAAGGCTC
ACTTTTGTGA ACGACCCTAA TGAAGAAGTC CAATTGGGTT GTCTTCCGAG
5951 GAGAAGGTAT ATTGCTGTTG ACAGTGAGCG CCGACACTAA TTCATCTGTA
CTCTTCCATA TAACGACAAC TGTCACTCGC GCCTGTGATT AAGTAGACAT
6001 ATTAGTGAAG CCACAGATGT AATTACAGAT GAATTAGTGT CCGTGCCTAC
TAATCACTTC GGTGTCTACA TTAATGTCTA CTTAATCACA GGCACGGATG

6051 TGCCTCGGAA TTCAAGGGGC TACTTTAGGA GCAATTATCT TGTTTTACTAA
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6101 AACTGAATAC CTTGCTATCT CTTTGATACA TTTTACAAA GCTGAATTAA
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6151 AATGGTATAA ATTAAATCAC TTTTTTCAAT TGGAAGACTA ATGCGGCCGG
TTACCATATT TAATTTAGTG AAAAAAGTTA ACCTTCTGAT TACGCCGGCC
6201 CCATTACTCC NTCNNGTGTC TTGTTGCATA TGTCTGCTGG TTTGTTTGTAT
GGTAATGAGG NAGNNCACAG AACAAACGTAT ACAGACGACC AAACAAACTA
6251 GTTGTGTTGCG GGCGGGCCCT ATAGTGAGTC GTATTACCTA GGACGCGTCC
CAACAAACGC CCGCCCAGGA TATCACTCAG CATAATGGAT CCTGCGCAGG
6301 TAGGGCGGCG AAACCTAATT AAGTCGAGGT CGACGGTATC GATAAGCTCG
ATCCCAGCCG TTTGAATTAA TTCAGCTCCA GCTGCCATAG CTATTGAGC
6351 CTTACGAGA TCATGTTTAA GGGTTCGGT TCCACTAGGT ACAATTCGAT
GAAGTGCTCT AGTACAAATT CCCAAGGCCA AGGTGATCCA TGTTAAGCTA
6401 ATCAAGCTTA TCGATAATCA ACCTCTGGAT TACAAAATTT GTGAAAGATT
TAGTTCGAAT AGCTATTAGT TGGAGACCTA ATGTTTTTAAA CACTTTCTAA
6451 GACTGGTATT CTTAACTATG TTGCTCCTTT TACGCTATGT GGATACGCTG
CTGACCATAA GAATTGATAC AACGAGGAAA ATGCGATACA CCTATGCGAC
6501 CTTTAATGCC TTTGTATCAT GCTATTGCTT CCCGTATGGC TTTTATTTTC
GAAATTACGG AAACATAGTA CGATAACGAA GGGCATAACG AAAGTAAAAG
6551 TCCTCCTTGT ATAAATCCTG GTTGCTGTCT CTTTATGAGG AGTTGTGGCC
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6601 CGTTGTCAGG CAACGTGGCG TGGTGTGCAC TGTGTTTGCT GACGCAACCC
GCAACAGTCC GTTGCACCGC ACCACACGTG ACACAAACGA CTGCGTTGGG
6651 CCACTGGTTG GGGCATTGCC ACCACCTGTC AGCTCCTTTC CGGGACTTTC
GGTGACCAAC CCCGTAACGG TGGTGGACAG TCGAGGAAAG GCCCTGAAAG
6701 GCTTTCCCCC TCCCTATTGC CACGGCGGAA CTCATCGCCG CCTGCCTTGC
CGAAAGGGGG AGGGATAACG GTGCCGCCTT GAGTAGCGGC GGACGGAACG
6751 CCGCTGCTGG ACAGGGGCTC GGCTGTTGGG CACTGACAAT TCCGTGGTGT
GGCGACGACC TGTCCCCGAG CCGACAACCC GTGACTGTTA AGGCACCACA
6801 TGTCGGGGAA ATCATCGTCC TTTCTTGGC TGCTCGCCTG TGTTGCCACC
ACAGCCCCTT TAGTAGCAGG AAAGGAACCG ACGAGCGGAC ACAACGGTGG
6851 TGGATTCTGC GCGGGACGTC CTTCTGCTAC GTCCCTTCGG CCCTCAATCC
ACCTAAGACG CGCCCTGCAG GAAGACGATG CAGGGAAGCC GGGAGTTAGG
6901 AGCGGACCTT CTTTCCCGC GCCTGCTGCC GGCTCTGCGG CCTCTTCCGC
TCGCTGGAA GGAAGGGCGC CGGACGACGG CCGAGACGCC GGAGAAGGCG
6951 GTCTTCGCCT TCGCCCTCAG ACGAGTCGGA TCTCCCTTTG GGCCGCCTCC

CAGAAGCGGA AGCGGGAGTC TGCTCAGCCT AGAGGGAAAC CCGGCGGAGG
 7001 CCGCATCGAT ACCGTCGACC TCGATCGAGA CCTAGAAAAA CATGGAGCAA
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 7051 TCACAAGTAG CAATACAGCA GCTACCAATG CTGATTGTGC CTGGCTAGAA
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 7101 GCACAAGAGG AGGAGGAGGT GGGTTTTCCA GTCACACCTC AGGTACCTTT
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 7151 AAGACCAATG ACTTACAAGG CAGCTGTAGA TCTTAGCCAC TTTTTAAAG
 TTCTGGTTAC TGAATGTTCC GTCGACATCT AGAATCGGTG AAAAAATTTT
 7201 AAAAGGGGGG ACTGGAAGGG CTAATTCACT CCCAACGAAG ACAAGATATC
 TTTTCCCCC TGACCTTCCC GATTAAGTGA GGGTTGCTTC TGTTCTATAG
 7251 CTTGATCTGT GGATCTACCA CACACAAGGC TACTTCCCTG ATTGGCAGAA
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 7301 CTACACACCA GGGCCAGGGA TCAGATATCC ACTGACCTTT GGATGGTGCT
 GATGTGTGGT CCCGGTCCCT AGTCTATAGG TGACTGGAAA CCTACCACGA
 7351 ACAAGCTAGT ACCAGTTGAG CAAGAGAAGG TAGAAGAAGC CAATGAAGGA
 TGTTTCGATCA TGGTCAACTC GTTCTCTTCC ATCTTCTTCG GTTACTTCTT
 7401 GAGAACACCC GCTTGTTACA CCCTGTGAGC CTGCATGGGA TGGATGACCC
 CTCTTGTGGG CGAACAAATGT GGGACACTCG GACGTACCCT ACCTACTGGG
 7451 GGAGAGAGAA GTATTAGAGT GGAGGTTTGA CAGCCGCCTA GCATTTTCATC
 CCTCTCTCTT CATAATCTCA CCTCCAAACT GTCGGCGGAT CGTAAAGTAG
 7501 ACATGGCCCG AGAGCTGCAT CCGGACTGTA CTGGGTCTCT CTGGTTAGAC
 TGTACCGGGC TCTCGACGTA GGCCTGACAT GACCCAGAGA GACCAATCTG
 7551 CAGATCTGAG CCTGGGAGCT CTCTGGCTAA CTAGGGAACC CACTGCTTAA
 GTCTAGACTC GGACCCTCGA GAGACCGATT GATCCCTTGG GTGACGAATT
 7601 GCCTCAATAA AGCTTGCCCT GAGTGCTTCA AGTAGTGTGT GCCCGTCTGT
 CGGAGTTATT TCGAACGGAA CTCACGAAGT TCATCACACA CGGGCAGACA
 7651 TGTGTGACTC TGGTAACTAG AGATCCCTCA GACCCTTTTA GTCAGTGTGG
 ACACACTGAG ACCATTGATC TCTAGGGAGT CTGGGAAAAT CAGTCACACC
 7701 AAAATCTCTA GCAGCATGTG AGCAAAAAGGC CAGCAAAAAG CCAGGAACCG
 TTTTAGAGAT CGTCGTACAC TCGTTTTCCG GTCGTTTTCC GGTCTTTGGC
 7751 TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCTGACG
 ATTTTTCCGG CGCAACGACC GCAAAAAGGT ATCCGAGGCG GGGGGACTGC
 7801 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA
 TCGTAGTGTT TTTAGCTGCG AGTTCAGTCT CCACCGCTTT GGGCTGTCTT
 7851 CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TCGCTCTCC
 GATATTTCTA TGGTCCGCAA AGGGGGACCT TCGAGGGAGC ACGCGAGAGG

7901 TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCTTCGG
ACAAGGCTGG GACGGCGAAT GGCCTATGGA CAGGCGGAAA GAGGGAAGCC

7951 GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG
CTTCGCACCG CGAAAGAGTA TCGAGTGC GAATCCATAGA GTCAAGCCAC

8001 TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC
ATCCAGCAAG CGAGGTTTGA CCCGACACAC GTGCTTGGGG GGCAAGTCGG

8051 CGACCCTGTC GCCTTATCCG GTAACATATCG TCTTGAGTCC AACCCGGTAA
GCTGGCGACG CGGAATAGGC CATTGATAGC AGAACTCAGG TTGGGCCATT

8101 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA
CTGTGCTGAA TAGCGGTGAC CGTCGTCTGGT GACCATTGTC CTAATCGTCT

8151 GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA
CGCTCCATAC ATCCGCCACG ATGTCTCAAG AACTTCACCA CCGGATTGAT

8201 CGGCTACACT AGAAGAACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG
GCCGATGTGA TCTTCTTGTC ATAAACCATA GACGCGAGAC GACTTCGGTC

8251 TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC
AATGGAAGCC TTTTTCTCAA CCATCGAGAA CTAGGCCGTT TGTTTTGGTGG

8301 GCTGGTAGCG GTGGTTTTTT TGTGTTGCAAG CAGCAGATTA CGCGCAGAAA
CGACCATCGC CACCAAAAAA ACAAACGTTT GTCGTCTAAT GCGCGTCTTT

8351 AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC
TTTTCTTAGA GTTCTTCTAG GAAACTAGAA AAGATGCCCC AGACTGCGAG

8401 AGTGAACGA AAACACAGT TAAGGGATTT TGGTCATGAG ATTATCAAAA
TCACCTTGCT TTTGAGTGCA ATTCCTTAAA ACCAGTACTC TAATAGTTTT

8451 AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT
TCCTAGAAGT GGATCTAGGA AAATTTAATT TTTACTTCAA AATTTAGTTA

8501 CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA
GATTTTCATAT ATACTCATT GAACCAGACT GTCAATGGTT ACGAATTAGT

8551 GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTTATC CATAGTTGCC
CACTCCGTGG ATAGAGTCGC TAGACAGATA AAGCAAGTAG GTATCAACGG

8601 TGAATCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG
ACTGAGGGGC AGCACATCTA TTGATGCTAT GCCCTCCCGA ATGGTAGACC

8651 CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT
GGGGTCACGA CGTTACTATG GCGCTCTGGG TGCGAGTGGC CGAGGTCTAA

8701 TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCTT
ATAGTCGTTA TTTGGTCTGGT CGGCCTTCCC GGCTCGCGTC TTCACCAGGA

8751 GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGAAGCTAG
CGTTGAAATA GCGGAGGTA GGTCAGATAA TTAACAACGG CCCTTCGATC

8801 AGTAAGTAGT TCGCCAGTTA ATAGTTTGGC CAACGTTGTT GCCATTGCTA
TCATTCATCA AGCGGTCAAT TATCAAACGC GTTGCAACAA CGGTAACGAT

8851 CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC
 GTCCGTAGCA CCACAGTGCG AGCAGCAAAC CATACCGAAG TAAGTCGAGG

8901 GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA
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8951 AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG
 TCGCCAATCG AGGAAGCCAG GAGGCTAGCA ACAGTCTTCA TTCAACCGGC

9001 CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC
 GTCACAATAG TGAGTACCAA TACCGTCGTG ACGTATTAAG AGAATGACAG

9051 ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC
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9101 ATTCTGAGAA TAGTGTATGC GGCACCGAG TTGCTCTTGC CCGGCGTCAA
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9151 TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT
 ATGCCCTATT ATGGCGCGGT GTATCGTCTT GAAATTTTCA CGAGTAGTAA

9201 GGAAAACGTT CTTCCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG
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9251 ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT
 TAGGTCAAGC TACATTGGGT GAGCACGTGG GTTGACTAGA AGTCGTAGAA

9301 TTACTTTTAC CAGCGTTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC
 AATGAAAGTG GTCGCAAAGA CCCACTCGTT TTTGTCCTTC CGTTTTACGG

9351 GCAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT
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9401 CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG
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9451 GATACATATT TGAATGTATT TAGAAAATA AACAAATAGG GGTTCCGCGC
 CTATGTATAA ACTTACATAA ATCTTTTTAT TTGTTTATCC CCAAGGCGCG

9501 ACATTTCCCC GAAAAGTGCC ACCTGAC
 TGTAAGGGG CTTTTACGG TGGACTG

Restriction analysis of pCMV-GIN-ZEO (9527bp)

Acc65I (G'GTAC_C) [KpnI,Asp718I]
 [dcm methylated]
 Cuts 2 times.
 Cuts at positions 4904, 7142.
 Fragment sizes 2238, 7289.

AfeI (AGC'GCT) [Eco47III, Aor51HI, FunI]
Cuts 1 time.
Cuts at position 3711.

AflII (C'TTAA_G) [BfrI, BspTI, Bst98I, MspCI, Vha464I]
Cuts 2 times.
Cuts at positions 902, 7596.
Fragment sizes 6694, 2833.

AgeI (A'CCGG_T) [AsiAI, BshTI, CspAI, PinAI]
Cuts 1 time.
Cuts at position 3715.

BbeI (G_GCGC'C) [NarI, EgeI, EheI, KasI, Mly113I, SfoI]
Cuts 2 times.
Cuts at positions 1026, 5181.
Fragment sizes 4155, 5372.

BfrBI (ATG'CAT) [EcoT22I, Mph1103I, NsiI, Zsp2I]
Cuts 1 time.
Cuts at position 3112.

BmtI (G_CTAG'C) [NheI, AsuNHI]
Cuts 1 time.
Cuts at position 3710.

BspEI (T'CCGG_A) [AccIII, BlfI, BseAI, Bsp13I, Kpn2I, MroI]
[dam methylated]
Cuts 2 times.
Cuts at positions 2623, 7520.
Fragment sizes 4897, 4630.

BspHI (T'CATG_A) [PagI, RcaI]
[dam methylated]
Cuts 2 times.
Cuts at positions 8434, 9442.
Fragment sizes 1008, 8519.

BsrGI (T'GTAC_A) [Bsp1407I, BstAUI, SspBI]
Cuts 1 time.
Cuts at position 4437.

EcoRI (G'AATT_C) [FunII]
Cuts 1 time.
Cuts at position 6058.

FspI (TGC'GCA) [Acc16I, AviII, NsbI]
Cuts 2 times.
Cuts at positions 5280, 8829.
Fragment sizes 3549, 5978.

HpaI (GTT' AAC) [KspAI]

Cuts 1 time.

Cuts at position 5933.

KasI (G'GCGC_C) [NarI, BbeI, EgeI, EheI, Mly113I, SfoI]

Cuts 2 times.

Cuts at positions 1022, 5177.

Fragment sizes 4155, 5372.

KpnI (G_GTAC'C) [Acc65I, Asp718I]

Cuts 2 times.

Cuts at positions 4908, 7146.

Fragment sizes 2238, 7289.

MluI (A'CGCG_T)

Cuts 1 time.

Cuts at position 6293.

MscI (TGG'CCA) [Bali, MlsI, MluNI, Msp20I]

[dcm methylated]

Cuts 1 time.

Cuts at position 3000.

NarI (GG'CG_CC) [BbeI, EgeI, EheI, KasI, Mly113I, SfoI]

Cuts 2 times.

Cuts at positions 1023, 5178.

Fragment sizes 4155, 5372.

NcoI (C'CATG_G) [Bsp19I]

Cuts 6 times.

Cuts at positions 617, 3001, 3475, 3726, 5048, 5610.

Fragment sizes 2384, 474, 251, 1322, 562, 4534.

NheI (G'CTAG_C) [AsuNHI, BmtI]

Cuts 1 time.

Cuts at position 3706.

NotI (GC'GGCC_GC) [CciNI]

Cuts 2 times.

Cuts at positions 4448, 5846.

Fragment sizes 1398, 8129.

NruI (TCG'CGA) [Bsp68I]

[dam methylated]

Cuts 2 times.

Cuts at positions 211, [1218].

Fragment sizes 1007, 8520.

NsiI (A_TGCA'T) [BfrBI, EcoT22I, Mph1103I, Zsp2I]

Cuts 1 time.
Cuts at position 3114.

PacI (TTA_AT'TAA)
Cuts 1 time.
Cuts at position 6319.

PciI (A'CATG_T) [BspLU11I]
Cuts 1 time.
Cuts at position 4953.

PmlI (CAC'GTG) [PmaCI,AcvI,BbrPI,Eco72I,PspCI]
Cuts 2 times.
Cuts at positions 3073, 4779.
Fragment sizes 1706, 7821.

PvuI (CG_AT'CG) [BspCI,MvrI,Ple19I]
Cuts 2 times.
Cuts at positions 7025, 8977.
Fragment sizes 1952, 7575.

PvuII (CAG'CTG)
Cuts 2 times.
Cuts at positions 5284, 7173.
Fragment sizes 1889, 7638.

SacII (CC_GC'GG) [Cfr42I,KspI,Sfr303I,SgrBI]
Cuts 1 time.
Cuts at position 6919.

ScaI (AGT'ACT) [AssI,ZrmI]
Cuts 2 times.
Cuts at positions 5872, 9087.
Fragment sizes 3215, 6312.

SfoI (GGC'GCC) [NarI,BbeI,EgeI,EheI,KasI,Mly113I]
Cuts 2 times.
Cuts at positions 1024, 5179.
Fragment sizes 4155, 5372.

SmaI (CCC'GGG) [Cfr9I,PspAI,XmaI,XmaCI]
Cuts 1 time.
Cuts at position 2913.

SnaBI (TAC'GTA) [BstSNI,Eco105I]
Cuts 2 times.
Cuts at positions 597, 3455.
Fragment sizes 2858, 6669.

SpeI (A'CTAG_T) [AhlI,BcuI]

Cuts 1 time.
Cuts at position 256.

SphI (G_CATG'C) [BbuI,PaeI,SpaHI]
Cuts 1 time.
Cuts at position 5583.

SspI (AAT'ATT)
Cuts 1 time.
Cuts at position 9411.

XbaI (T'CTAG_A)
[dam methylated]
Cuts 1 time.
Cuts at position 3092.

XhoI (C'TCGA_G) [BssHI,Paer7I,Sfr274I,SlaI,StrI,TliI]
Cuts 1 time.
Cuts at position 5948.

XmaI (C'CCGG_G) [SmaI,Cfr9I,PspAI,XmaCI]
Cuts 1 time.
Cuts at position 2911.

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