

pGIPZ lentiviral shRNAmir empty vector

RHS4349

The pGIPZ lentiviral shRNAmir empty vector is the pGIPZ lentiviral vector without a shRNAmir insert. The pGIPZ empty vector can be used to clone in shRNAmir constructs from the pSM2 retroviral vector.

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 (turbo green florescent protein) 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 empty lentiviral vector is shipped as bacterial cultures 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

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 <i>E.coli</i>
SIN-LTR	3' Self inactivating long terminal repeat
RRE	Rev response element
ZEO _r	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
Puromycin	variable	Mammalian selectable marker

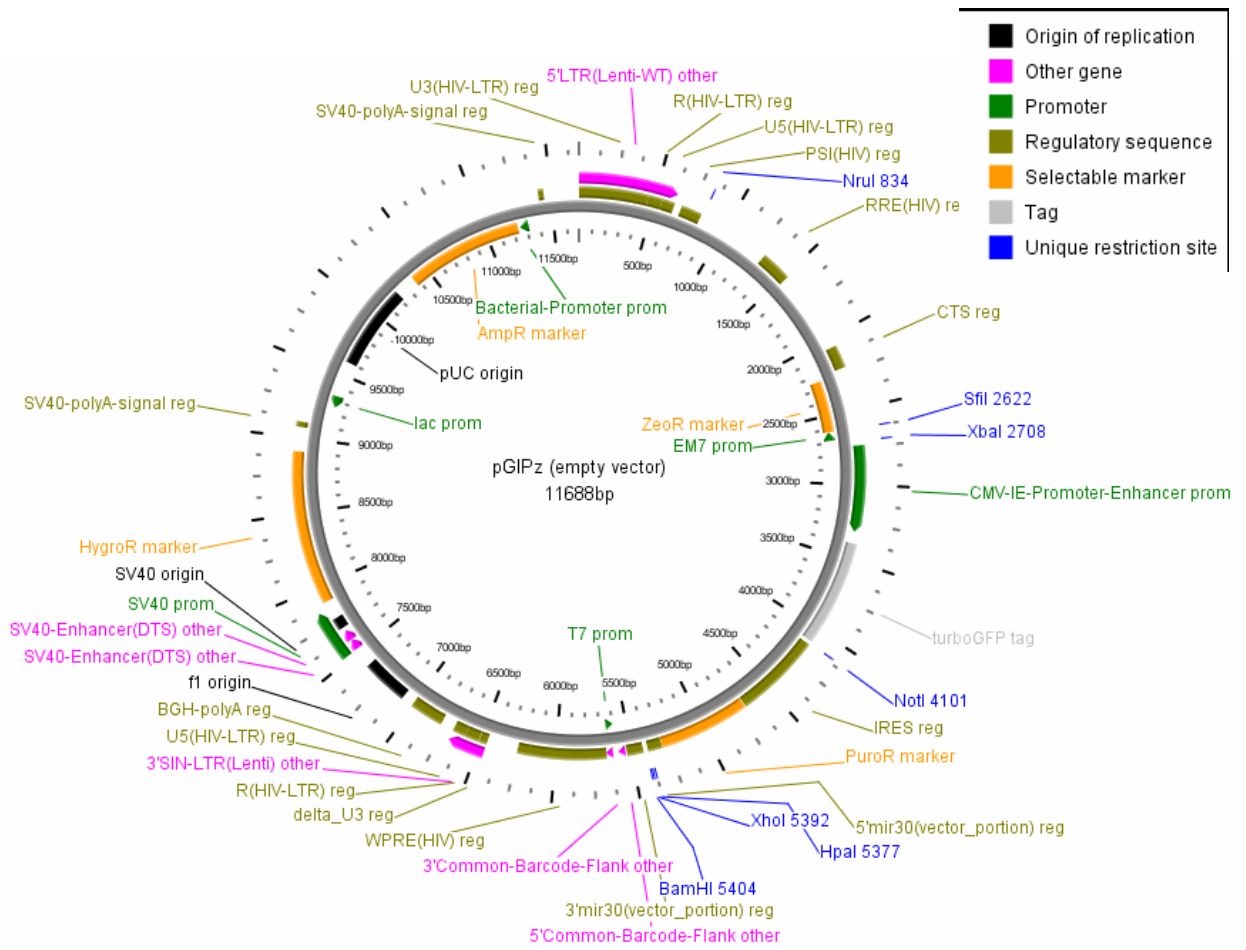


Figure 1: Detailed Vector Map of pGIPZ empty vector

Moving shRNAmir constructs from pSM2 to pGIPZ

- (1) Order the pSM2 vector already expressing the shRNAmir of interest from [Open Biosystems](#).
- (2) Order the following PCR primers:
 - pSM2 forward - 5' aagcccttgtacaccctaagcct 3'
 - pSM2 reverse - 5' actggtgaaactcaccaggatt 3'
- (3) Order a KOD Hotstart Polymerase kit from Novagen (cat no71086-5 for 20U)
- (4) Resuspend the PCR primers at a stock concentration of 50pmol/μl in sterile DEPC water. Dilute the stock 1:10 for a working concentration of 5pmol/μl in sterile DEPC water.
- (5) Set up the following PCR reaction at room temperature. Add the components in the order listed. The following is for one 50ul reaction. To do more reactions simply multiply the master mix components by the desired number of reactions plus 10%. We recommend doing 4 reactions to ensure enough fragment will be available for cloning.

Component	volume in μl
H2O (DEPC)	25
5M Betaine	5
10X PCR buffer for KOD Hotstart Polymerase	5
dNTP's (2mM each)	5
MgSO ₄ 25mM	2
pSM2 Forward Primer (5pmol/μl)	3
pSM2 Reverse Primer (5pmol/μl)	3
KOD Hotstart Polymerase (1U)	1
Total volume	49
Template (1ul of glycerol stock from your pSM2 clone of interest)	1

- (6) Input the following program into your thermocycler:

PCR Program HS KOD		
	Temperature °C	Time
Hot Start Enzyme Activation	94	2min
Melt	94	15sec
Anneal	58	30sec
Extension	72	25sec
Cycles	30	
Expected Product Size	1735bp	

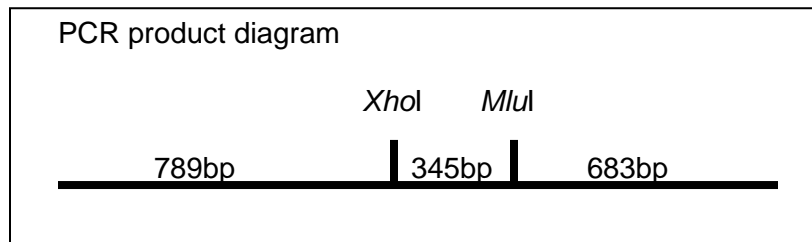
- (7) Put the four PCR reactions through a Wizard SV Gel and PCR Clean-up System column according to the kit directions (Promega cat noA9281 for 50 preps), with the exception of eluting with 110µl of the provided nuclease free water. All four reactions can be run on a single SV Gel and PCR Clean-up column.
- (8) Set up the following restriction digest using the Clean-up column eluant.

Component	volume in µl
PCR elutant	100
10X Restriction Enzyme Buffer	20
<i>Mlu</i> I	2
<i>Xho</i> I	2
Sterile Water	76
Total volume	200

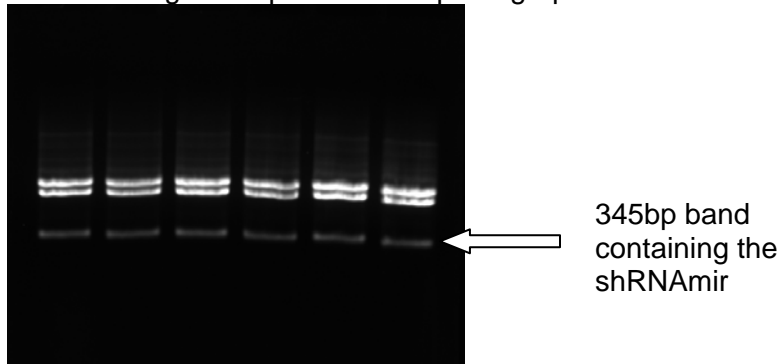
Incubate at 37°C for 3 hours.

- (9) Run the entire digest on a 1.2%-1.5% agarose gel. Three bands should be seen (789bp, 683bp, 345bp). Three bands will appear only if both *Mlu*I and *Xho*I have cut. Therefore the digest is diagnostic of the enzyme cuts in the following fashion.

Band sizes seen	Conclusion
1735bp only	Neither <i>Mlu</i> I or <i>Xho</i> I cut
789bp, 683bp, and 345bp	Both <i>Mlu</i> I and <i>Xho</i> I cut
1028bp and 789bp	Only <i>Xho</i> I cut. <i>Mlu</i> I did not cut.
1134bp and 683bp	Only <i>Mlu</i> I cut. <i>Xho</i> I did not cut.



The following is a representative photograph.



- (10) Excise the 345bp band containing the shRNAmir of interest and purify on a Wizard SV Gel and PCR Clean-up System column according to the kit directions (Promega cat no A9281 for 50 preps). Elute in 50µl nuclease free water.

- (11) Quantitate the insert fragment.
- (12) Prepare the pGIPZ empty vector for ligation to the shRNAmir insert. Set up the following restriction digest.

Component	volume in μl
pGIPZ empty vector (250ng/ μl)	12
10X Restriction Enzyme Buffer	10
<i>Mlu</i> I	2
<i>Xho</i> I	2
Sterile Water	74
Total volume	100

Mix the solution by pipetting and then gently spin the reaction for approximately 10 seconds to collect all the solution in the bottom of the tube. This will aid in decreasing contamination of uncut vector in your vector prep to follow. Incubate at 37°C for 3 hours.

- (13) Run the entire digest on a 0.8% agarose gel. Make sure to run the gel through no less than 3cm length of agarose. This will also aid in decreasing contamination of uncut vector in your vector prep.
- (14) Gel isolate the 13087bp band using a Wizard SV Gel and PCR Clean-up System column according to the kit directions (Promega cat noA9281 for 50 preps). Elute in 50 μl nuclease free water. You will likely not see a band representing the excised portion of the vector as it is too small.
- (15) Quantitate the amount of cut vector per μl you have isolated.
- (16) Set up the following ligation reactions:

Component	No Insert control	shRNAmir ligation
shRNAmir insert cut with <i>Mlu</i> I and <i>Xho</i> I (total 7.4ng)	XXXXXXXXXX	____ μl
pGIPZ vector cut with <i>Mlu</i> I and <i>Xho</i> I (total 250ng)	____ μl	____ μl
DEPC water	____ μl	____ μl
10X ligase buffer	2 μl	2 μl
Ligase	0.5 μl	0.5 μl
Total volume	20 μl	20 μl

This setup yields a molar ratio of 1 vector to 1 insert.

- (17) Ligate for 3 hours at room temperature. Dilute the ligation mix by adding 160 μl DEPC water.
- (18) Transform 5 μl of the diluted ligation mix into Prime+ competent *E. coli* (MBC4246). Follow the transformation protocol for the competent cells. Plate the

transformed cells onto agar plates containing 100µg/ml ampicillin (carbenicillin) and 25µg/ml zeocin. Be sure to transform the same volume of ligation mix and plate the same volume of cells for both the control and the experimental sample. Plating 100µl, 50µl, and 10µl aliquots is recommended.

(19) Incubate plates at 30°C overnight. Count colonies and determine the ratio of colonies on the control plate versus the experimental plates. Determine the number of colonies to screen.

(20) Order the following PCR primers to screen your clones for insertion of the shRNAmir sequence of interest.

X76 Forward - 5' acgtcgaggtgccccgaagga -3'

M100 Reverse - 5' aagcagcgtatccacatagcgt -3'

(21) Set up the following PCR reaction. For template simply pick a colony with a toothpick, swirl in a small broth culture containing ampicillin and zeocin to maintain a stock and then swirl the same toothpick into your PCR well containing the appropriate amount of master mix. For a no insert control simply use 1ng of empty pGIPZ vector. The amounts in the table below are for a single 50µl reaction.

Component	volume in µl
H2O (DEPC)	26
5M Betaine	5
10X PCR buffer for KOD Hotstart Polymerase	5
dNTP's (2mM each)	5
MgSO ₄ 25mM	2
pSM2 Forward Primer (5pmol/µl)	3
pSM2 Reverse Primer (5pmol/µl)	3
KOD Hotstart Polymerase (1U)	1
Total volume	49
Template colony picked with toothpick	---

(22) Run the following PCR program. Note the annealing temperature has changed.

PCR Program HS KOD#2		
	Temperature °C	Time
Hot Start Enzyme Activation	94	2min
Melt	94	15sec
Anneal	56	30sec
Extention	72	25sec
Cycles	30	

Expected band sizes are as follows:

shRNA inserted = 497bp
 No shRNA inserted = 414bp

Sequence verify clones

The recommended sequencing primer is: 5'- acagaatcgttgctgcaca -3'

Sequence of pGIPZ empty vector (11688bp)

```
5'LTR(Lenti-WT) other(1,635)>>>
|
U3(HIV-LTR) reg(1,455)>>>
|
1   tggaaagggctaattcactcccaaagaagacaagatatccttgatctgtggatctaccaca 60
   ACCTTCCCGATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT
61   cacaaggctacttccctgattagcagaactacacaccagggccaggggtcagatatccac 120
   GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCCAGTCTATAGGTG
121  tgacctttggatgggtgctacaagctagtagcagttgagccagataaggtagaagaggcca 180
   ACTGGAAACCTACCACGATGTTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT
181  ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgaccgag 240
   TATTTCTCTCTTGTGGTTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC
241  agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccgag 300
   TCTCTCTTCACAATCTCACCTCCAAACTGTGGCGGATCGTAAAGTAGTGCACCGGGCTC
301  agctgcatccggagtagtacttcaagaactgctgatatcgagcttgctacaagggactttccg 360
   TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC
361  ctggggactttccagggaggcgtggcctgggcgggactggggagtgggcgagccctcagat 420
   GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA
                                     R(HIV-LTR) reg(456,550)>>>
                                     |
421  cctgcatataagcagctgctttttgctgtactgggtctctctggttagaccagatctga 480
   GGACGTATATTCGTTCGACGAAAAACGGACATGACCCAGAGAGACCAATCTGGTCTAGACT
481  gcctgggagctctctgggtaactagggaaaccactgcttaagcctcaataaagcttgct 540
   CGGACCCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTTCGAACGGA
                                     U5(HIV-LTR) reg(551,635)>>>
                                     |
541  tgagtgcttcaagtagtgtgtgcccgtctgttgtgtgactctggtaactagagatccctc 600
   ACTCACGAAGTTCATCACACACGGGCAGACAACACACTGAGACCATTGATCTCTAGGGAG
601  agacccttttagtcagtggtgaaaatctctagcagtgggcggcgaacagggacttgaaag 660
   TCTGGGAAAATCAGTCACACCTTTTTCAGAGATCGTCACCGGGGCTTGTCCCTGAACTTTC
                                     PSI(HIV) reg(685,822)>>>
                                     |
661  cgaaagggaaaccagaggagctctctcgacgcaggactcggcttgctgaagcgcgcacgg 720
   GCTTTCCCTTTGGTCTCCTCGAGAGAGCTGCGTCTGAGCCGAACGACTTCGCGCGTGCC
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721 caagagggcgagggggcgactggtgagtagcgcacaaaatTTTgactagcggaggctaga 780
    GTTCTCCGCTCCCCGCCGCTGACCACTCATGCGTTTTTTAAACTGATCGCCTCCGATCT

                                                                    NruI
                                                                    |
781 aggagagagatgggtgagagagcgtcagtattaagcgggggagaattagatcgcgatggg 840
    TCCTCTCTCTACCCACGCTCTCGCAGTCATAATTGCCCCCTCTTAATCTAGCGCTACCC

841 aaaaaattcgggtaaggccagggggaaagaaaaatataaattaaaacatatagtatggg 900
    TTTTTTAAGCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATAACCC

901 caagcagggagctagaacgattcgcagttaatcctggcctgtagaaacatcagaaggct 960
    GTTCGTCCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTTCCGA

961 gtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagat 1020
    CATCTGTTTTATGACCCTGTGATGTTGGTAGGGAAGTCTGTCCTAGTCTTCTTGAATCTA

1021 cattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagaca 1080
    GTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTTCTGT

1081 ccaaggaagcttagacaagatagaggaagagcaaaacaaaagtaagaccaccgcacagc 1140
    GGTTCCCTTCGAAATCTGTTCTATCTCCTTCTCGTTTTGTTCATTCTGGTGGCGTGTGC

1141 aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag 1200
    TTCGCCGGCCGGCGACTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTAACTCTTC

1201 tgaattatataaatataaagtagtaaaaattgaaccattaggagtagcaccaccaaggc 1260
    ACTTAATATATTTATATTTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTCCG

                                                                    RRE (HIV)
                                                                    |
reg(1314,1518)>>>
1261 aaagagaagagtggtgagagagaaaaaagagcagtggggaataggagctttgttccttgg 1320
    TTTCTCTTCTCACCACGTCTCTTTTTTTCTCGTCACCCTTATCCTCGAAACAAGGAACC

1321 gttcttgggagcagcaggaagcactatgggagcagcgtcaatgacgctgacggtacaggc 1380
    CAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGTTACTGCGACTGCCATGTCCG

1381 cagacaattattgtctggtatagtgagcagcagaacaatttgctgagggctattgaggc 1440
    GTCTGTTAATAACAGACCATATCACGTCGTCGTCCTTGTTAAACGACTCCCGATAACTCCG

1441 gcaacagcatctgttgcaactcacagctctggggcatcaagcagctccaggcaagaatcct 1500
    CGTTGTGCTAGACAACGTTGAGTGTGACACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGA

1501 ggctgtggaaagatacctaaggatcaacagctcctggggatttgggggtgctctggaaa 1560
    CCGACACCTTTCTATGGATTTCTAGTTGTGAGGACCCCTAAACCCCAACGAGACCTTT

1561 actcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctggaaca 1620
    TGAGTAAACGTGGTGACGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGT

1621 gatttgggaatcacacgacctggatggagtgggacagagaaattaacaattacacaagctt 1680
    CTAACCTTAGTGTGCTGGACCTACCTCACCTGTCTCTTTAATTGTTAATGTGTTCCGAA

1681 aatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattatt 1740
    TTATGTGAGGAATTAACCTTCTTAGCGTTTTGGTCGTTCTTTTCTTACTTGTTCTTAATAA

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1741 ggaattagataaatgggcaagtttgtggaattggtttaacataacaaattggctgtggta 1800
    CCTTAATCTATTTACCCGTTCAAACACCTTAACCAAATTGTATTGTTTAACCGACACCAT

1801 tataaaattattcataatgatagtaggaggcttggtaggtttaagaatagtttttgctgt 1860
    ATATTTTAATAAGTATTACTATCATCCTCCGAACCATCCAAATTCTTATCAAAAACGACA

1861 acttttctatagtgaatagagtttaggcagggatattcaccattatcgtttcagaccacct 1920
    TGAAAGATATCACTTATCTCAATCCGTCCTATAAGTGGTAATAGCAAAGTCTGGGTGGA

1921 cccaacccccgaggggacccgcagaggcccgaaggaatagaagaagaaggtggagagagaga 1980
    GGGTTGGGGCTCCCCTGGGCTGTCCGGGCTTCCTTATCTTCTTCTTCCACCTCTCTCTCT

1981 cagagacagatccattcgattagtgaaacggatcggcactgctgcgccaattctgcagac 2040
    GTCTCTGTCTAGGTAAGCTAATCACTTGCCTAGCCGTGACGCACGCGTTAAGACGTCTG

                                CTS reg(2064,2214)>>>
                                |
2041 aaatggcagttatccacaaattttaaagaaaaggggggattggggggtacagtgcag 2100
    TTTACCGTCATAAGTAGGTGTTAAAATTTTCTTTTCCCCCTAACCCCCATGTCACGTC

2101 gggaaagaatagtagacataatagcaacagacatacaaaactaaagaattacaaaaacaaa 2160
    CCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTAATGTTTTTGT

2161 ttacaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220
    AATGTTTTTAAGTTTTAAAAGCCAAATAATGTCCCTGTCGTCTCTAGGTCAAACCAATC

                                ZeoR marker(2245,2619)<<<
                                |
2221 taccgggcccgtctagtcggaatcagtcctgctcctcggccacgaagtgcacgcagtt 2280
    ATGGCCCGGGCAGATCAGGCCTTAGTCAGGACGAGGAGCCGGTGCTTCACGTGCGTCAA

2281 gccggccgggtcgcgagggcgaactcccggccacggctgctcgccgatctcggtcat 2340
    CGGCCGGCCAGCGCGTCCCGCTTGAGGGCGGGGTGCCGACGAGCGGCTAGAGCCAGTA

2341 ggccggccgggagggcgtcccggaagttcgtggacacgacctccgaccactcggcgtacag 2400
    CCGGCCGGCCCTCCGACGGCCTTCAAGCACCTGTGCTGGAGGCTGGTGAGCCGCATGTC

2401 ctcgtccaggccgcgacccacacccaggccaggggtgtgtccggcaccacctggtcctg 2460
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2461 gaccgcgctgatgaacagggtcacgtcgtcccggaccacaccggcgaagtcgtcctccac 2520
    CTGGCGGACTACTTGTCCCAGTGCAGCAGGGCCTGGTGTGGCCGCTTACAGCAGGAGGTG

2521 gaagtcccgggagaacccgagccggtcgggtccagaactcgaccgctccggcgacgtcgcg 2580
    CTTACAGGCCCTCTTGGGCTCGGCCAGCCAGGTCTTGAGCTGGCGAGGCCGCTGCAGCGC

                                SfiI
                                |
                                EM7 prom(2620,2683)<<<
                                | |
2581 cgcggtgagcaccggaacggcactggtcaacttggccatggtggccctcctatagtgagt 2640
    GCGCCACTCGTGGCCTTGCCGTGACCAGTTGAACCGGTACCACCGGGAGGATATCACTCA

2641 cgtattatactatgccgatatactatgccgatgattaattgtcaacacgtgctgcaggtc 2700
    GCATAATATGATACGGCTATATGATACGGCTACTAATTAACAGTTGTGCACGACGTCCAG

```

XbaI CMV-IE-Promoter-Enhancer

prom(2738,3311)>>> | |

2701 cgaggttctagacgtattaccgccatgcattagttattaatagtaatcaattacggggtc 2760
GCTCCAAGATCTGCATAATGGCGGTACGTAATCAATAATTATCATTAGTTAATGCCCCAG

2761 attagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcc 2820
TAATCAAGTATCGGGTATATACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGG

2821 tggctgaccgcccacgacccccgccattgacgtcaataatgacgtatgttcccatagt 2880
ACCGACTGGCGGGTTGCTGGGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATCA

2881 aacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaaactgccca 2940
TTGCGGTTATCCCTGAAAGGTAAGTGCAGTTACCCACCTCATAAATGCCATTTGACGGGT

2941 cttggcagtacatcaagtgtatcatatgcccaagtacgccccctattgacgtcaatgacgg 3000
GAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGGGGGATAACTGCAGTTACTGCC

3001 taaatggccccgcctggcattatgccagtacatgaccttatgggactttcctacttggca 3060
ATTTACCGGGCGGACCGTAATACGGGTTCATGTACTGGAATACCCTGAAAGGATGAACCGT

3061 gtacatctacgtattagtcacgctattaccatgggtgatgcggttttggcagtacatcaa 3120
CATGTAGATGCATAATCAGTAGCGATAATGGTACCCTACGCCAAAACCGTCATGTAGTT

3121 tgggctggatagcgggttgactcacggggatttccaagtctccaccccattgacgtcaa 3180
ACCCGCACCTATCGCCAAACTGAGTGCCCTAAAGGTTTCAGAGGTGGGGTAACTGCAGTT

3181 tgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtacaactccgc 3240
ACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTGTTGAGGCG

3241 ccattgacgcaaatgggcggtaggcgtgtacgggtgggaggtctatataagcagagctcg 3300
GGGTAAGTGCCTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTTCGTCTCGAGC

3301 tttagtgaaaccgtcagatcgccctggagacgccatccacgctgttttgacctccatagaag 3360
AAATCACTTGGCAGTCTAGCGGACCTCTGCGGTAGGTGCGACAAAACCTGGAGGTATCTTC

turboGFP tag(3390,4088)>>>
|

3361 acaccgactctactagaggatctgccaccatggagagcagcagagcggcctgcccgccca 3420
TGTGGCTGAGATGATCTCCTAGACGGTGGTACCTCTCGCTGCTCTCGCCGGACGGGCGGT

3421 tggagatcgagtgccgcatcaccggcaccctgaacggcgtggagttcgagctggtgggcg 3480
ACCTCTAGCTCACGGCGTAGTGGCCGTGGGACTTGCCGCACCTCAAGCTCGACCACCCGC

3481 gcggagagggcacccccagcagggccgcatgaccaacaagatgaagagcaccaaagggcg 3540
CGCCTCTCCCGTGGGGGCTCGTCCCGCGTACTGGTTGTTCTACTTCTCGTGGTTTTCCGC

3541 ccctgaccttcagcccctacctgctgagccacgtgatgggctacggcttctaccacttcg 3600
GGGACTGGAAGTGGGGATGGACGACTCGGTGCACTACCCGATGCCGAAGATGGTGAAGC

3601 gcacctaccccagcggctacgagaacccttctgacgccatcaacaacggcggttaca 3660
CGTGGATGGGGTCCCGATGCTCTTGGGGAAGGACGTGCGGTAGTTGTTGCCGCCGATGT

3661 ccaacaccgcatcgagaagtagcaggacggcggtgctgacgtgagcttcagctacc 3720
GGTTGTGGGCGTAGCTCTTCATGCTCCTGCCGCCGACGACGTGCACTCGAAGTCGATGG


```

4741  ccccgggccgtacgcaccctcgccgcccgcgttcgccgactaccccgccacgcgccacacc 4800
      GGGGCCCGGCATGCGTGGGAGCGGGCGGCAAGCGGCTGATGGGGCGGTGCGCGGTGTGG

4801  gtcgaccgaccgaccacatcgagcgggtcaccgagctgcaagaactcttcctcacgcgc 4860
      CAGCTGGGCCTGGCGGTGTAGCTCGCCAGTGGCTCGACGTTCTTGAGAAGGAGTGCAGCG

4861  gtcgggctcgacatcggcaaggtgtgggtcgcggaacgacggcgccgcgggtggcggtctgg 4920
      CAGCCCGAGCTGTAGCCGTTCCACACCCAGCGCCTGCTGCCGCGGCGCCACCGCCAGACC

4921  accacgcccggagagcgtcgaagcggggggcggtgttcgccgagatcggctcgcgcatggcc 4980
      TGGTGCGGCCTCTCGCAGCTTCGCCCCCGCCACAAGCGGCTCTAGCCGAGCGCGTACCGG

4981  gagttgagcggttcccggctggccgcgagcaacagatggaaggcctcctggcgccgcac 5040
      CTCAACTCGCAAGGGCCGACCGGCGCGTTCGTTGTCTACCTTCCGGAGGACCGCGGCGTG

5041  cggcccaaggagcccgcgtggttcctggccaccgctcggcgtctcgcccgaccaccagggc 5100
      GCCGGGTTTCTCGGGCGCACCAAGGACCGGTGGCAGCCGAGAGCGGGCTGGTGGTCCCCG

5101  aagggtctgggagcgcgcgtcgtgctccccggagtgaggcgccgagcgcgctgggggtg 5160
      TTCCAGACCCGTCGCGGCAGCACGAGGGGCTCACCTCCGCCGGCTCGCGGACCCAC

5161  cccgccttcctggagacctccgcgccccgcaacctccccttctacgagcggctcggcttc 5220
      GGGCGGAAGGACCTCTGGAGGCGCGGGGCGTTGGAGGGGAAGATGCTCGCCGAGCCGAAG

5221  accgtcaccgcccagcgtcgaggtgcccgaaggaccgcgcacctgggtgcatgaccgcaag 5280
      TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCTGGCGCGTGGACCACGTACTGGGCGTTC

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

      HpaI XhoI
      | |
5341  tcttgaaacacttgctgggattacttcttcagggttaaccaacagaaggctcgaggtaa 5400
      AGAACCTTTGTGAACGACCCTAATGAAGAAGTCCAATTGGGTTGTCTTCCGAGCTCCATT

      BamHI 3'mir30(vector_portion) reg(5421,5528)>>>
      | |
5401  ccggatcctgatcagaattcaaggggctactttaggagcaattatcttgtttactaaaac 5460
      GGCCTAGGACTAGTCTTAAGTTCCCCGATGAAATCCTCGTTAATAGAACAAATGATTTTG

5461  tgaataccttgctatctcttttgatacatttttacaaagctgaattaaaatggtataaatt 5520
      ACTTATGGAACGATAGAGAACTATGTAAAAATGTTTCGACTTAATTTTACCATATTTAA

      5'Common-Barcode-Flank other(5540,5560)>>>
      |
5521  aaatcacttttttcaattggaagactaatgcccggccattactccgtctcgtgcttg 5580
      TTTAGTGAAAAAAGTTAACCTTCTGATTACGCCGGCCGTAATGAGGCAGAGCACAGAAC

      T7 prom(5624,5643)<<<
      |
      3'Common-Barcode-Flank
other(5621,5643)>>>
      | |
5581  ttgcatatgtctgctggtttggttgatggtggttgccgggcccctatagtgagtcgta 5640

```

```

AACGTATACAGACGACCAAACAAACTACAACAAACGCCCGCCCGGGATATCACTCAGCAT
                                WPRE(HIV) reg(5663,6251)>>>
                                |
5641 ttacctaggacgcgtctggaacaatcaacctctggattacaaaatttgtgaaagattgac 5700
    AATGGATCCTGCGCAGACCTTGTTAGTTGGAGACCTAATGTTTTAAACACTTTTCTAACTG

5701 tggattcttaactatgttgctccttttacgctatgtggatacgtgctttaatgccttt 5760
    ACCATAAGAATTGATACAACGAGGAAAATGCGATACACCTATGCGACGAAATTACGGAAA

5761 gtatcatgctattgcttcccgtatggctttcattttctcctccttgataaatcctgggt 5820
    CATAGTACGATAACGAAGGGCATACCGAAAAGTAAAAGAGGAGGAACATATTTAGGACCAA

5821 gctgtctctttatgaggagtgtggtggccgtgtcaggcaacgtggcgtgggtgtgcactgt 5880
    CGACAGAGAAATACTCCTCAACACCGGGCAACAGTCCGTTGCACCGCACACACGTGACA

5881 gtttgctgacgcaacccccactgggtggggcattgccaccacctgtcagctcctttccgg 5940
    CAAACGACTGCGTTGGGGGTGACCAACCCCGTAACGGTGGTGGACAGTCGAGGAAAGGCC

5941 gactttcgctttccccctcctattgccacggcggaactcatcgccgcctgccttgcccg 6000
    CTGAAAGCGAAAGGGGGAGGGATAACGGTGCCGCCTTGAGTAGCGGCGGACGGAACGGGC

6001 ctgctggacaggggctcggctggtgggactgacaattccgtgggtgtgtcggggaagct 6060
    GACGACCTGTCCCCGAGCCGACAACCCGTGACTGTTAAGGCACCACAACAGCCCCCTTCGA

6061 gacgtcctttccatggctgctcgctgtgttgccacctggattctgcgcgggacgtcctt 6120
    CTGCAGGAAAGGTACCGACGAGCGGACACAACGGTGGACCTAAGACGCGCCCTGCAGGAA

6121 ctgctacgtcccttcggccctcaatccagcggaccttccttcccgcggcctgctgcccgc 6180
    GACGATGCAGGGAAGCCGGGAGTTAGGTGCGCCTGGAAGGAAGGGCGCCGGACGACGGCCG

6181 tctgcccctcctccgcgtcttcgccttcgcctcagacgagtcggatctccctttggggc 6240
    AGACGCCGGAGAAGGCGCAGAAGCGGAAGCGGGAGTCTGCTCAGCCTAGAGGGAAACCCG

6241 cgcctccccgcctggaattaattctgcagtcgagacctagaaaaacatggagcaatcaca 6300
    GCGGAGGGGCGGACCTTAATTAAGACGTCAGCTCTGGATCTTTTTGTACCTCGTTAGTGT

6301 agtagcaatacagcagctaccaatgctgattgtgcctggctagaagcacaagaggaggag 6360
    TCATCGTTATGTGTCGATGGTTACGACTAACACGGACCGATCTTCGTGTTCTCCTCCTC

6361 gaggtggggtttccagtcacacctcaggtaccttaagaccaatgacttacaaggcagct 6420
    CTCCACCCAAAAGGTGAGTGTGGAGTCCATGGAAATTCTGGTACTGAATGTTCCGTCGA

                                delta_U3 reg(6458,6510)>>>
                                |
                                3' SIN-LTR(Lenti)
other(6458,6693)>>>
                                |
6421 gtagatcttagccactttttaaaagaaaagaggggactggaagggctaattcactcccaa 6480
    CATCTAGAATCGGTGAAAATTTTCTTTTCTCCCCTGACCTTCCCGATTAAGTGAGGGTT

                                R(HIV-LTR) reg(6513,6607)>>>
                                |
6481 cgaagacaagatctgctttttgcttgactgggtctctctggttagaccagatctgagcc 6540
    GCTTCTGTTCTAGACGAAAAACGAACATGACCCAGAGAGACCAATCTGGTCTAGACTCGG

```

6541 tgggagctctctggctaactaggggaacccactgcttaagcctcaataaagcttgcccttga 6600
ACCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTTCGAACGGAAC

U5(HIV-LTR) reg(6608,6692)>>>

6601 gtgcttcaagtagtggtgccccgtctggttggtgactctggtaactagagatccctcaga 6660
CACGAAGTTCATCACACACGGGCAGACAACACACTGAGACCATTGATCTCTAGGGAGTCT

6661 cccttttagtcagtggtgaaaatctctagcagtagtagttcatgtcatcttattattcag 6720
GGGAAAATCAGTCACACCTTTTAGAGATCGTCATCATCAAGTACAGTAGAATAATAAGTC

6721 tatttataaacttgcaagaaatgaatatcagagagtgagaggccttgacattgtttaaac 6780
ATAAATATTGAACGTTTTCTTTACTTATAGTCTCTCACTCTCCGGAACGTAAACAAATTTG

BGH-polyA reg(6782,7005)>>>

6781 ccgctgatcagcctcgactgtgaccttctagttgccagccatctggtggttggccctcccc 6840
GGCGACTAGTCGGAGCTGACACGGAAGATCAACGGTCCGGTAGACAACAAACGGGGAGGGG

6841 cgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgagga 6900
GCACGGAAGGAACTGGGACCTTCCACGGTGAGGGTGACAGGAAAGGATTATTTTACTCTCT

6901 aattgcatcgcattgtctgagtaggtgtcattctattctgggggggtgggggtggggcgagga 6960
TTAACGTAGCGTAACAGACTCATCCACAGTAAGATAAGACCCCCACCCACCCCGTCTCT

6961 cagcaagggggaggattgggaagacaatagcaggcatgctggggatgctgggtgggctctat 7020
GTCGTTCCCCCTCCTAACCTTCTGTTATCGTCCGTACGACCCCTACGCCACCCGAGATA

7021 ggcttctgagggcggaaagaaccagctggggctctaggggggatccccacgcccctgtag 7080
CCGAAGACTCCGCCTTTCTTGGTCGACCCCGAGATCCCCATAGGGGTGCGCGGGACATC

f1 origin(7085,7391)>>>

7081 cggcgccattaagcgcggcggggtggtggttacgcgcagcgtgaccgctacacttgccag 7140
GCCGCGTAATTCGCGCCGCCACACCACCAATGCGCGTCGCACTGGCGATGTGAACGGTC

7141 cgccctagcgcggcctcctttcgctttcttcccttctcttctcgccacgcttcgcccggctt 7200
GCGGGATCGCGGGCGAGGAAAGCGAAAGAAGGGAAGGAAAGAGCGGTGCAAGCGGCCGAA

7201 tccccgtcaagctctaaatcgggggctccctttaggggtccgatttagtgctttacggca 7260
AGGGGCAGTTCGAGATTTAGCCCCCGAGGGAAATCCCAAGGCTAAATCACGAAATGCCGT

7261 cctcgacccccaaaaaacttgattaggggtgatgggttcacgtagtgggccatcgccctgata 7320
GGAGCTGGGGTTTTTTTGAACATAATCCCACTACCAAGTGCATCACCCGGTAGCGGGACTAT

7321 gacgggtttttcgccctttgacggttgaggtccacggttctttaaagtggactcttgttcca 7380
CTGCCAAAAAGCGGGAAACTGCAACCTCAGGTGCAAGAAATTATCACCTGAGAACAAGGT

7381 aactggaacaacactcaaccctatctcgggtctattcttttgatttataagggattttgcc 7440
TTGACCTTGTTGTGAGTTGGGATAGAGCCAGATAAGAAAATAAATATTCCCTAAAACGG

7441 gatttcggcctattgggttaaaaaatgagctgatttaacaaaaatttaacgcgaattaatt 7500
CTAAAGCCGGATAACCAATTTTTTACTCGACTAAATTGTTTTTAAATTGCGCTTAATTA

SV40 prom(7523,7844)>>>

SV40-Enhancer (DTS) other (7523, 7594) >>>

|

7501 ctgtggaatgtgtgtcagttaggggtgtggaaagtccccagggtccccagcagggcagaagt 7560
GACACCTTACACACAGTCAATCCCACACCTTTTCAGGGGTCCGAGGGGTTCGTCCGTCTTCA

SV40-Enhancer (DTS)

other (7595, 7666) >>>

|

7561 atgcaaagcatgcatctcaattagtcagcaaccagggtgtggaaagtccccagggtcccca 7620
TACGTTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTTCAGGGGTCCGAGGGGT

7621 gcaggcagaagtatgcaaagcatgcatctcaattagtcagcaaccatagtcgccccccta 7680
CGTCCGTCTTCATACGTTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGAT

SV40 origin (7690, 7767) >>>

|

7681 actccgcccattcccccccctaactccgcccagttccgcccattctccgcccattggctga 7740
TGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACT

7741 ctaatTTTTTTTTatTTatgCagagggccgagggccgcctctgcctctgagctattccagaag 7800
GATTAATAAATAAATACGTCTCCGGCTCCGGCGGAGACGGAGACTCGATAAGGTCTTC

7801 tagtgaggaggctTTTTTTggaggcctaggctTTTTgcaaaaagctcccgggagcttgata 7860
ATCACTCCTCCGAAAAACCTCCGGATCCGAAAACGTTTTTCGAGGGCCCTCGAACATAT

HygroR marker (7893, 8910) >>>

|

7861 tccattttcggatctgatcagcacgtgatgaaaaagcctgaactcaccgcgacgtctgtc 7920
AGGTAAAAGCCTAGACTAGTCGTGCACTACTTTTTTCGGACTTGAGTGGCGCTGCAGACAG

7921 gagaagtttctgatcgaaaagttcgacagcgtctccgacctgatgcagctctcggagggc 7980
CTCTTCAAAGACTAGCTTTTTCAAGCTGTGCGAGAGGCTGGACTACGTGAGAGCCTCCCG

7981 gaagaatctcgtgctttcagcttcgatgtaggagggcgtggatatgtcctgcgggtaaat 8040
CTTCTTAGAGCACGAAAGTCAAGCTACATCCTCCCGCACCTATACAGGACGCCATTTA

8041 agctgcgcccgatggtttctacaaagatcgttatgtttatcggcactttgcatcggccgcg 8100
TCGACGCGGCTACCAAAGATGTTTCTAGCAATACAAATAGCCGTGAAACGTAGCCGGCGC

8101 ctcccgatccggaagtgccttgacattggggaattcagcgagagcctgacctattgcatc 8160
GAGGGCTAAGGCCTTACGAACTGTAACCCCTTAAGTCGCTCTCGGACTGGATAACGTAG

8161 tcccgcgctgcacaggggtgtcacggtgcaagacctgcctgaaaccgaactgcccgtggt 8220
AGGGCGGCACGTGTCCCACAGTGCAACGTTCTGGACGGACTTTGGCTTGACGGGCGACAA

8221 ctgcagccggtcgccgaggccatggatgcatcgctgcggccgatcttagccagacgagc 8280
GACGTCCGCCAGCGCCTCCGGTACCTACGCTAGCGACGCCGGCTAGAATCGGTCTGCTCG

8281 ggggtccgcccattcggaccgcaaggaatcgggtcaatacactacatggcgtgatttcata 8340
CCCAAGCCGGGTAAGCCTGGCGTTCCTTAGCCAGTTATGTGATGTACCGCACTAAAGTAT

8341 tgcgcgattgctgatccccatgtgtatcactggcaaacctgtgatggacgacaccgtcagt 8400
ACGCGCTAACGACTAGGGGTACACATAGTGACCGTTTTGACACTACCTGCTGTGGCAGTCA

8401 gcgtccgtcgcgcaggctctcgatgagctgatgctttgggcccaggactgccccgaagtc 8460
CGCAGGCAGCGCTCCGAGAGCTACTCGACTACGAAACCCGGCTCCTGACGGGGCTTCAG

8461 cggcacctcgtgcacgcggatttcggctccaacaatgtcctgacggacaatggccgcata 8520
GCCGTGGAGCACGTGCGCCTAAAGCCGAGGTTGTTACAGGACTGCCTGTTACCGGCGTAT

8521 acagcgggtcattgactggagcggagcgatggttcggggattcccaatacagaggtcgccaac 8580
TGTCGCCAGTAACTGACCTCGCTCCGCTACAAGCCCCTAAGGGTTATGCTCCAGCGGTTG

8581 atcttcttctggaggccgtgggttgcttgatggagcagcagacgcgctacttcgagcgg 8640
TAGAAGAAGACCTCCGGCACCAACCGAACATACTCGTCGTCTGCGCGATGAAGCTCGCC

8641 aggcattccggagcttgaggatcgccgcggctccgggctatatgctccgcattggtcct 8700
TCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGGCCGCATATACGAGGCGTAACCAGAA

8701 gaccaactctatcagagcttggttgacggcaatttcgatgatgcagcttgggagcaggggt 8760
CTGGTTGAGATAGTCTCGAACCAACTGCCGTTAAAGCTACTACGTCGAACCCGCGTCCCA

8761 cgatgcgacgcaatcgtccgatccggagccgggactgtcgggctacacaaatcgcccgc 8820
GCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCTGACAGCCCGCATGTGTTTAGCGGGCG

8821 agaagcgcggccgtctggaccgatggctgtgtagaagtactcgccgatagtggaaaccga 8880
TCTTCGCGCCGGCAGACCTGGCTACCGACACATCTTCATGAGCGGCTATCACCTTTGGCT

8881 cgccccagcactcgtccgagggcaaaggaatagcacgtgctacgagatctcgattccacc 8940
GCGGGGTCGTGAGCAGGCTCCCGTTTCCTTATCGTGCACGATGCTCTAAAGCTAAGGTGG

8941 gccgccttctatgaaaggttgggcttcggaatcgttttcgggagcgcgggtggatgatc 9000
CGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAG

9001 ctccagcgcggggatctcatgctggagttcttcgcccaccccaacttgtttattgcagct 9060
GAGGTGCGCCCCCTAGAGTACGACCTCAAGAAGCGGGTGGGGTTGAACAAATAACGTCGA

SV40-polyA-signal reg(9074,9108)>>>

9061 tataatggttacaaataaagcaatagcatcacaaatctcacaaataaagcatttttttca 9120
ATATTACCAATGTTTATTTTCGTTATCGTAGTGTAAAGTGTATTTTCGTAAAAAAGT

9121 ctgcattctagttgtggtttgtccaaactcatcaatgtatcttatcatgtctgtataccg 9180
GACGTAAGATCAACACCAAACAGGTTTGGAGTAGTTACATAGAATAGTACAGACATATGGC

9181 tcgacctctagctagagcttggcgtaatcatgggtcatagctgtttcctgtgtgaaattgt 9240
AGCTGGAGATCGATCTCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACA

lac prom(9256,9339)<<<

9241 tatccgctcacaattccacacaacatacagagccggaagcataaagtgtaaagcctgggggt 9300
ATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGCCCTTCGTATTTACATTTTCGGACCCCA

9301 gcctaattgagtgagctaactcacattaattgcggttgcgctcactgcccgctttccagtcg 9360
CGGATTACTCACTCGATTGAGTGTAATTAACGCAACGCGAGTGACGGGCGAAAGGTCAGC

9361 ggaaacctgtcgtgccagctgcattaatgaatcggcccaacgcgcggggagagggcggtttg 9420
CCTTTGGACAGCACGGTCGACGTAATTACTTAGCCGGTTGCGCGCCCCTCTCCGCCAAAC

9421 cgtattggggcgtcttccgcttccctcgctcactgactcgctgcgctcgggtcggttcggctg 9480
GCATAACCCGCGAGAAGGCGAAGGAGCGAGTGACTGAGCGACGCGAGCCAGCAAGCCGAC

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9481  cggcgagcggtatcagctcactcaaagggcggaatacggttatccacagaatcaggggat 9540
      GCCGCTCGCCATAGTCGAGTGAGTTTTCCGCCATTATGCCAATAGGTGTCTTAGTCCCCTA

9541  aacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggcc 9600
      TTGCGTCCTTTCTTGTACTCGTTTTCCGGTCGTTTTCCGGTCCTTGGCATTMTTCCGG

      pUC origin(9600,10219)<<<
      |
9601  gcggttgctggcggtttttccataggctccgccccctgacgagcatcacaaaaatcgacgc 9660
      CGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAGCTGCG

9661  tcaagtcagaggtggcgaaacccgacaggactataaagataaccaggcgtttccccctgga 9720
      AGTTCAGTCTCCACCGCTTTGGGCTGTCTGATATTTCTATGGTCCGCAAAGGGGGACCT

9721  agctccctcgtgcgctctcctggttccgaccctgccgcttaccggatacctgtccgccttt 9780
      TCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGCGGAAA

9781  ctcccttcgggaagcgtggcgcttttctcatagctcacgctgtaggtatctcagttcggtg 9840
      GAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTGCGACATCCATAGAGTCAAGCCAC

9841  taggtcgttcgctccaagctgggctgtgtgcacgaacccccggttcagcccgaccgctgc 9900
      ATCCAGCAAGCGAGGTTTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACG

9901  gccttatccggtaactatcgtccttgagtccaacccggtaagacacgacttatcgccactg 9960
      CGGAATAGGCCATTGATAGCAGAACTCAGGTTGGGCCATTCTGTGCTGAATAGCGGTGAC

9961  gcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttc
10020  CGTCGTGGTGACCATTGTCTAATCGTCTCGCTCCATACATCCGCCACGATGTCTCAAG

10021  ttgaagtgggcttaactacggctacactagaagaacagtatctgggtatctgcgctctg
10080  AACTTCACCACCGGATTGATGCCGATGTGATCTTCTTGTGCATAAACCATAGACGCGAGAC

10081  ctgaagccagttaccttcggaaaaagagttggtagctccttgatccggcaaaacaaaccacc
10140  GACTTCGGTCAATGGAAGCCTTTTTCTCAACCATCGAGAACTAGGCCGTTTTGTTTGGTGG

10141  gctggtagcgggtgggttttttggtttgcaagcagcagattacgcgcagaaaaaaaggatct
10200  CGACCATCGCCACCAAAAAACAAACGTTTCGTGCTAATGCGCGTCTTTTTTTTCTCTAGA

10201  caagaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgt
10260  GTTCTTCTAGGAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGGAGTGCA

10261  taagggatcttgggtcatgagattatcaaaaaggatcttcacctagatccttttaaattaa
10320  ATTCCCTAAAACCAGTACTCTAATAGTTTTTCTCTAGAAGTGGATCTAGGAAAATTTAATT

                                                                 AmpR
marker(10374,11234)<<<
      |
10321  aaatgaagtttttaaatcaatctaaagtatatatgagtaaacttgggtctgacagttaccaa
10380  TTTACTTCAAATTTAGTTAGATTTTCATATATACTCATTGTAACCAGACTGTCAATGGTT

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10381 tgcttaatcagtgaggcacctatctcagcgatctgtctatcttgcgttcatccatagttgcc
10440
ACGAATTAGTCACTCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTATCAACGG
10441 tgactccccgctcgtgtagataactacgatacgggagggcttaccatctggccccagtgct
10500
ACTGAGGGGCAGCACATCTATTGATGCTATGCCCTCCCGAATGGTAGACCGGGGTCACGA
10501 gcaatgataccgcgagacccacgctcaccggctccagatttatcagcaataaaccagcca
10560
CGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAGGTCTAAATAGTCGTTATTTGGTCGGT
10561 gccggaagggccgagcgcagaagtggctcctgcaactttatccgcctccatccagtctatt
10620
CGGCCTTCCCGGCTCGCGTCTTACCAGGACGTTGAAATAGGCGGAGGTAGGTCAGATAA
10621 aattggttgcgggaagctagagtaagtagttcgccagttaatagtttgcgcaacggttgtt
10680
TTAACAAACGGCCCTTCGATCTCATTTCATCAAGCGGTCAATTATCAAACGCGTTGCAACAA
10681 gccattgctacaggcatcgtgggtgtcacgctcgtcgtttggatggcttcattcagctcc
10740
CGGTAACGATGTCCGTAGCACCACAGTGCAGCAGCAAACCATAACCGAAGTAAGTCGAGG
10741 ggttcccaacgatcaaggcgagttacatgatccccatggttgcaaaaaagcggtagc
10800
CCAAGGGTTGCTAGTTCGCTCAATGTACTAGGGGGTACAACACGTTTTTTTCGCCAATCG
10801 tccttcggctcctccgatcgttgtcagaagtaagttggccgcagtggtatcactcatggtt
10860
AGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCAACCGGCGTCACAATAGTGAGTACCAA
10861 atggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgact
10920
TACCGTCGTGACGTATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAGACACTGA
10921 ggtgagtactcaaccaagtcatctctgagaatagtgtatgcggcgaccgagttgctcttgc
10980
CCACTCATGAGTTGGTTCAGTAAGACTCTTATCACATACGCCGCTGGCTCAACGAGAACG
10981 ccggcgtcaatacgggataataccgcgccacatagcagaactttaaagtgctcatcatt
11040
GGCCGCAGTTATGCCCTATTATGGCGCGGTGTATCGTCTTGAAATTTTCACGAGTAGTAA
11041 ggaaaacggttcttcggggcgaaaactctcaaggatcttaccgctggtgagatccagttcg
11100
CCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCTAGAATGGCGACAACCTCTAGGTCAAGC
11101 atgtaaccactcgtgcaccaactgatcttcagcatcttttactttcaccagcgtttct
11160
TACATTGGGTGAGCACGTGGGTTGACTAGAAGTCGTAGAAAATGAAAGTGGTCGCAAAGA
11161 gggtgagcaaaaacaggaaggcaaaaatgccgcaaaaaaggaataagggcgacacggaaa
11220
CCCACTCGTTTTTTGTCCTTCCGTTTTACGGCGTTTTTTCCCTTATTCCCGCTGTGCCTTT

Bacterial-

Promoter prom(11276,11314)<<<

11221 |
11280 tgttgaatactcatactcttcctttttcaatattattgaagcatttatcaggggttattgt
ACAACCTTATGAGTATGAGAAGGAAAAAGTTATAATAACTTCGTAAATAGTCCCAATAACA
11281 ctcatgagcgggatacatatattgaatgtatntagaaaaataaacaataggggttccgcgc
11340 GAGTACTCGCCTATGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCCAAGGCGCG
11341 acatttccccgaaaagtgccacctgacgtcgacggatcgggagatcaacttgtttattgc
11400 TGTAAGGGGCTTTTTCACGGTGGACTGCAGCTGCCTAGCCCTCTAGTTGAACAAATAACG

SV40-polyA-signal reg(11418,11452)>>>

11401 |
11460 agcttataatgggttacaaataaagcaatagcatcacaatttcacaaataaagcattttt
TCGAATATTACCAATGTTTATTTTCGTTATCGTAGTGTTTAAAGTGTTTATTTTCGTAAAAA
11461 ttcactgcattctagttgtgggtttgtccaaactcatcaatgtatcttatcatgtctggat
11520 AAGTGACGTAAGATCAACACCAAACAGGTTTGGAGTAGTTACATAGAATAGTACAGACCTA
11521 caactggataactcaagctaacccaaatcatcccaaacttcccacccataccctattac
11580 GTTGACCTATTGAGTTCGATTGGTTTTAGTAGGGTTTGAAGGGTGGGGTATGGGATAATG
11581 cactgcccaattacctgtgggtttcatttactctaaacctgtgattcctctgaattattttc
11640 GTGACGGTTAATGGACACCAAAGTAAATGAGATTTGGACACTAAGGAGACTTAATAAAAG
11641 attttaaagaaattgtatattgttaaataatgtactacaaacttagtagt 11688
TAAAATTTCTTTAACATAAACAATTTTATACATGATGTTTGAATCATCA

Restriction Analysis of pGIPZ

AhdI (GACnn_n'nnGTC) [Eam1105I,AspEI,DriI,EclHKI]

Cuts 1 time.
Cuts at position 10447.
Fragment sizes 10447, 1241.

AleI (CACnn'nnGTG) [OliI]

Cuts 1 time.
Cuts at position 1577.
Fragment sizes 1577, 10111.

AloI (GAACnnnnnnTCCnnnnnnn_nnnnn')

Cuts 1 time.
Cuts at position 7337.
Fragment sizes 7337, 4351.

AloI (GGAnnnnnnGTTcnnnnnnn_nnnnn')

Cuts 1 time.

Cuts at position 7369.

Fragment sizes 7369, 4319.

AsiSI (GCG_AT'CGC) [SgfI]

Cuts 1 time.

Cuts at position 8252.

Fragment sizes 8252, 3436.

BamHI (G'GATC_C)

Cuts 1 time.

Cuts at position 5403.

Fragment sizes 5403, 6285.

BbvCI (CC'TCA_GC)

Cuts 1 time.

Cuts at position 1424.

Fragment sizes 1424, 10264.

BlpI (GC'TnA_GC) [Bpu1102I,Bsp1720I,CelIII]

Cuts 1 time.

Cuts at position 3564.

Fragment sizes 3564, 8124.

Bpu10I (CC'TnA_GC)

Cuts 1 time.

Cuts at position 1424.

Fragment sizes 1424, 10264.

BsiWI (C'GTAC_G) [Pfl123II,PspLI,SunI]

Cuts 1 time.

Cuts at position 4749.

Fragment sizes 4749, 6939.

BsrGI (T'GTAC_A) [Bsp1407I,BstAUI,SspBI]

Cuts 1 time.

Cuts at position 4089.

Fragment sizes 4089, 7599.

BstZ17I (GTA'TAC) [BssNAI,Bst1107I]

Cuts 1 time.

Cuts at position 9175.

Fragment sizes 9175, 2513.

Bsu36I (CC'TnA_GG) [AxyI,Bse21I,Eco81I]

Cuts 1 time.

Cuts at position 6383.

Fragment sizes 6383, 5305.

CspCI (CAAnnnnnGTGGnnnnnnnnnnn_nn')
Cuts 1 time.
Cuts at position 3141.
Fragment sizes 3141, 8547.

CspCI (CCACnnnnnTTGnnnnnnnnnnn_nn')
Cuts 1 time.
Cuts at position 3106.
Fragment sizes 3106, 8582.

EcoNI (CCTnn'n_nnAGG) [BstENI,XagI]
Cuts 1 time.
Cuts at position 1170.
Fragment sizes 1170, 10518.

FspI (TGC'GCA) [Acc16I,AviII,NsbI]
Cuts 1 time.
Cuts at position 10669.
Fragment sizes 10669, 1019.

HpaI (GTT'AAAC) [KspAI]
Cuts 1 time.
Cuts at position 5376.
Fragment sizes 5376, 6312.

MluI (A'CGCG_T)
Cuts 1 time.
Cuts at position 5650.
Fragment sizes 5650, 6038.

NotI (GC'GGCC_GC) [CciNI]
Cuts 1 time.
Cuts at position 4100.
Fragment sizes 4100, 7588.

NruI (TCG'CGA) [Bsp68I]
[dam methylated]
Cuts 1 time.
Cuts at position [833].
Fragment sizes 833, 10855.

PmeI (GTTT'AAAC) [MssI]
Cuts 1 time.
Cuts at position 6776.
Fragment sizes 6776, 4912.

PpuMI (rG'GwC_Cy) [PpuXI,Psp5II,PspPPI]
[dcm methylated]
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9754.

PshAI (GACnn'nnGTC) [BoxI,BstPAI]

Cuts 1 time.
Cuts at position 7915.
Fragment sizes 7915, 3773.

PspXI (vC'TCGA_Gb)

Cuts 1 time.
Cuts at position 5391.
Fragment sizes 5391, 6297.

SanDI (GG'GwC_CC)

Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9754.

SfiI (GGCCn_nnn'nGGCC)

[dcm methylated]
Cuts 1 time.
Cuts at position 2621.
Fragment sizes 2621, 9067.

SgrAI (Cr'CCGG_yG)

Cuts 1 time.
Cuts at position 2500.
Fragment sizes 2500, 9188.

SnaBI (TAC'GTA) [BstSNI,Eco105I]

Cuts 1 time.
Cuts at position 3070.
Fragment sizes 3070, 8618.

SspI (AAT'ATT)

Cuts 1 time.
Cuts at position 11251.
Fragment sizes 11251, 437.

XbaI (T'CTAG_A)

[dam methylated]
Cuts 1 time.
Cuts at position 2707.
Fragment sizes 2707, 8981.

XhoI (C'TCGA_G) [BssHI,Paer7I,Sfr274I,SlaI,StrI,TliI]

Cuts 1 time.
Cuts at position 5391.
Fragment sizes 5391, 6297.

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