Facility for Life Science Automation

LISA

Technical Datasheet 001.02 - GIPZ

pGIPZ shRNAmir plasmids

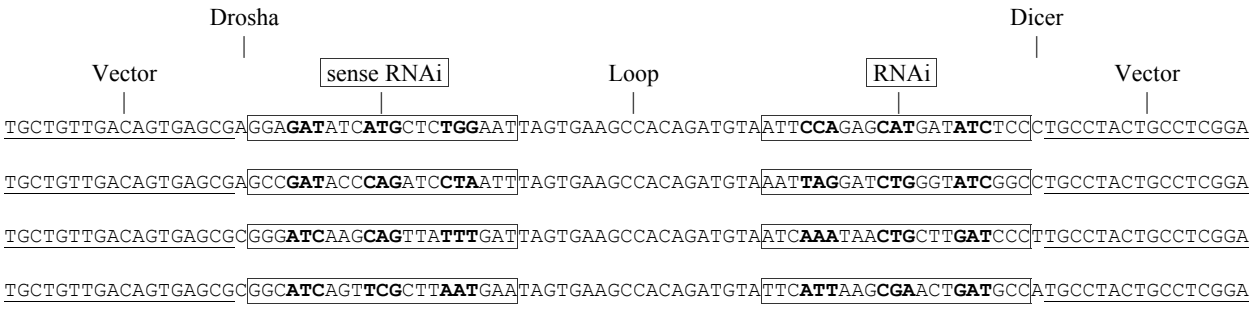
Open Biosystems provides a plasmid map for pGIPZ on page 4 of the GIPZ manual.

Size with shRNAmir	11774 bp
Markers	AmpR, ZeoR, PuroR, HygroR
Replication	pUC ori, f1 ori, SV40 ori
	pGIPZ is a high copy plasmid derived from the pUC origin of replication (pMB1, rop deleted)
Promoter	Bacterial promotor prom (AmpR), EM7 promotor (ZeoR), SV40 promotor (HygroR), T7 promotor (PuroR), CMV-IE Promotor Enhancer promotor (turboGFP, shRNAmir)
Unique Sites	NruI (834), SfiI (2622), XbaI (2708), NotI (4101), HpaI (5377), XhoI (5392)
shRNAmir	Hairpin sequence cloned into MluI (ACGCGT) (5650-5655) and XhoI (CTCGAG) (5391-5396). Distance between MluI and XhoI is 345nt.

pGIPZ sequencing primer

OBS	5'- GCATTAAAGCAGCGTATC -3' (6A+4C+4G+4T = 18; Tm = 52.7°C, M=5508).
	OBS gives the binding site as bases 5820-5842. Other users have found it to bind bases 5756-5739 on pGIPZ. Please note that this primer runs in the reverse complement direction. You should find the MluI cloning site some 70 nt from the start of your sequencing result and the antisense strand of the hairpin should start another 240-260 nt from there.
Alternative	5'- ACAGAATCGTTGCCTGCACA -3' (6A+6C+4G+4T = 20; Tm = 60°C).

shRNAmir example reference



Restriction analysis of pGIPZ lentiviral vector

AhdI (GACnn_n'nnGTC) [Eam1105I,AspEI,DriI,EclHKI]
Cuts 1 time.
Cuts at position 10533.
Fragment sizes 10533, 1241.
AleI (CACnn'nnGTG) [OliI]
Cuts 1 time.
Cuts at position 1577.
Fragment sizes 1577, 10197.
AloI (GAACnnnnnnTCCnnnnnnn_nnnnn')
Cuts 1 time.
Cuts at position 7423.
Fragment sizes 7423, 4351.
AloI (GGAnnnnnnGTTCnnnnnnn_nnnnn')
Cuts 1 time.
Cuts at position 7455.
Fragment sizes 7455, 4319.
AsiSI (GCG_AT'CGC) [SgfI]
Cuts 1 time.
Cuts at position 8338.
Fragment sizes 8338, 3436.
BbvCI (CC'TCA_GC)
Cuts 1 time.
Cuts at position 1424.
Fragment sizes 1424, 10350.
BlpI (GC'TnA_GC) [Bpu1102I,Bsp1720I,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) [Pfl23II,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 (CAAnnnnnGTGGnnnnnnnnnn_nn')
Cuts 1 time.
Cuts at position 3141.
Fragment sizes 3141, 8633.
CspCI (CCACnnnnnTTGnnnnnnnnnnn_nn')
Cuts 1 time.
Cuts at position 3106.
Fragment sizes 3106, 8668.

EcoNI (CCTnn'n_nnAGG) [BstENI,XagI]
Cuts 1 time.
Cuts at position 1170.
Fragment sizes 1170, 10604.
FspI (TGC'GCA) [Acc16I,AviII,NsbI]
Cuts 1 time.
Cuts at position 10755.
Fragment sizes 10755, 1019.
HpaI (GTT'AAC) [KspAI]
Cuts 1 time.
Cuts at position 5376.
Fragment sizes 5376, 6398.
MluI (A'CGCG_T)
Cuts 1 time.
Cuts at position 5736.
Fragment sizes 5736, 6038.
NotI (GC'GGCC_GC) [CciNI]
Cuts 1 time.
Cuts at position 4100.
Fragment sizes 4100, 7674.
NruI (TCG'CGA) [Bsp68I]
[dam methylated]
Cuts 1 time.
Cuts at position [833].
Fragment sizes 833, 10941.
PmeI (GTTT'AAAC) [MssI]
Cuts 1 time.
Cuts at position 6862.
Fragment sizes 6862, 4912.
PpuMI (rG'GwC_Cy) [PpuXI,Psp5II,PspPPI]
[dcm methylated]
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9840.
PshAI (GACnn'nnGTC) [BoxI,BstPAI]
Cuts 1 time.
Cuts at position 8001.
Fragment sizes 8001, 3773.
SanDI (GG'GwC_CC)
Cuts 1 time.
Cuts at position 1934.
Fragment sizes 1934, 9840.
SfiI (GGCCn_nnn'nGGCC)
[dcm methylated]
Cuts 1 time.
Cuts at position 2621.
Fragment sizes 2621, 9153.
SgrAI (Cr'CCGG_yG)
Cuts 1 time.
Cuts at position 2500.
Fragment sizes 2500, 9274.
SnaBI (TAC'GTA) [BstSNI,Eco105I]
Cuts 1 time.
Cuts at position 3070.
Fragment sizes 3070, 8704.
SspI (AAT'ATT)
Cuts 1 time.
Cuts at position 11337.
Fragment sizes 11337, 437.
XbaI (T'CTAG_A)
[dam methylated]
Cuts 1 time.
Cuts at position 2707.
Fragment sizes 2707, 9067.
XhoI (C'TCGA_G) [BssHI,PaeR7I,Sfr274I,SlaI,StrI,TliI]
Cuts 1 time.
Cuts at position 5391.

Sequence of pGIPZ lentiviral vector (11774bp)

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

61      cacaaggctacttccctgattagcagaactacacaccagggccaggggtcagatatccac     120
        GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCCAGTCTATAGGTG

121     tgacctttggatggtgctacaagctagtaccagttgagccagataaggtagaagaggcca     180
        ACTGGAAACCTACCACGATGTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT

181     ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgacccgg     240
        TATTTCCTCTCTTGTGGTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC

241     agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccgag     300
        TCTCTCTTCACAATCTCACCTCCAAACTGTCGGCGGATCGTAAAGTAGTGCACCGGGCTC

301     agctgcatccggagtacttcaagaactgctgatatcgagcttgctacaagggactttccg     360
        TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC

361     ctggggactttccagggaggcgtggcctgggcgggactggggagtggcgagccctcagat     420
        GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA

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

481     gcctgggagctctctggctaactagggaacccactgcttaagcctcaataaagcttgcct     540
        CGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTCGAACGGA

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

601     agacccttttagtcagtgtggaaaatctctagcagtggcgcccgaacagggacttgaaag     660
        TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC

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

721     caagaggcgaggggcggcgactggtgagtacgccaaaaattttgactagcggaggctaga     780
        GTTCTCCGCTCCCCGCCGCTGACCACTCATGCGGTTTTTAAAACTGATCGCCTCCGATCT

                                                              NruI
                                                              |
781     aggagagagatgggtgcgagagcgtcagtattaagcgggggagaattagatcgcgatggg     840
        TCCTCTCTCTACCCACGCTCTCGCAGTCATAATTCGCCCCCTCTTAATCTAGCGCTACCC

841     aaaaaattcggttaaggccaggggaaagaaaaaatataaattaaaacatatagtatggg     900
        TTTTTTAAGCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATACCC
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901     caagcagggagctagaacgattcgcagttaatcctggcctgttagaaacatcagaaggct     960
        GTTCGTCCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTTCCGA

961     gtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagat     1020
        CATCTGTTTATGACCCTGTCGATGTTGGTAGGGAAGTCTGTCCTAGTCTTCTTGAATCTA

1021    cattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagaca     1080
        GTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTTCTGT

1081    ccaaggaagctttagacaagatagaggaagagcaaaacaaaagtaagaccaccgcacagc     1140
        GGTTCCTTCGAAATCTGTTCTATCTCCTTCTCGTTTTGTTTTCATTCTGGTGGCGTGTCG

1141    aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag     1200
        TTCGCCGGCCGGCGACTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTTAACCTCTTC

1201    tgaattatataaatataaagtagtaaaaattgaaccattaggagtagcacccaccaaggc     1260
        ACTTAATATATTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTTCCG

                                                          RRE(HIV)
                                                          reg(1314,1518)>>
>
                                                          |
1261    aaagagaagagtggtgcagagagaaaaaagagcagtgggaataggagctttgttccttgg     1320
        TTTCTCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCTCGAAACAAGGAACC

1321    gttcttgggagcagcaggaagcactatgggcgcagcgtcaatgacgctgacggtacaggc     1380
        CAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGTTACTGCGACTGCCATGTCCG

1381    cagacaattattgtctggtatagtgcagcagcagaacaatttgctgagggctattgaggc     1440
        GTCTGTTAATAACAGACCATATCACGTCGTCGTCTTGTTAAACGACTCCCGATAACTCCG

1441    gcaacagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaagaatcct     1500
        CGTTGTCGTAGACAACGTTGAGTGTCAGACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGA

1501    ggctgtggaaagatacctaaaggatcaacagctcctggggatttggggttgctctggaaa     1560
        CCGACACCTTTCTATGGATTTCCTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTT

1561    actcatttgcaccactgctgtgccttggaatgctagttggagtaataaatctctggaaca     1620
        TGAGTAAACGTGGTGACGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGT

1621    gatttggaatcacacgacctggatggagtgggacagagaaattaacaattacacaagctt     1680
        CTAAACCTTAGTGTGCTGGACCTACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGAA

1681    aatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattatt     1740
        TTATGTGAGGAATTAACTTCTTAGCGTTTTGGTCGTTCTTTTCTTACTTGTTCTTAATAA

1741    ggaattagataaatgggcaagtttgtggaattggtttaacataacaaattggctgtggta     1800
        CCTTAATCTATTTACCCGTTCAAACACCTTAACCAAATTGTATTGTTTAACCGACACCAT

1801    tataaaattattcataatgatagtaggaggcttggtaggtttaagaatagtttttgctgt     1860
        ATATTTTAATAAGTATTACTATCATCCTCCGAACCATCCAAATTCTTATCAAAAACGACA

1861    actttctatagtgaatagagttaggcagggatattcaccattatcgtttcagacccacct     1920
        TGAAAGATATCACTTATCTCAATCCGTCCCTATAAGTGGTAATAGCAAAGTCTGGGTGGA

1921    cccaaccccgaggggacccgacaggcccgaaggaatagaagaagaaggtggagagagaga     1980
        GGGTTGGGGCTCCCCTGGGCTGTCCGGGCTTCCTTATCTTCTTCTTCCACCTCTCTCTCT

1981    cagagacagatccattcgattagtgaacggatcggcactgcgtgcgccaattctgcagac     2040
        GTCTCTGTCTAGGTAAGCTAATCACTTGCCTAGCCGTGACGCACGCGGTTAAGACGTCTG

                                CTS reg(2064,2214)>>>
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2041    aaatggcagtattcatccacaattttaaaagaaaaggggggattgggggtacagtgcag    2100
        TTTACCGTCATAAGTAGGTGTTAAAATTTTCTTTTCCCCCCTAACCCCCCATGTCACGTC

2101    gggaaagaatagtagacataatagcaacagacatacaaactaaagaattacaaaaacaaa    2160
        CCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTAATGTTTTTGTTT

2161    ttacaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag    2220
        AATGTTTTTAAGTTTTAAAAGCCCAAATAATGTCCCTGTCGTCTCTAGGTCAAACCAATC

                                ZeoR marker(2245,2619)<<<
                                |
2221    taccgggcccgctctagtccggaatcagtcctgctcctcggccacgaagtgcacgcagtt    2280
        ATGGCCCGGGCGAGATCAGGCCTTAGTCAGGACGAGGAGCCGGTGCTTCACGTGCGTCAA

2281    gccggccgggtcgcgcagggcgaactcccgcccccacggctgctcgccgatctcggtcat    2340
        CGGCCGGCCCAGCGCGTCCCGCTTGAGGGCGGGGGTGCCGACGAGCGGCTAGAGCCAGTA

2341    ggccggcccggaggcgtcccggaagttcgtggacacgacctccgaccactcggcgtacag    2400
        CCGGCCGGGCCTCCGCAGGGCCTTCAAGCACCTGTGCTGGAGGCTGGTGAGCCGCATGTC

2401    ctcgtccaggccgcgcacccacacccaggccagggtgttgtccggcaccacctggtcctg    2460
        GAGCAGGTCCGGCGCGTGGGTGTGGGTCCGGTCCCACAACAGGCCGTGGTGGACCAGGAC

2461    gaccgcgctgatgaacagggtcacgtcgtcccggaccacaccggcgaagtcgtcctccac    2520
        CTGGCGCGACTACTTGTCCCAGTGCAGCAGGGCCTGGTGTGGCCGCTTCAGCAGGAGGTG

2521    gaagtcccgggagaacccgagccggtcggtccagaactcgaccgctccggcgacgtcgcg    2580
        CTTCAGGGCCCTCTTGGGCTCGGCCAGCCAGGTCTTGAGCTGGCGAGGCCGCTGCAGCGC

                                                  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    tggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagt    2880
        ACCGACTGGCGGGTTGCTGGGGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATCA

2881    aacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgccca    2940
        TTGCGGTTATCCCTGAAAGGTAACTGCAGTTACCCACCTCATAAATGCCATTTGACGGGT

2941    cttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg    3000
        GAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGGGGGATAACTGCAGTTACTGCC

3001    taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggca    3060
        ATTTACCGGGCGGACCGTAATACGGGTCATGTACTGGAATACCCTGAAAGGATGAACCGT

3061    gtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaa    3120
        CATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTT
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3121    tgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaa    3180
        ACCCGCACCTATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTT

3181    tgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgc    3240
        ACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTGTTGAGGCG

3241    cccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcg    3300
        GGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGAGC

3301    tttagtgaaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaag    3360
        AAATCACTTGGCAGTCTAGCGGACCTCTGCGGTAGGTGCGACAAAACTGGAGGTATCTTC

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

3421    tggagatcgagtgccgcatcaccggcaccctgaacggcgtggagttcgagctggtgggcg    3480
        ACCTCTAGCTCACGGCGTAGTGGCCGTGGGACTTGCCGCACCTCAAGCTCGACCACCCGC

3481    gcggagagggcacccccgagcagggccgcatgaccaacaagatgaagagcaccaaaggcg    3540
        CGCCTCTCCCGTGGGGGCTCGTCCCGGCGTACTGGTTGTTCTACTTCTCGTGGTTTCCGC

3541    ccctgaccttcagcccctacctgctgagccacgtgatgggctacggcttctaccacttcg    3600
        GGGACTGGAAGTCGGGGATGGACGACTCGGTGCACTACCCGATGCCGAAGATGGTGAAGC

3601    gcacctaccccagcggctacgagaaccccttcctgcacgccatcaacaacggcggctaca    3660
        CGTGGATGGGGTCGCCGATGCTCTTGGGGAAGGACGTGCGGTAGTTGTTGCCGCCGATGT

3661    ccaacacccgcatcgagaagtacgaggacggcggcgtgctgcacgtgagcttcagctacc    3720
        GGTTGTGGGCGTAGCTCTTCATGCTCCTGCCGCCGCACGACGTGCACTCGAAGTCGATGG

3721    gctacgaggccggccgcgtgatcggcgacttcaaggtgatgggcaccggcttccccgagg    3780
        CGATGCTCCGGCCGGCGCACTAGCCGCTGAAGTTCCACTACCCGTGGCCGAAGGGGCTCC

3781    acagcgtgatcttcaccgacaagatcatccgcagcaacgccaccgtggagcacctgcacc    3840
        TGTCGCACTAGAAGTGGCTGTTCTAGTAGGCGTCGTTGCGGTGGCACCTCGTGGACGTGG

3841    ccatgggcgataacgatctggatggcagcttcacccgcaccttcagcctgcgcgacggcg    3900
        GGTACCCGCTATTGCTAGACCTACCGTCGAAGTGGGCGTGGAAGTCGGACGCGCTGCCGC

3901    gctactacagctccgtggtggacagccacatgcacttcaagagcgccatccaccccagca    3960
        CGATGATGTCGAGGCACCACCTGTCGGTGTACGTGAAGTTCTCGCGGTAGGTGGGGTCGT

3961    tcctgcagaacgggggccccatgttcgccttccgccgcgtggaggaggatcacagcaaca    4020
        AGGACGTCTTGCCCCCGGGGTACAAGCGGAAGGCGGCGCACCTCCTCCTAGTGTCGTTGT

4021    ccgagctgggcatcgtggagtaccagcacgccttcaagaccccggatgcagatgccggtg    4080
        GGCTCGACCCGTAGCACCTCATGGTCGTGCGGAAGTTCTGGGGCCTACGTCTACGGCCAC

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

4141    ttactggccgaagccgcttggaataaggccggtgtgcgtttgtctatatgttattttcca    4200
        AATGACCGGCTTCGGCGAACCTTATTCCGGCCACACGCAAACAGATATACAATAAAAGGT

4201    ccatattgccgtcttttggcaatgtgagggcccggaaacctggccctgtcttcttgacga    4260
        GGTATAACGGCAGAAAACCGTTACACTCCCGGGCCTTTGGACCGGGACAGAAGAACTGCT

4261    gcattcctaggggtctttcccctctcgccaaaggaatgcaaggtctgttgaatgtcgtga    4320
        CGTAAGGATCCCCAGAAAGGGGAGAGCGGTTTCCTTACGTTCCAGACAACTTACAGCACT
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4321    aggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgacccctttgca     4380
        TCCTTCGTCAAGGAGACCTTCGAAGAACTTCTGTTTGTTGCAGACATCGCTGGGAAACGT

4381    ggcagcggaacccccccacctggcgacaggtgcctctgcggccaaaagccacgtgtataag     4440
        CCGTCGCCTTGGGGGGTGGACCGCTGTCCACGGAGACGCCGGTTTTCGGTGCACATATTC

4441    atacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtggaaa     4500
        TATGTGGACGTTTCCGCCGTGTTGGGGTCACGGTGCAACACTCAACCTATCAACACCTTT

4501    gagtcaaatggctctcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtac     4560
        CTCAGTTTACCGAGAGGAGTTCGCATAAGTTGTTCCCCGACTTCCTACGGGTCTTCCATG

4561    cccattgtatgggatctgatctggggcctcggtgcacatgctttacatgtgtttagtcga     4620
        GGGTAACATACCCTAGACTAGACCCCGGAGCCACGTGTACGAAATGTACACAAATCAGCT

4621    ggttaaaaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaacac     4680
        CCAATTTTTTTGCAGATCCGGGGGGCTTGGTGCCCCTGCACCAAAAGGAAACTTTTTGTG

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

4741    ccccgggccgtacgcaccctcgccgccgcgttcgccgactaccccgccacgcgccacacc     4800
        GGGGCCCGGCATGCGTGGGAGCGGCGGCGCAAGCGGCTGATGGGGCGGTGCGCGGTGTGG

4801    gtcgacccggaccgccacatcgagcgggtcaccgagctgcaagaactcttcctcacgcgc     4860
        CAGCTGGGCCTGGCGGTGTAGCTCGCCCAGTGGCTCGACGTTCTTGAGAAGGAGTGCGCG

4861    gtcgggctcgacatcggcaaggtgtgggtcgcggacgacggcgccgcggtggcggtctgg     4920
        CAGCCCGAGCTGTAGCCGTTCCACACCCAGCGCCTGCTGCCGCGGCGCCACCGCCAGACC

4921    accacgccggagagcgtcgaagcgggggcggtgttcgccgagatcggctcgcgcatggcc     4980
        TGGTGCGGCCTCTCGCAGCTTCGCCCCCGCCACAAGCGGCTCTAGCCGAGCGCGTACCGG

4981    gagttgagcggttcccggctggccgcgcagcaacagatggaaggcctcctggcgccgcac     5040
        CTCAACTCGCCAAGGGCCGACCGGCGCGTCGTTGTCTACCTTCCGGAGGACCGCGGCGTG

5041    cggcccaaggagcccgcgtggttcctggccaccgtcggcgtctcgcccgaccaccagggc     5100
        GCCGGGTTCCTCGGGCGCACCAAGGACCGGTGGCAGCCGCAGAGCGGGCTGGTGGTCCCG

5101    aagggtctgggcagcgccgtcgtgctccccggagtggaggcggccgagcgcgctggggtg     5160
        TTCCCAGACCCGTCGCGGCAGCACGAGGGGCCTCACCTCCGCCGGCTCGCGCGACCCCAC

5161    cccgccttcctggagacctccgcgccccgcaacctccccttctacgagcggctcggcttc     5220
        GGGCGGAAGGACCTCTGGAGGCGCGGGGCGTTGGAGGGGAAGATGCTCGCCGAGCCGAAG

5221    accgtcaccgccgacgtcgaggtgcccgaaggaccgcgcacctggtgcatgacccgcaag     5280
        TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCCTGGCGCGTGGACCACGTACTGGGCGTTC

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

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

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

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

5521   ggagcaattatcttgtttactaaaactgaataccttgctatctctttgatacatttttac    5580
       CCTCGTTAATAGAACAAATGATTTTGACTTATGGAACGATAGAGAAACTATGTAAAAATG

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

5641   cggccattactccgtctcgtgtcttgttgcatatgtctgctggtttgtttgatgttgttt    5700
       GCCGGTAATGAGGCAGAGCACAGAACAACGTATACAGACGACCAAACAAACTACAACAAA

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

5761   gattacaaaatttgtgaaagattgactggtattcttaactatgttgctcctttttacgcta    5820
       CTAATGTTTTAAACACTTTCTAACTGACCATAAGAATTGATACAACGAGGAAAATGCGAT

5821   tgtggatacgctgctttaatgcctttgtatcatgctattgcttcccgtatggctttcatt    5880
       ACACCTATGCGACGAAATTACGGAAACATAGTACGATAACGAAGGGCATACCGAAAGTAA

5881   ttctcctccttgtataaatcctggttgctgtctctttatgaggagttgtggcccgttgtc    5940
       AAGAGGAGGAACATATTTAGGACCAACGACAGAGAAATACTCCTCAACACCGGGCAACAG

5941   aggcaacgtggcgtggtgtgcactgtgtttgctgacgcaacccccactggttggggcatt    6000
       TCCGTTGCACCGCACCACACGTGACACAAACGACTGCGTTGGGGGTGACCAACCCCGTAA

6001   gccaccacctgtcagctcctttccgggactttcgctttccccctccctattgccacggcg    6060
       CGGTGGTGGACAGTCGAGGAAAGGCCCTGAAAGCGAAAGGGGGAGGGATAACGGTGCCGC

6061   gaactcatcgccgcctgccttgcccgctgctggacaggggctcggctgttgggcactgac    6120
       CTTGAGTAGCGGCGGACGGAACGGGCGACGACCTGTCCCCGAGCCGACAACCCGTGACTG

6121   aattccgtggtgttgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgcc    6180
       TTAAGGCACCACAACAGCCCCTTCGACTGCAGGAAAGGTACCGACGAGCGGACACAACGG

6181   acctggattctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggac    6240
       TGGACCTAAGACGCGCCCTGCAGGAAGACGATGCAGGGAAGCCGGGAGTTAGGTCGCCTG

6241   cttccttcccgcggcctgctgccggctctgcggcctcttccgcgtcttcgccttcgccct    6300
       GAAGGAAGGGCGCCGGACGACGGCCGAGACGCCGGAGAAGGCGCAGAAGCGGAAGCGGGA

6301   cagacgagtcggatctccctttgggccgcctccccgcctggaattaattctgcagtcgag    6360
       GTCTGCTCAGCCTAGAGGGAAACCCGGCGGAGGGGCGGACCTTAATTAAGACGTCAGCTC
```

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6361    acctagaaaaacatggagcaatcacaagtagcaatacagcagctaccaatgctgattgtg     6420
        TGGATCTTTTTGTACCTCGTTAGTGTTCATCGTTATGTCGTCGATGGTTACGACTAACAC

6421    cctggctagaagcacaagaggaggaggaggtgggttttccagtcacacctcaggtacctt     6480
        GGACCGATCTTCGTGTTCTCCTCCTCCTCCACCCAAAAGGTCAGTGTGGAGTCCATGGAA

6481    taagaccaatgacttacaaggcagctgtagatcttagccacttttttaaaagaaaagaggg     6540
        ATTCTGGTTACTGAATGTTCCGTCGACATCTAGAATCGGTGAAAAATTTTCTTTTCTCCC

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

6601    ctctctggttagaccagatctgagcctgggagctctctggctaactagggaacccactgc     6660
        GAGAGACCAATCTGGTCTAGACTCGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACG

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

6721    actctggtaactagagatccctcagacccttttagtcagtgtggaaaatctctagcagta     6780
        TGAGACCATTGATCTCTAGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCAT

6781    gtagttcatgtcatcttattattcagtatttataacttgcaaagaaatgaatatcagaga     6840
        CATCAAGTACAGTAGAATAATAAGTCATAAATATTGAACGTTTCTTTACTTATAGTCTCT

                                     BGH-polyA reg(6868,7091)>>>
                                     |
6841    gtgagaggccttgacattgtttaaacccgctgatcagcctcgactgtgccttctagttgc     6900
        CACTCTCCGGAACTGTAACAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACG

6901    cagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactccc     6960
        GTCGGTAGACAACAAACGGGGAGGGGGCACGGAAGGAACTGGGACCTTCCACGGTGAGGG

6961    actgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattct     7020
        TGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGA

7021    attctgggggtggggtggggcaggacagcaaggggaggattgggaagacaatagcagg      7080
        TAAGACCCCCCACCCCACCCCGTCCTGTCGTTCCCCCTCCTAACCCTTCTGTTATCGTCC

7081    catgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctct     7140
        GTACGACCCCTACGCCACCCGAGATACCGAAGACTCCGCCTTTCTTGGTCGACCCCGAGA

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

7201    cgcagcgtgaccgctacacttgccagcgccctagcgcccgctcctttcgctttcttccct     7260
        GCGTCGCACTGGCGATGTGAACGGTCGCGGGATCGCGGGCGAGGAAAGCGAAAGAAGGGA

7261    tcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctcccttta     7320
        AGGAAAGAGCGGTGCAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGCCCCCGAGGGAAAT

7321    gggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggt     7380
        CCCAAGGCTAAATCACGAAATGCCGTGGAGCTGGGGTTTTTTGAACTAATCCCACTACCA
```

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7381    tcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacg    7440
        AGTGCATCACCCGGTAGCGGGACTATCTGCCAAAAAGCGGGAAACTGCAACCTCAGGTGC

7441    ttctttaatagtggactcttgttccaaactggaacaacactcaaccctatctcggtctat    7500
        AAGAAATTATCACCTGAGAACAAGGTTTGACCTTGTTGTGAGTTGGGATAGAGCCAGATA

7501    tcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatt    7560
        AGAAAACTAAATATTCCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

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

7621    ccccaggctccccagcaggcagaagtatgcaaagcatgcatctcaattagtcagcaacca    7680
        GGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGT

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

                                          SV40 origin(7776,7853)>>>
                                          |
7741    agtcagcaaccatagtcccgcccctaactccgcccatcccgcccctaactccgcccagtt    7800
        TCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAA

7801    ccgcccattctccgccccatggctgactaatttttttatttatgcagaggccgaggccg    7860
        GGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGC

7861    cctctgcctctgagctattccagaagtagtgaggaggctttttggaggcctaggctttt    7920
        GGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAA

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

7981    agcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtct    8040
        TCGGACTTGAGTGGCGCTGCAGACAGCTCTTCAAAGACTAGCTTTTCAAGCTGTCGCAGA

8041    ccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggag    8100
        GGCTGGACTACGTCGAGAGCCTCCCGCTTCTTAGAGCACGAAAGTCGAAGCTACATCCTC

8101    ggcgtggatatgtcctgcgggtaaatagctgcgccgatggtttctacaaagatcgttatg    8160
        CCGCACCTATACAGGACGCCCATTTATCGACGCGGCTACCAAAGATGTTTCTAGCAATAC

8161    tttatcggcactttgcatcggccgcgctcccgattccggaagtgcttgacattggggaat    8220
        AAATAGCCGTGAAACGTAGCCGGCGCGAGGGCTAAGGCCTTCACGAACTGTAACCCCTTA

8221    tcagcgagagcctgacctattgcatctcccgccgtgcacagggtgtcacgttgcaagacc    8280
        AGTCGCTCTCGGACTGGATAACGTAGAGGGCGGCACGTGTCCCACAGTGCAACGTTCTGG

8281    tgcctgaaaccgaactgcccgctgttctgcagccggtcgcggaggccatggatgcgatcg    8340
        ACGGACTTTGGCTTGACGGGCGACAAGACGTCGGCCAGCGCCTCCGGTACCTACGCTAGC

8341    ctgcggccgatcttagccagacgagcgggttcggcccattcggaccgcaaggaatcggtc    8400
        GACGCCGGCTAGAATCGGTCTGCTCGCCCAAGCCGGGTAAGCCTGGCGTTCCTTAGCCAG
```

```
8401    aatacactacatggcgtgatttcatatgcgcgattgctgatccccatgtgtatcactggc    8460
        TTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCG

8461    aaactgtgatggacgacaccgtcagtgcgtccgtcgcgcaggctctcgatgagctgatgc    8520
        TTTGACACTACCTGCTGTGGCAGTCACGCAGGCAGCGCGTCCGAGAGCTACTCGACTACG

8521    tttgggccgaggactgccccgaagtccggcacctcgtgcacgcggatttcggctccaaca    8580
        AAACCCGGCTCCTGACGGGGCTTCAGGCCGTGGAGCACGTGCGCCTAAAGCCGAGGTTGT

8581    atgtcctgacggacaatggccgcataacagcggtcattgactggagcgaggcgatgttcg    8640
        TACAGGACTGCCTGTTACCGGCGTATTGTCGCCAGTAACTGACCTCGCTCCGCTACAAGC

8641    gggattcccaatacgaggtcgccaacatcttcttctggaggccgtggttggcttgtatgg    8700
        CCCTAAGGGTTATGCTCCAGCGGTTGTAGAAGAAGACCTCCGGCACCAACCGAACATACC

8701    agcagcagacgcgctacttcgagcggaggcatccggagcttgcaggatcgccgcggctcc    8760
        TCGTCGTCTGCGCGATGAAGCTCGCCTCCGTAGGCCTCGAACGTCCTAGCGGCGCCGAGG

8761    gggcgtatatgctccgcattggtcttgaccaactctatcagagcttggttgacggcaatt    8820
        CCCGCATATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAA

8821    tcgatgatgcagcttgggcgcagggtcgatgcgacgcaatcgtccgatccggagccggga    8880
        AGCTACTACGTCGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGCTAGGCCTCGGCCCT

8881    ctgtcgggcgtacacaaatcgcccgcagaagcgcggccgtctggaccgatggctgtgtag    8940
        GACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATC

8941    aagtactcgccgatagtggaaaccgacgccccagcactcgtccgagggcaaaggaatagc    9000
        TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTCGTGAGCAGGCTCCCGTTTCCTTATCG

9001    acgtgctacgagatttcgattccaccgccgccttctatgaaaggttgggcttcggaatcg    9060
        TGCACGATGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061    ttttccgggacgccggctggatgatcctccagcgcggggatctcatgctggagttcttcg    9120
        AAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGC

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

9181    atttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactcatca    9240
        TAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGT

9241    atgtatcttatcatgtctgtataccgtcgacctctagctagagcttggcgtaatcatggt    9300
        TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCA

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

9361    gaagcataaagtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcgt    9420
        CTTCGTATTTCACATTTCGGACCCCACGGATTACTCACTCGATTGAGTGTAATTAACGCA

9421    tgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcg    9480
        ACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTCGACGTAATTACTTAGC

9481    gccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactg    9540
        CGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGAC
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9541    actcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaa    9600
        TGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTCCGCCATT

9601    tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc    9660
        ATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCG

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

9721    ctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactat    9780
        GACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATA

9781    aaagataccaggcgtttcccccctggaagctccctcgtgcgctctcctgttccgaccctgc    9840
        TTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACG

9841    cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct    9900
        GCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901    cacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacg    9960
        GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTCGACCCGACACACGTGC

9961    aacccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacc    10020
        TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021   cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga    10080
        GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081   ggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaa    10140
        CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141   gaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggta    10200
        CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTCTCAACCAT

10201   gctcttgatccggcaaacaaaccaccgctggtagcggtggttttttgtttgcaagcagc    10260
        CGAGAACTAGGCCGTTTGTTTGGTGGCGACCATCGCCACCAAAAAAACAAACGTTCGTCG

10261   agattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctg    10320
        TCTAATGCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGAC

10321   acgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaagga    10380
        TGCGAGTCACCTTGCTTTTGAGTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCT

10381   tcttcacctagatcctttaaattaaaaatgaagttttaaatcaatctaaagtatatatg    10440
        AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATATAC

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

10501   gtctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacgatacggg    10560
        CAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCCC

10561   agggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctc    10620
        TCCCGAATGGTAGACCGGGGTCACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAG

10621   cagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaa    10680
        GTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGGCTCGCGTCTTCACCAGGACGTT

10681   ctttatccgcctccatccagtctattaattgttgccgggaagctagagtaagtagttcgc    10740
        GAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCCCTTCGATCTCATTCATCAAGCG
```

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10741   cagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgt    10800
        GTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACCACAGTGCGAGCA

10801   cgtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatccc    10860
        GCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGG

10861   ccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagt    10920
        GGTACAACACGTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCA

10921   tggccgcagtgttatcactcatggttatggcagcactgcataattctcttactgtcatgc    10980
        ACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACG

10981   catccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatagt    11040
        GTAGGCATTCTACGAAAAGACACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCA

11041   gtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacata    11100
        CATACGCCGCTGGCTCAACGAGAACGGGCCGCAGTTATGCCCTATTATGGCGCGGTGTAT

11101   gcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaagga    11160
        CGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCT

11161   tcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcag    11220
        AGAATGGCGACAACTCTAGGTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTC

11221   catcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaa    11280
        GTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTGTCCTTCCGTTTTACGGCGTT

11281   aaaagggaataagggcgacacggaaatgttgaatactcatactcttccttttcaatatt    11340
        TTTTCCCTTATTCCCGCTGTGCCTTTACAACTTATGAGTATGAGAAGGAAAAAGTTATAA

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

11401   aaaataaacaaataggggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg    11460
        TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGCTGC

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

11521   acaaatttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactc    11580
        TGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG

11581   atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc    11640
        TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTCGATTGGTTTTAGTAGGG

11641   aaacttcccaccccataccctattaccactgccaattacctgtggtttcatttactctaa    11700
        TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT

11701   acctgtgattcctctgaattattttcattttaaagaaattgtatttgttaaatatgtact    11760
        TGGACACTAAGGAGACTTAATAAAAGTAAAATTTCTTTAACATAAACAATTTATACATGA

11761   acaaacttagtagt                                                  11774
        TGTTTGAATCATCA
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Jose M Silva *et al*.: "Second-generation shRNA libraries covering the mouse and human genomes", Nature Genetics 37 (2005): 1281-88.