

Product: Expression Arrest™ pSM2 empty vector

Catalog #: RHS1763

The laboratory of Dr. Greg Hannon at Cold Spring Harbor Laboratory (CSHL) has created an RNAi Clone Library comprised of multiple short-hairpin RNAs (shRNAs) specifically targeting annotated human and mouse genes. The shRNA Library permits rapid, cost efficient, loss-of-function genetic screens and rapid tests for genetic interactions to be performed in mammalian cells. Each shRNA has been cloned and sequence verified to ensure a match to the target gene.

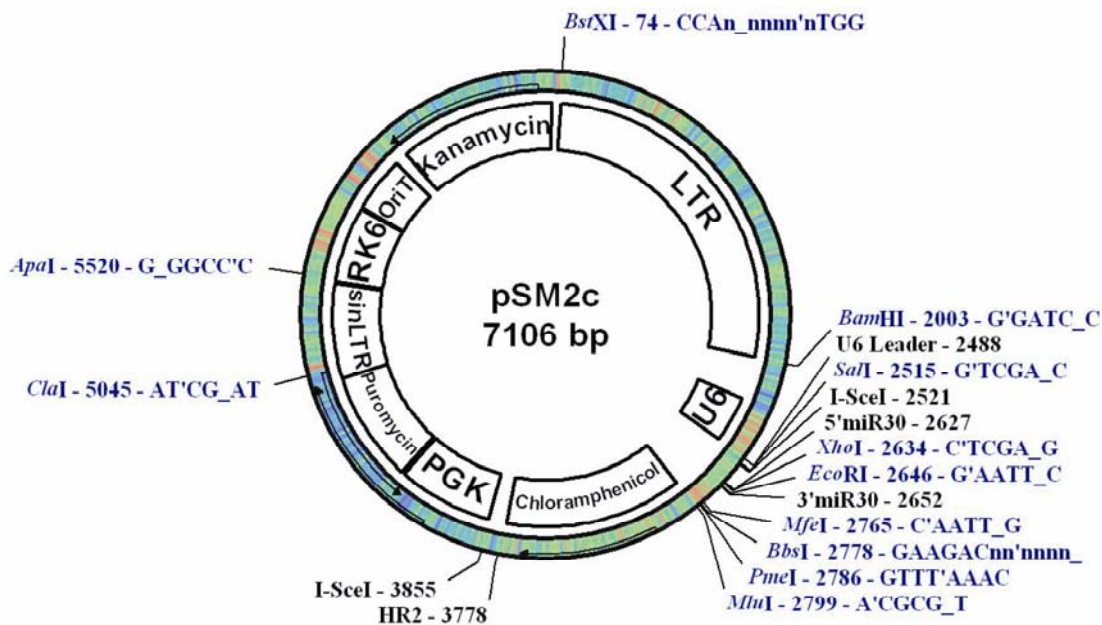
The shRNA Control Vector (See Figure 1) is a negative control for any transfection experiment performed using the Expression Arrest™ shRNA-containing expression vectors. This control vector is pSHAG-MAGIC 2 without an insert.

Each vial of the shRNA Control Vector is shipped at a concentration of 0.25µg/µl in a total volume of 40µl of Tris buffer (pH 8.0), thus providing a total amount of 10µg vector DNA.

shRNA vector storage

The vector DNA is shipped in a microfuge tube at room temperature and should be stored at -20°C or -80°C.

Figure 1: Vector Map of pSHAG-MAGIC 2



Note: The pSM vectors must be transformed into PIR1 competent bacteria. These plasmids harbor a conditional bacterial origin of replication, which requires the expression of the “pir1” gene to be rendered functional.

Antibiotic Resistance

pSHAG-MAGIC 2 contains 3 antibiotic resistance markers. (See Table 1)

Table 1: Antibiotic Resistance Conveyed by pSHAG-MAGIC 2

Antibiotic	Concentration	Utility
Chloramphenicol	50µg/ml	Bacterial selection marker (shRNA insert)
Kanamycin	optional	Bacterial selection marker (vector)
Puromycin		Mammalian selectable marker

The pSHAG-MAGIC expression vector is a self-inactivating (SIN) MSCV retroviral vector, containing a NheI/XbaI deletion in the U3 region of the 3' LTR. Self-inactivating MSCV particles can be produced by transfection into commonly available retroviral packaging lines.

The sequence of pSHAG-MAGIC2

5' MSCV LTR- ~80-2000

Packaging signal-

U6 promoter + 27nt leader sequence...2003-2515

5' mir30 context...2515-2634 Cloning site [XhoI-EcoRI] 2634-2646

3' mir30 context...2646-2760

U6 terminator...2760-2764

Barcode cloning site [MfeI-MluI]... 2765-2799

Chloramphenicol resistance gene...2809-3763

PGK-PURO-3862-4987 (3862-4374-pgk, 4391-4987-puro)

3' MSCV-“SIN”-LTR-4988-5500

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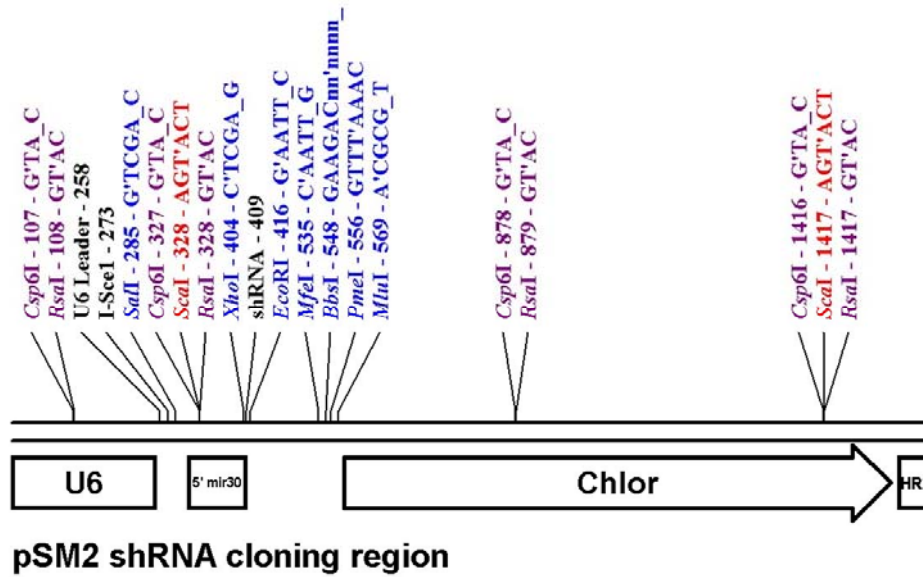
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See Appendix 1 for restriction analysis

Figure 5: pSM2 cloning region



See Appendix 2 for restriction analysis

Appendix1

Restriction analysis of pSM2 vector

SspI

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GAAGGGTTGG AATGGTCTCC CGCGGGGTCG ACAGGCTTA TAATATTTAA

BstXI

51 ATCGCACACA TAAAACCAT GCTGTTGGTG TGTCTATTAA ATCGGCAACT
TAGCGTGTGT ATTTTGGTA CGACAACCAC ACAGATAATT TAGCCGTTGA

PvuI

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AfeI BspHI

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GCTTTGTTTCG CGAGTACTCG GGCTTACCAG CTCGGGCTAG AAGGGGTAGC

SgrAI

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EcoRV

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AfIII

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AfeI

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AfIII Bsu36I

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1601 GAAAGATGTC GAGCGGATCG CTCACAACCA GTCGGTAGAT GTCAAGAAGA
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BstEII

1651 GACGTTGGGT TACCTTCTGC TCTGCAGAAT GGCCAACCTT TAACGTCGGA
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1701 TGGCCGCGAG ACGGCACCTT TAACCGAGAC CTCATCACCC AGGTTAAGAT
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SexAI

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BsrGI

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EcoNI

BglII

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CspCI

BamHI

CspCI

XcmI

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BtsI

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PmlI

DrallI

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AseI

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NdeI

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Sall

HincII

AccI

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Scal

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HpaI

HincII

XhoI

PspXI

EcoRI

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MluI

MfeI

BbsI

PmeI

AflIII

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MslI

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SnaBI

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AclI

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PfIMI

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SspI

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AgeI

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4001 TCCGTTCTTT GGTGGCCCCT TCGCGCCACC TTCTACTCCT CCCCTAGTCA
AGGCAAGAAA CCACCGGGGA AGCGCGGTGG AAGATGAGGA GGGGATCAGT

4051 GGAAGTTCCC CCCC GCCCCG CAGCTCGCGT CGTGCAGGAC GTGACAAATG
CCTTCAAGGG GGGGCGGGG GTCGAGCGCA GCACGTCCTG CACTGTTTAC

BssSI

B1pI

4101 GAAGTAGCAC GTCTACTAG TCTCGTGCAG ATGGACAGCA CCGCTGAGCA
CTTCATCGTG CAGAGTGATC AGAGCACGTC TACCTGTCGT GGCGACTCGT

StuI

4151 ATGGAAGCGG GTAGGCCTTT GGGGCAGCGG CCAATAGCAG CTTTGCTCCT
TACCTTCGCC CATCCGGAAA CCCCCTCGCC GGTATCGTC GAAACGAGGA

4201 TCGCTTTCTG GGCTCAGAGG CTGGGAAGGG GTGGGTCCGG GGGCGGGCTC
AGCGAAAGAC CCGAGTCTCC GACCCTTCCC CACCCAGGCC CCCGCCGAG

4251 AGGGGCGGGC TCAGGGGCGG GCGGGCGCC CGAAGGTCCT CCGGAGGCC
TCCCCGCCG AGTCCCCGCC CCGCCCGCGG GCTTCCAGGA GGCCTCCGGG

4301 GGCATTCTGC ACGTTCAAAG AGCGCACGTC TGCCGCGCTG TTCTCCTCTT
CCGTAAGACG TCGAAGTTT TCGCGTGCAG ACGGCGCGAC AAGAGGAGAA

HindIII

4351 CCTCATCTCC GGGCCTTTTCG ACCTGCAGCC CAAGCTTACC ATGACCGAGT
GGAGTAGAGG CCCGGAAGC TGGACGTCGG GTTTCGAATGG TACTGGCTCA

BsiWI

4401 ACAAGCCAC GGTGCGCCTC GCCACCCGCG ACGACGTCCC CAGGGCCGTA
TGTTCCGGTG CCACGCGGAG CGGTGGGCGC TGCTGCAGGG GTCCCCGCAT

4451 CGCACCTCG CCGCCGCGTT CGCCGACTAC CCCGCCACGC GCCACACCGT
GCGTGGGAGC GCGGCGCAA GCGGCTGATG GGGCGGTGCG CCGTGTGGCA

Ppil

RsrII BstEII Ppil

4501 CGATCCGGAC CGCCACATCG AGCGGGTCAC CGAGCTGCAA GAACTCTTCC
GCTAGGCCTG GCGGTGTAGC TCGCCAGTG GCTCGACGTT CTTGAGAAGG

4551 TCACGCGCGT CGGGCTCGAC ATCGGCAAGG TGTGGGTCGC GGACGACGGC
AGTGCGCGCA GCCCGAGCTG TAGCCGTTCC ACACCCAGCG CCTGCTGCC

SacII

4601 GCCGCGGTGG CGGTCTGGAC CACGCCGGAG AGCGTCGAAG CGGGGGCGGT
CGGCGCCACC GCCAGACCTG GTGCGGCCTC TCGCAGCTTC GCCCCCGCCA

4651 GTTCGCCGAG ATCGGCCCGC GCATGGCCGA GTTGAGCGGT TCCCGGCTGG
CAAGCGGCTC TAGCCGGGCG CGTACCGGCT CAACTCGCCA AGGGCCGACC

BsaXI

StuI BsaXI

4701 CCGCGCAGCA ACAGATGGAA GGCCTCCTGG CGCCGCACCG GCCCAAGGAG
GGCGCGTCGT TGTCTACCTT CCGGAGGACC GCGGCGTGGC CGGGTTCTC

4751 CCCGCGTGGT TCCTGGCCAC CGTCGGCGTC TCGCCCGACC ACCAGGGCAA
GGGCGACCA AGGACCGGTG GCAGCCGCAG AGCGGGCTGG TGTCCCGTT

Bcgl

Bcgl

4801 GGGTCTGGGC AGCGCCGTCG TGCTCCCCGG AGTGGAGGCG GCCGAGCGCG
CCCAGACCCG TCGCGGCAGC ACGAGGGGCC TCACCTCCGC CGGCTCGCGC

PfoI

4851 CCGGGGTGCC CGCCTTCTG GAGACCTCCG CGCCCCGCAA CCTCCCCTTC
GGCCCCACGG GCGGAAGGAC CTCTGGAGGC GCGGGGCGTT GGAGGGGAAG

4901 TACGAGCGGC TCGGCTTAC CGTCACCGCC GACGTGAGG TGCCCGAAGG
ATGCTCGCCG AGCCGAAGTG GCAGTGGCGG CTGCAGCTCC ACGGGCTTCC

SexAI

DraIII

4951 ACCGCGCACC TGGTGCATGA CCCGCAAGCC CCGTGCCTGA CGCCCGCCCC
TGGCGCGTGG ACCACGTACT GGGCGTTCGG GCCACGGACT GCGGGCGGGG

NsiI ClaI

BfrBI

5001 ACGACCCGCA GCGCCCGACC GAAAGGAGCG CACGACCCCA TGCATCGATA
TGCTGGGCGT CGCGGGCTGG CTTTCTCGC GTGCTGGGGT ACGTAGCTAT

5051 AAATAAAAGA TTTTATTTAG TCTCCAGAAA AAGGGGGGAA TGAAAGACCC
TTTATTTTCT AAAATAAATC AGAGGTCTTT TTCCCCCTT ACTTTCTGGG

5101 CACCTGTAGG TTTGGCAAGC TAGAGAACCA TCAGATGTTT CCAGGGTGCC

GTGGACATCC AAACCGTTCG ATCTCTTGGT AGTCTACAAA GGTCCCACGG

5151 CCAAGGACCT GAAATGACCC TGTGCCTTAT TTGAACTAAC CAATCAGTTC
GGTTCCTGGA CTTTACTGGG ACACGGAATA AACTTGATTG GTTAGTCAAG

Sacl
EcoICRI

5201 GCTTCTCGCT TCTGTTGCGC CGCTTCTGCT CCCCAGCTC AATAAAAGAG
CGAAGAGCGA AGACAAGCGC GCGAAGACGA GGGGCTCGAG TTATTTTCTC

Xmal
SmaI

AscI

5251 CCCACAACCC CTCACTCGGC GCGCCAGTCC TCCGATAGAC TCGTTCGCCC
GGGTGTTGGG GAGTGAGCCG CGCGGTCAGG AGGCTATCTG ACGCAGCGGG

BaeI
BaeI
KpnI
BaeI
BaeI
Acc65I

5301 GGGTACCCGT GTATCCAATA AACCTCTTG CAGTTGCATC CGACTTGTGG
CCCATGGGCA CATAGGTTAT TTGGGAGAAC GTCAACGTAG GCTGAACACC

5351 TCTCGCTGTT CTTGGGAGG GTCTCCTCTG AGTGATTGAC TACCCGTCAG
AGAGCGACAA GGAACCCTCC CAGAGGAGAC TACTAACTG ATGGGCAGTC

5401 CGGGGGTCTT TCATGGGTAA CAGTTTCTTG AAGTTGGAGA ACAACATTCT
GCCCCAGAA AGTACCCATT GTCAAAGAAC TTCAACCTCT GTTTGTAAGA

5451 GAGGGTAGGA GTCGAATCGA GAGAGAGAGA GAGAGAGAGA GAGAGAGAGA
CTCCATCCT CAGCTTAGCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT

PspOMI
ApaI

5501 GAGAGAGAGA GACGTGGGCC CAATTCTGTC AGCCGTTAAG TGTTCTGTG
CTCTCTCTCT CTGCACCCGG GTTAAGACAG TCGGCAATTC ACAAGGACAC

5551 TCACTGAAAA TTGCTTTGAG AGGCTCTAAG GGCTTCTCAG TCGTTACAT
AGTGACTTTT AACGAACTC TCCGAGATTC CCGAAGAGTC ACGCAATGTA

HindIII

5601 CCCTGGCTTG TTGTCCACAA CCGTTAAACC TTAAAAGCTT TAAAAGCCTT
GGGACCGAAC AACAGGTGTT GGCAATTTGG AATTTTCGAA ATTTTCGGAA

5651 ATATATTCTT TTTTTCTTA TAAAACCTAA AACCTTAGAG GCTATTTAAG
TATATAAGAA AAAAAAGAAT ATTTTGAATT TTGGAATCTC CGATAAATTC

AseI

5701 TTGCTGATTT ATATTAATTT TATTGTTCAA ACATGAGAGC TTAGTACGTG
AACGACTAAA TATAATTAATA ATAACAAGTT TGTACTCTCG AATCATGCAC

5751 AAACATGAGA GCTTAGTACG TTAGCCATGA GAGCTTAGTA CGTTAGCCAT

TTGTACTCT CGAATCATGC AATCGGTACT CTCGAATCAT GCAATCGGTA

5801 GAGGGTTT TAG TTCGTAAAC ATGAGAGCTT AGTACGTAA ACATGAGAGC
CTCCCAAATC AAGCAATTTG TACTCTCGAA TCATGCAATT TGTACTCTCG

SnaBI

5851 TTAGTACGTG AACATGAGA GCTTAGTACG TACTATCAAC AGGTTGAACT
AATCATGCAC TTTGTACTCT CGAATCATGC ATGATAGTTG TCCAACCTGA

BclI

5901 GCTGATCAAC AGATCCTCTA CACTAGAAGG GACGCACCGC TAGCAGCGCC
CGACTAGTTG TCTAGGAGAT GTGATCTTCC CTGCGTGGCG ATCGTCGCGG

5951 CCTAGCGGTA TCCTATAAAA AAACACACCG CGCCGCTAGC AGCACCCCTA
GGATCGCCAT AGGATATTTT TTTGTGTGGC GCGGCGATCG TCGTGGGGAT

6001 ATATAAATA ATGTTTTTTA TAAAAATAGT CAGTACCACC CCTACAAAAC
TATATTTTAT TACAAAAAAT ATTTTTATCA GTCATGGTGG GGATGTTTTG

6051 GGTGTCGGCG CGTTGTTGTA GCCGCGCCGA CACCGCTTTT TAAATATCA
CCACAGCCGC GCAACAACAT CGGCGCGGCT GTGGCGAAAA AATTTATAGT

6101 TAAAGAGAGT AAGAGAACT AATTTTTCAT AACACTCTAT TTATAAAGAA
ATTTCTCTCA TTCTCTTGA TAAAAAAGTA TTGTGAGATA AATATTTCTT

6151 AAATCAGCAA AAACCTGTTT TTGCGTGGGG TGTGGTGCTT TTGGTGGTGA
TTTAGTCGTT TTTGAACAAA AACGCACCCC ACACCACGAA AACCACCACT

6201 GAACCACCAA CCTGTTGAGC CTTTTTGTGG AGTGGGTAA ATTATACTAG
CTTGGTGGTT GGACAACCTG GAAAAACACC TCACCCAATT TAATATGATC

BstBI BpII

6251 CGCGTTTCGA ACCCCAGAGT CCCGCTCAGA AGAACTCGTC AAGAAGGCGA
GCGCAAAGCT TGGGGTCTCA GGGCGAGTCT TCTTGAGCAG TTCTTCCGCT

BssSI

6301 TAGAAGGCGA TCGCTGCGA ATCGGGAGCG GCGATACCGT AAAGCACGAG
ATCTTCCGCT ACGCGACGCT TAGCCCTCGC CGCTATGGCA TTTCTGTCTC

SapI

6351 GAAGCGGTCA GCCCATTGCG CGCCAAGCTC TTCAGCAATA TCACGGGTAG
CTTCGCCAGT CGGTAAGCG GCGGTTGAG AAGTCGTTAT AGTGCCCATC

RsrII

6401 CCAACGCTAT GTCCTGATAG CGGTCCGCCA CACCCAGCCG GCCACAGTCG
GGTTGCGATA CAGGACTATC GCCAGGCGGT GTGGGTCGGC CGGTGTCAGC

MsiI

6451 ATGAATCCAG AAAAGCGGCC ATTTTCCACC ATGATATTCG GCAAGCAGGC
TACTTAGGTC TTTTCGCCGG TAAAAGGTGG TACTATAAGC CGTTCGTCCG

BpuEI

6501 ATCGCCATGT GTCACGACGA GATCCTCGCC GTCGGGCATG CGCGCCTTGA
TAGCGGTACA CAGTGCTGCT CTAGGAGCGG CAGCCCGTAC GCGCGGAACT

SapI

6551 GCCTGGCGAA CAGTTCGGCT GGC GCGAGCC CCTGATGCTC TTCGTCCAGA
CGGACCGCTT GTCAAGCCGA CCGCGCTCGG GGACTACGAG AAGCAGGTCT

FalI

6601 TCATCCTGAT CGACAAGACC GGCTTCCATC CGAGTACGTG CTCGCTCGAT
AGTAGGACTA GCTGTTCTGG CCGAAGGTAG GCTCATGCAC GAGCGAGCTA

6651 GCGATGTTTC GCTTGGTGGT CGAATGGGCA GGTAGCCGGA TCAAGCGTAT
CGCTACAAAG CGAACCACCA GCTTACCCGT CCATCGGCCT AGTTCGCATA

6701 GCAGCCGCCG CATTGCATCA GCCATGATGG ATACTTTCTC GGCAGGAGCA
CGTCGGCGGC GTAACGTAGT CGGTACTACC TATGAAAGAG CCGTCCTCGT

6751 AGGTGAGATG ACAGGAGATC CTGCCCCGGC ACTTCGCCCA ATAGCAGCCA
TCCACTCTAC TGTCTCTAG GACGGGGCCG TGAAGCGGGT TATCGTCGGT

FspI

6801 GTCCCTTCCC GCTTCAGTGA CAACGTCGAG CACAGCTGCG CAAGGAACGC
CAGGGAAGGG CGAAGTCACT GTTGCGAGCTC GTGTCGACGC GTTCCTTGCG

6851 CCGTCGTGGC CAGCCACGAT AGCCGCGCTG CCTCGTCCTG CAGTTCATTC
GGCAGCACCG GTCGGTGCTA TCGGCGCGAC GGAGCAGGAC GTCAAGTAAG

DrdI

6901 AGGGCACCGG ACAGGTCGGT CTTGACAAAA AGAACCGGGC GCCCTGCGC
TCCCGTGGCC TGTCAGCCA GAACTGTTTT TCTTGCCCG CGGGGACGCG

6951 TGACAGCCGG AACACGGCGG CATCAGAGCA GCCGATTGTC TGTTGTGCC
ACTGTCGGCC TTGTGCCGCC GTAGTCTCGT CGGCTAACAG ACAACACGGG

7001 AGTCATAGCC GAATAGCCTC TCCACCCAAG CGGCCGGAGA ACCTGCGTGC
TCAGTATCGG CTTATCGGAG AGGTGGGTTT GCCGGCCTCT TGGACGCACG

BsaBI

BclI

7051 AATCCATCTT GTTCAATCAT GC GAAACGAT CCTCATCCTG TCTCTTGATC
TTAGGTAGAA CAAGTTAGTA CGCTTTGCTA GGAGTAGGAC AGAGA ACTAG

BglII

7101 AGATCT

Appendix 2: Restriction Analysis of pSM2 cloning region

EcoO109I

EarI Sau96I

1 GCAGGAAGAG GGCCTATTC CCATGATTCC TTCATATTTG CATATACGAT
CGTCCTTCTC CCGGATAAAG GGTACTAAGG AAGTATAAAC GTATATGCTA

AseI

51 ACAAGGCTGT TAGAGAGATA ATTAGAATTA ATTTGACTGT AAACACAAAG
TGTTCCGACA ATCTCTCTAT TAATCTTAAT TAAACTGACA TTTGTGTTTC

Tsp45I

101 ATATTAGTAC AAAATACGTG ACGTAGAAAG TAATAATTTT TTGGGTAGTT
TATAATCATG TTTTATGCAC TGCATCTTTC ATTATTAAAG AACCCATCAA

NdeI

151 TGCAGTTTTT AAAATTATGT TTTAAAATGG ACTATCATAT GCTTACCGTA
ACGTCAAAAA TTTTAATACA AAATTTTACC TGATAGTATA CGAATGGCAT

201 ACTTCAAAGT ATTTGATTT CTTGGCTTTA TATATCTTGT GGAAAGGACG
TGAACCTTCA TAAAGCTAAA GAACCGAAAT ATATAGAACA CCTTTCCTGC

Sall

HincII

AccI

Bsp1286I

TseI

BfaI

BsiHKAI

BbvI

SpeI

BfaI

251 AACACCGTG CTCGCTTCGG CAGCACATAT ACTAGTCGAC TAGGGATAAC
TTTGTGGCAC GAGCGAAGCC GTCGTGTATA TGATCAGCTG ATCCCTATTG

Scal

AcuI

BsgI

301 AGGGTAATTG TTTGAATGAG GCTTCAGTAC TTTACAGAAT CGTTGCCTGC
TCCCATTAAC AAACCTACTC CGAAGTCATG AAATGTCTTA GCAACGGACG

HpaI

BseYI

AcuI HincII

351 ACATCTTGA AACACTTGCT GGGATTACTT CTTCAAGTTA ACCCAACAGA
TGTAGAACCT TTGTGAACGA CCCTAATGAA GAAGTCCAAT TGGGTTGTCT

XhoI

SmlI

AvaI

EcoRI

PspXI

ApoI

401 AGGCTCGAGC AACCAGAATT CAAGGGGCTA CTTTAGGAGC AATTATCTTG
TCCGAGCTCG TTGGTCTTAA GTTCCCCGAT GAAATCCTCG TTAATAGAAC

451 TTTACTAAAA CTGAATACCT TGCTATCTCT TTGATACATT TTTACAAAGC
AAATGATTTT GACTTATGGA ACGATAGAGA AACTATGTAA AAATGTTTCG

MfeI BbsI

501 TGAATTAATA TGGTATAAAT TAAATCACTT TTTTCAATTG GAAGACTAAT
ACTTAATTTT ACCATATTTA ATTTAGTGAA AAAAGTTAAC CTTCTGATTA

MluI
AflIII
HgaI TaqII TspGWI
PmeI Hpy99I BsiEI Tsp45I
551 GCGTTTAAAC ACGCGGCGAC GCGTTCGACC GAATAAAACC TGTGACGGAA
CGCAAATTTG TGCGCCGCTG CGCAAGCTGG CTTATTTTGG ACACTGCCTT

BsmFI
BsiFI
BsiFI NciI
601 GATCACTTCG CAGAATAAAT AAATCCTGGT GTCCCTGTTG ATACCGGGAA
CTAGTGAAGC GTCTTATTTA TTTAGGACCA CAGGGACAAC TATGGCCCTT

Sau96I BsmBI
PaeI PfiMI BsmAI
651 GCCCTGGGCC AACTTTTGGC GAAAATGAGA CGTTGATCGG CACGTAAGAG
CGGGACCCGG TTGAAAACCG CTTTACTCT GCAACTAGCC GTGCATTCTC

MmeI MspI NciI
701 GTTCCAATT TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT
CAAGGTTGAA AGTGGTATTA CTTTATTCTA GTGATGGCCC GCATAAAAAA

DdeI
Bpu10I TspRI
751 GAGTTGTCGA GATTTTCAGG AGCTAAGGAA GCTAAAATGG AGAAAAAAT
CTCAACAGCT CTAAGTCC TCGATTCTT CGATTTTACC TCTTTTTTTA
M E K K I Frame 1

BsrI
TspRI SfaNI
801 CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT AAAGAACATT
GTGACCTATA TGGTGGCAAC TATATAGGGT TACCGTAGCA TTTCTTGTA
T G Y T T V D I S Q W H R K E H F Frame 1

PvuII
MspA1I
851 TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG
AACTCCGTAA AGTCAGTCAA CGAGTTACAT GGATATTGGT CTGGCAAGTC
E A F Q S V A Q C T Y N Q T V Q Frame 1

BceAI
901 CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT
GACCTATAAT GCCGGAAAAA TTTCTGGCAT TTCTTTTTAT TCGTGTTCAA
L D I T A F L K T V K K N K H K F Frame 1

BspEI
BsaWI
FokI
FauI BsmI BstF5I
951 TTATCCGGCC TTTATTCACA TTCTTGCCCG CCTGATGAAT GCTCATCCGG
AATAGGCCGG AAATAAGTGT AAGAACGGGC GGACTACTTA CGAGTAGGCC
Y P A F I H I L A R L M N A H P E Frame 1

SnaBI BsrDI
1001 AATTACGTAT GGCAATGAAA GACGGTGAGC TGGTGATATG GGATAGTGTT
TTAATGCATA CCGTTACTTT CTGCCACTCG ACCACTATAC CCTATCACAA
L R M A M K D G E L V I W D S V Frame 1

AclI BtgZI
1051 CACCCTTGTT ACACCGTTTT CCATGAGCAA ACTGAAACGT TTTCATCGCT
GTGGGAACAA TGTGGCAAAA GGTACTCGTT TGACTTTGCA AAAGTAGCGA
H P C Y T V F H E Q T E T F S S L Frame 1

BpmI Hpy99I
1101 CTGGAGTGAA TACCACGACG ATTTCCGGCA GTTCTACAC ATATATTCGC
GACCTCACTT ATGGTGCTGC TAAAGGCCGT CAAAGATGTG TATATAAGCG
W S E Y H D D F R Q F L H I Y S Q Frame 1

1151 AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC TAAAGGGTTT
TTCTACACCG CACAATGCCA CTTTGGACC GGATAAAGGG ATTTCCCAA
D V A C Y G E N L A Y F P K G F Frame 1

DdeI
BspCNI
BseMII
BsmAI TaqII
BsmBI PfiMI PstI BsrI
1201 ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG
TAACTCTTAT ACAAAAAGCA GAGTCGGTTA GGGACCCACT CAAAGTGGTC
I E N M F F V S A N P W V S F T S Frame 1

MscI
EaeI
1251 TTTTGATTTA AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA
AAAATAAAT TTGCACCGGT TATACCTGTT GAAGAAGCGG GGGCAAAAGT
F D L N V A N M D N F F A P V F T Frame 1

StyI
NcoI MspA1I
BtgI SspI SfaNI
1301 CCATGGGCAA ATATTATACG CAAGGCGACA AGGTGCTGAT GCCGCTGGCG
GGTACCCGTT TATAATATGC GTTCCGCTGT TCCACGACTA CGGCGACCGC
M G K Y Y T Q G D K V L M P L A Frame 1

BceAI BclI BsmI
1351 ATTCAGGTTT ATCATGCCGT TTGTGATGGC TTCCATGTCG GCAGAATGCT
TAAGTCCAAG TAGTACGGCA AACACTACCG AAGGTACAGC CGTCTTACGA
I Q V H H A V C D G F H V G R M L Frame 1

Scal BtgZI Faul
1401 TAATGAATTA CAACAGTACT GCGATGAGTG GCAGGGCGGG GCGTAATTTT
ATTACTTAAT GTTGTGATGA CGTACTCAC CGTCCCGCCC CGCATTAAAA
N E L Q Q Y C D E W Q G G A * Frame 1

Bsp1286I
Bme1580I
BanI

1451 TTTAAGGCAG TTATTGGTGC CCTTAAACGC CTGGTTGCTA CGCCTGAATA
AAATTCCGTC AATAACCACG GGAATTTGCG GACCAACGAT GCGGACTTAT

FokI BstYI
BstF5I ApeI AlwI BsiEI

1501 AGTGATAATA AGCGGATGAA TGGCAGAAAT TCGGATCTCG ACCGCGTTTG
TCACTATTAT TCGCCTACTT ACCGTCTTTA AGCCTAGAGC TGGCGCAAAC

AlwNI EarI

1551 GGCGGTGGCT CCCTGCCACG CGGCTCCGAA CAGAAGCTGA TCTCCGAAGA
CCGCCACCGA GGGACGGTGC GCCGAGGCTT GTCTTCGACT AGAGGCTTCT

BstYI
AlwI

1601 GGATCTGATT
CCTAGACTAA

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