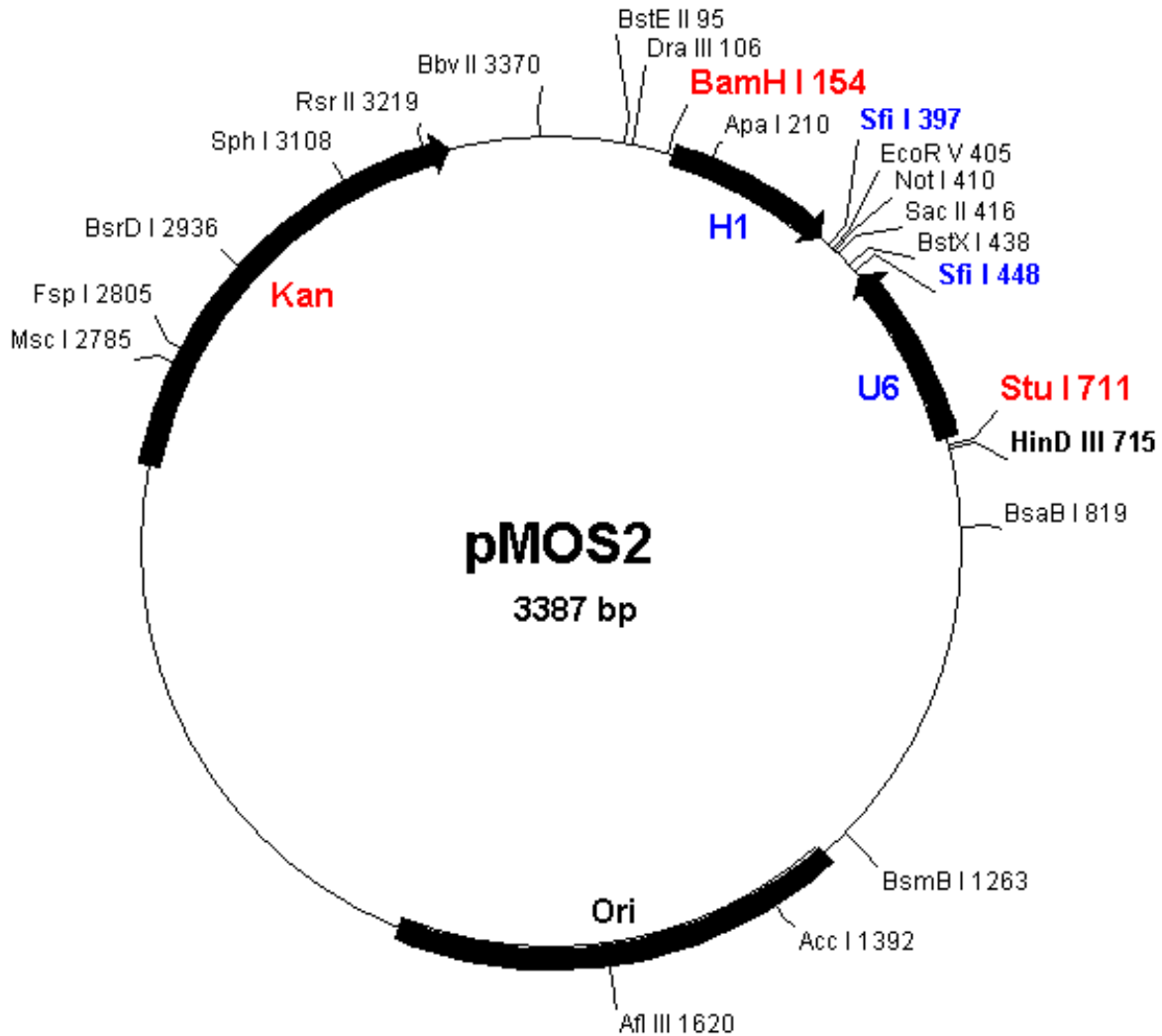


Vector: pMOS2 (for stepwise cloning of pMPBOS knockdown vector)

Antibiotic Selection: Kan

Creator(s): Chen Zhao, MD, Molecular Oncology Lab of University of Chicago Medical Center

Date of Construction: March 2012



Sfi I Site Stuffer and siRNA Cassette Design:

```
h1>>> ggccaaaacGGCCgatatcGCGGCCGCGgaaacagctatgaCCAtgacgcTGGCCgtttttggcc
cgggttttgCCGGctatagcgcgcgcctttgtcgatactggtactgccaacgggcaaaaccgg <<<U6
```

Sfi I Digestion

5' -GGCCAAAA	A (n19-27nt) TTTT	TGGCC-3'
3' -CCGGT	TTTT (n19-27nt) A	AAAACGG-5'

pMOS2 Full-Length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCCTACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTG
 GAGCCATGGCTGGTGACCACGTCGTGGAATGCCCTTC**GaattTAATTC**AGCACCTGCACATGGGAC**GTCGA****CGATC**agctta
 attcgaacgctgacgtcatcaaccgctccaaggaatcgccggccagtgctactaggcgggaacacccagcgcgctgccc
 cctggcaggaagatggctgtgagggacaggggagtgccgacctgcaatatttgcattgctgctatgtgttctgggaaatcacc
 ataaacgtgaaatgtctttggatttgggaatcttataagttctgtatgagaccacagatcgccaaaacggccgatatcGCC
 GCCCGgaaacagctatgaCCAtgacgcTggccgttttggcctcctttccacaagatatataaagccaagaaatcgaatac
 tttcaagttagcgttaagcatatgatagttccatttttaaacataaatttttaaaactgcaaaactaccaagaatattactttc
 tacgtcacgtattttgtaactaatatctttgtgttttaacagtaaaatttaacttctctctaacagccttctgtatcgtat
 atgcaaatatgaaggaatcatgggaaataggccctcttctctgcccagcctt**AGGCCTAAGCTT**GCCTAATCGGACGAAAAAA
 TGACCATGATTACGCCAAGCTCCAATTTCGCCCTATAGTGAAGTTCGATTACAATTCAGTGGCCGTCGTTTTTACCCGGATCTGC
 ATCGCAGGATGCTGCTGGCTACCCCTGTGGAACACCTACATCTGTATTAAACGAAGCGCTGGCATTGACCCCTGAGTGATTTTTTC
 TCTGGTCCCGCCGCATCCATACCGCCAGTTGTTTTACCCCTCACAACTCCAGTAACCGGGCATGTTTCATCATCAGTAACCCG
 TATCGTGAGCATCCTCTCTCGTTTTCATCGGTATCATTACCCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGTGACC
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 TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAA
 GCCCGTCAGGGCGCTCAGCGGGTGTGGCGGGTGTGGGGCGCAGCCATGACCCAGTACGTAGCGATAGCGGAGTGTATA
 CTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGG
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 CAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCA
 AAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
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 CTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTG
 TAGGTATCTCAGTTCCGTTGAGTTCGTTCCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCCAGCCGACCGCTGCGCC
 TTATCCGGTAACTATCGTCTTGAAGTCCAACCCGGTAAAGACACAGCTTATCGCCACTGGCAGCAGCCATGGTAACAGGATTA
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 TATCTGCGCTCTGCTGAAGCGGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC
 GGTGGTTTTTTTTGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTGATCTTTTTCTACGGGGT
 CTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTTGGTTCATGAGATTATCAAAAAGGATCTTCCACTAGATCCTTTTT
 AAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
 GCACCTATCTCAGCGATCTGTCTATTTCTGTTTCCATAGTTGCC**GAC**TCCC**GTC**ATTCAAATATGTATCCGCTCATGAG
 ACAATAACCCGTATAAATGCTTCAATAATAT**ATG**ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAG
 AGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCCGCGTGTTCGGCTGTCAGCGCAGGGGCGCC
 CGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCAC
 GACGGCGTTCCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
 CAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTG
 ATCCGGCTACCTGCCAATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTGCGA
 TCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACGTTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGC
 GAGGATCTCGTGTGACCCATGGCGATGCCCTGCTTGGCCGAATATCATGGTGGAAAATGGCCGCTTTTTCTGGATTTCATCGACT
 GTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
 GGCTGACCGCTTCTCGTCTTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCTTCTATCGCTTCTTGCAGAGTTCT
 TTCT**TGACCTTCTGCTTCA**AAGaatt

Unique enzymes in pMOS2:

BstE II	G`GNAC,C	95
PflI I	CCAN,NNN`NTGG	106
Dra III	CAC,NNN`GTG	106
Bsm I	GAATG,C 7	116
Bsg I	GTGCAG 22/20	120
BamH I	G`GATC,C	154
Bsp120 I	G`GGCC,C	206
Apa I	G,GGCC`C	210
BssH II	G`CGCC,C	236
Ssp I	AAT ATT	295
Bsa I	GGTCTC 7/11	371
EcoR V	GAT ATC	405
Not I	GC`GGCC,GC	410
Sac II	CC,GC`GG	416
BstX I	CCAN,NNNN`NTGG	438
EcoO109 I	RG`GNC,CY	687
Bsu36 I	CC`TNA,GG	706
Stu I	AGG CCT	711
Hind III	A`AGCT,T	715
BsaB I	GATNN NNATC	819
Eco47 III	AGC GCT	876
Psp1406 I	AA`CG,TT	947
BsmB I	CGTCTC 7/11	1263
Acc I	GT`MK,AC	1392
Bst1107 I	GTA TAC	1393
Xca I	GTA TAC	1393

Afl III	A`CRYG,T	1620
Msc I	TGG CCA	2785
Fsp I	TGC GCA	2805
BsrD I	GCAATG, 8	2936
Bsp1286 I	G,DGCH`C	3006
Sph I	G,CATG`C	3108
NgoM I	G`CCGG,C	3203
Nae I	GCC GGC	3205
Rsr II	CG`GWC,CG	3219
Bbs I	GAAGAC 8/12	3369
Bbv II	GAAGAC 7/11	3370
Number of enzymes = 37		

The following enzymes do not cut in pMOS2:

Acc65 I	Afl II	Age I	Apo I	Asc I
Asp718	Ava I	Avr II	Bcl I	Bgl II
Blp I	BseR I	BsiW I	BspM II	BsrG I
Cla I	Eco72 I	EcoN I	EcoR I	Esp I
Fse I	Hinc II	Hind II	Hpa I	Kpn I
Mlu I	Mun I	Nhe I	Nru I	Nsi I
PaeR7 I	Pme I	Pml I	PpuM I	PspA I
Pst I	Pvu I	Sac I	Sal I	Sca I
Sma I	SnaB I	Spe I	Spl I	Srf I

