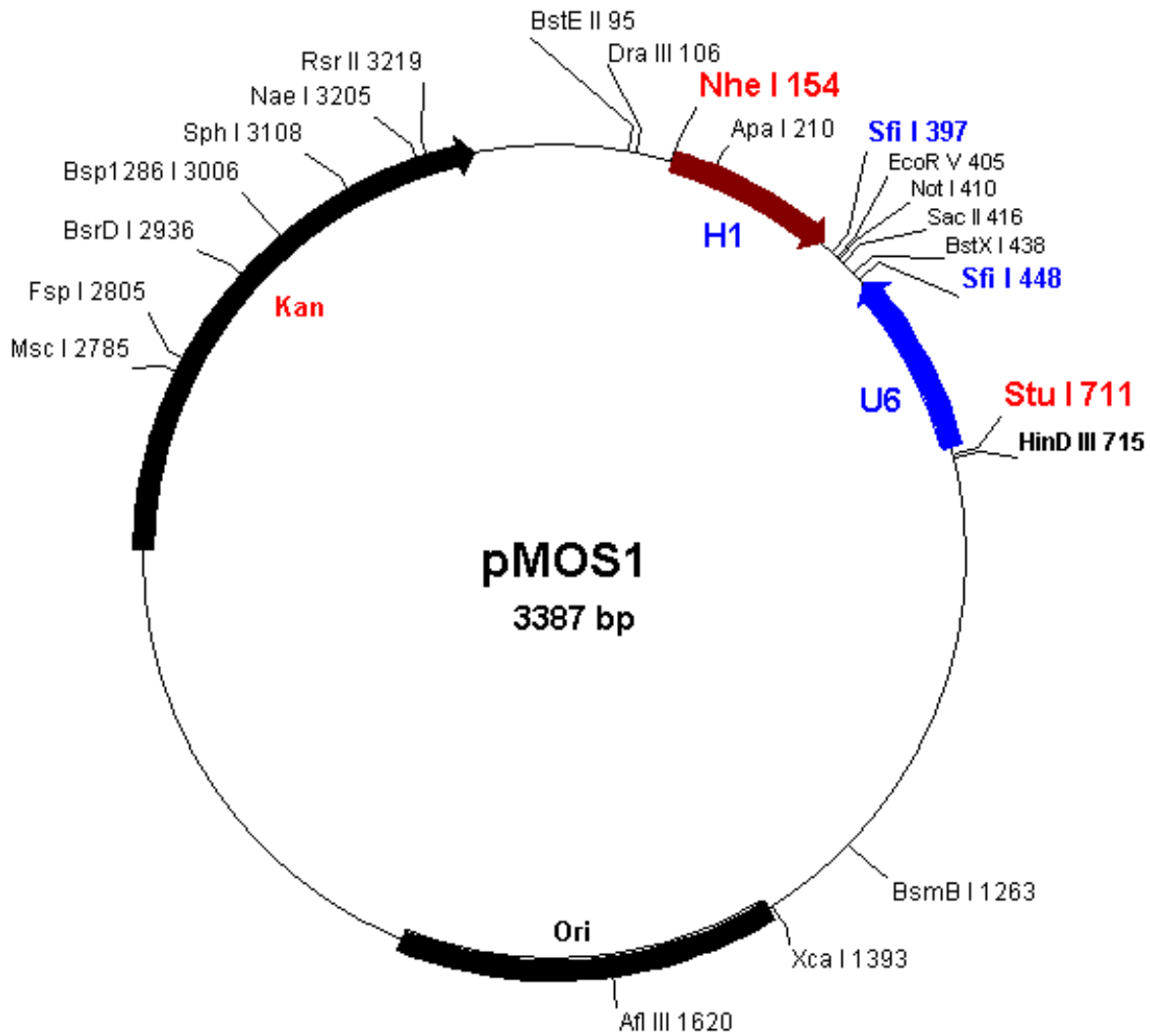


Vector: pMOS1 (for stepwise cloning of pMPBOS knockdown vector)

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>>> ggccaaaacGGCCgatatacGCGGCCGCggaacagctatgaCCAtgacgcTGGCCgtttttggcc
 cccgtttttGCGGctatagcgcggcgccctttgtogatactggtactgogaccggcaaaaaccgg <<<U6

Sfi I Digestion

5' -GGCCAAA	A (n19-27nt) TTTT	TGGCC-3'
3' -CCGGT	TTTT (n19-27nt) A	AAAACCGG-5'

pMOS1 Full-Length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTG
 GAGCCATGGCTGGTACCACGTCGTGGAATGCCCTTC**GaattAATTC**AGCACCTGCACATGGGAC**GTCGA****CTAGC**agctta
 attcgaacgctgacgtcatcaaccgctccaaggaatcgccggccagtgctactaggcgggaacaccagcgcgctgccc
 cctggcaggaagatggctgtgagggacaggggagtgccgacctgcaatatttgcattgctgctatgtgttctgggaaatcacc
 ataaacgtgaaatgtctttggatttgggaatcttataagttctgtatgagaccacagatcgccaaaacggccgatatcGCCG
 GCCCGgaaacagctatgaCCAtgacgcTggccgttttggcctcctttccacaagatatataaagccaagaaatcgaatac
 tttcaagttagcgttaagcatatgatagtcatttttaaaacataattttaaaactgcaaaactcccaagaaattattactttc
 tacgtcacgtattttgtactaataatctttgtgttttacagtcataatttctctctctaacagaccttctgattcgtat
 atgcaaatatgaaggaatcatgggaaatagccctcttctcctgcccagcctt**AGGCCTAAGCTT**GCCTAATCGGACGAAAAAA
 TGACCATGATTACGCCAAGCTCCAATTGCCCCTATAGTGAGTCGTATTACAATTCAGTGGCCGTCGTTTTTACCCGGATCTGC
 ATCGCAGGATGCTGCTGGCTACCCCTGTGGAACACCTACATCTGTATTAAACGAAGCGCTGGCATTGACCCCTGAGTGATTTTTTC
 TCTGGTCCC GCCGATCCATAACCGCCAGTTGTTTACCCCTCACAACTCCAGTAACCGGGCATGTTTCATCATCAGTAACCCG
 TATCGTGAGCATCCTCTCTCGTTTTATCGGTATCATTACCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGTGACC
 AACAGGAAAAAACCGCCCTTAAACATGGCCCGCTTTATCAGAAGCCAGACATTAACGCTTCTGGAGAACTCAACGAGCTGG
 ACGCGGATGAACAGGCAGACATCTGTGAATCGCTTACGACCACGCTGATGAGCTTTACCGCAGCTGCCCTCGCGCTTTCCG
 TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAA
 GCCCGTCAGGGCGCTCAGCGGGTGTGGCGGGTGTGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATA
 CTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGG
 AGAAAATACCGCATCAGGCGCTCTTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCCGGCTGCGGCGAGCGGTAT
 CAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCA
 AAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
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 CTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTG
 TAGGTATCTCAGTTCCGTTGAGTTCGTTCCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCCAGCCGACCGCTGCGCC
 TTATCCGGTAACTATCGTCTTGTAGTCCAACCCGGTAAAGACAGCACTTATCGCCACTGGCAGCAGCCATGGTAAACAGGATTA
 CGAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCCCTAACACTAGCGGTACACTAGAAGGACAGTATTTGG
 TATCTGCGCTCTGCTGAAGCCGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGTATCCGGCAAACAAACCACCGCTGGTAGC
 GGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTTCTACGGGGT
 CTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTTGGTTCATGAGATTATCAAAAAGGATCTTCCACTAGATCCTTTTT
 AAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
 GCACCTATCTCAGCGATCTGTCTATTTCTGTTTCCATAGTTGCC**GAC**TCCC**GTC**ATTCAAATATGTATCCGCTCATGAG
 ACAATAACCCGTATAAATGCTTCAATAATAT**ATG**ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAG
 AGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCCGCGTGTCCGGCTGTCAGCGCAGGGGCGCC
 CGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCAC
 GACGGCGTTCCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
 CAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTG
 ATCCGGCTACCTGCCAATTGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTGCGA
 TCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACGTTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGC
 GAGGATCTCGTGTGACCCATGGCGATGCCCTGCTTGGCCGAATATCATGGTGGAAAATGGCCGCTTTTTCTGGATTATCGACT
 GTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
 GGCTGACCGCTTCTCGTGTCTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCTTCTATCGCTTCTTACAGAGTTCT
 TTCT**TGACCTTCTGCTTC**AAGaatt

Unique enzymes in pMOS1:

BstE II	G`GTNAC,C	95	Afl III	A`CRYG,T	1620		
PflI I	CCAN,NNN`NTGG	106	Msc I	TGG CCA	2785		
Dra III	CAC,NNN`GTG	106	Fsp I	TGC GCA	2805		
Bsm I	GAATG,C 7	116	BsrD I	GCAATG, 8	2936		
Bsg I	GTGCAG 22/20	120	Bsp1286 I	G,DGCH`C	3006		
Nhe I	G`CTAG,C	154	Sph I	G,CATG`C	3108		
Bsp120 I	G`GGCC,C	206	NgoM I	G`CCGG,C	3203		
Apa I	G,GGCC`C	210	Nae I	GCC GGC	3205		
BssH II	G`CGCC,C	236	Rsr II	CG`GWC,CG	3219		
Ssp I	AAT ATT	295	Bbs I	GAAGAC 8/12	3369		
Bsa I	GGTCTC 7/11	371	Bbv II	GAAGAC 7/11	3370		
EcoR V	GAT ATC	405	Number of enzymes = 37				
Not I	GC`GGCC,GC	410	The following enzymes do not cut in pMOS1:				
Sac II	CC,GC`GG	416	Acc65 I	Afl II	Age I	Apo I	Asc I
BstX I	CCAN,NNNN`NTGG	438	Asp718	Ava I	Avr II	BamH I	Bcl I
EcoO109 I	RG`GNC,CY	687	Bgl II	Blp I	BseR I	BsiW I	BspM II
Bsu36 I	CC`TNA,GG	706	BsrG I	Clal I	Eco72 I	EcoN I	EcoR I
Stu I	AGG CCT	711	Esp I	Fse I	HinC II	Hind II	Hpa I
HinD III	A`AGCT,T	715	Kpn I	Mlu I	Mun I	Nru I	Nsi I
BsaB I	GATNN NNATC	819	PaeR7 I	Pme I	Pml I	PpuM I	PspA I
Eco47 III	AGC GCT	876	Pst I	Pvu I	Sac I	Sal I	Sca I
Psp1406 I	AA`CG,TT	947	Sma I	SnaB I	Spe I	Spl I	Srf I
BsmB I	CGTCTC 7/11	1263					
Acc I	GT`MK,AC	1392					
Bst1107 I	GTA TAC	1393					
Xca I	GTA TAC	1393					

