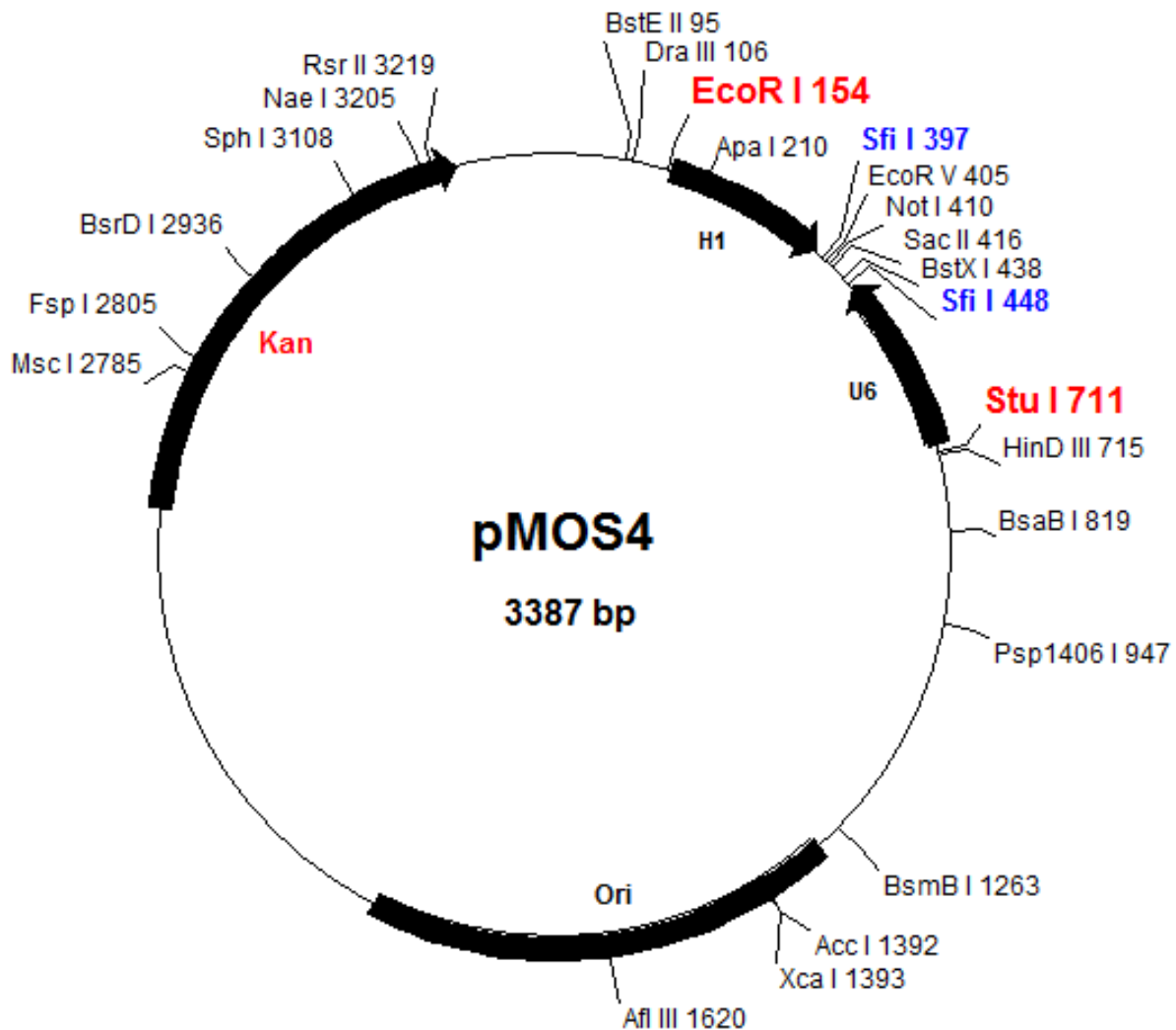


Vector: pMOS4 (for stepwise cloning of pMPBOS knockdown vector)

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>>> ggccaaaacGGCCgatataCGGGCCGCggaaacagctatgaCCAtgaagcTGGCCgttttggcc  
ccggttttgCCGGctatagcgcggcgccctttgtcgatactggtactggaacggcaaaaccgg <<<U6
```

Sfi I Digestion

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

pMOS4 Full-Length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTG
 GAGCCATGGCTGGTACCACGTCGTGGAATGCCCTTC**GaattAATTC**AGCACCTGCACATGGGAC**GTCGA****GAATTC**agctta
 attcgaacgctgacgtcatcaaccgctccaaggaatcgccggccagtgctactaggcgggaacacccagcgcgctgccc
 cctggcaggaagatggctgtgagggacaggggagtgccgacctgcaatatttgcattgctgctatgtgttctgggaaatcacc
 ataaacgtgaaatgtctttggatttgggaatcttataagttctgtatgagaccacagatcgcccaaacggccgatatcGCCG
 GCCCGgaaacagctatgaCCAtgacgcTggccgttttggcctcctttccacaagatatataaagccaagaaatcgaatac
 tttcaagttacggttaagcatatgatagtcatttttaaaacataatttttaaaactgcaaacctaccaagaaattattactttc
 tacgtcacgtattttgtactaataatctttgttttacagtcaaattaatttctaattatctctctaacagccttctgtagat
 atgcaaatatgaaggaatcatgggaaataggccctcttctctgcccagcctt**AGGCCTAAGCTT**GCCTAATCGGACGAAAAAA
 TGACCATGATTACGCCAAGCTCCAATTTCGCCCTATAGTGAGTCGTATTACAATTCACCTGGCCGTCGTTTTTACCCGGATCTGC
 ATCGCAGGATGCTGCTGGCTACCCCTGTGGAACACCTACATCTGTATTAAACGAAGCGCTGGCATTGACCCCTGAGTGATTTTTTC
 TCTGGTCCCGCCGCATCCATACCGCCAGTTGTTTTACCCCTCACAACTCCAGTAACCGGGCATGTTTCATCATCAGTAACCCG
 TATCGTGAGCATCCTCTCTCGTTTTATCGGTATCATTACCCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGTGACC
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 TATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGTATCCGGCAAACAAACCACCGTGGTAGC
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 GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCC**GAC**TCCC**GTC**ATTCAAATATGTATCCGCTCATGAG
 ACAATAACCCGTATAAATGCTTCAATAATAT**ATG**ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAG
 AGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTTGTTCCGGCTGTCAGCGCAGGGGCGCC
 CGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCAC
 GACGGCGTTCCCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
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 GAGGATCTCGTGTGACCCATGGCGATGCCCTGCTTGCCTGAATATCATGGTGGAAAAATGGCCGCTTTTTCTGGATTTCATCGACT
 GTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATG
 GGCTGACCGCTTCTCGTCTTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCTTCTATCGCTTCTTGCAGAGTTCT
 TTCT**TGACCTTCTGCTTCA**AAGaatt

Unique enzymes in pMOS4:

BstE II	G`GTNAC,C	95
PflM I	CCAN,NNN`NTGG	106
Dra III	CAC,NNN`GTG	106
Bsm I	GAATG,C 7	116
Bsg I	GTGCAG 22/20	120
Apo I	R`AATT,Y	154
EcoR I	G`AATT,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
BerD 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 = 38		

The following enzymes do not cut in pMOS4:

Acc65 I	Afl II	Age I	Asc I	Asp718
Ava I	Avr II	BamH I	Bcl I	Bgl II
Blp I	BseR I	BsiW I	BspM II	BsrG I
Cla I	Eco72 I	EcoN 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	Xba I

		2383	2397	2450		Apo I	R`AATT,Y	1	Asc I	GG`CGCG,CC	-
Msl I	(2)	333	3140			Ase I	AT`TA,AT	2	Asp718	G`GTAC,C	-
Msp I	(16)	812	959	1266	1300	Ava I	C`YCGR,G	-	Ava II	G`GWC,C	2
		1827	1974	2000	2190	Avr II	C`CTAG,G	-	BamH I	G`GATC,C	-
		2608	2685	2707	2735	Ban I	G`GYRC,C	4	Ban II	G,RCGY`C	2
MspAl I	(6)	2866	2956	3023	3204	Bbe I	G,CGGC`C	2	Bbs I	GAAGAC 8/12	1
		415	1213	1332	1962	Bbv I	GCAGC 13/17	9	Bbv II	GAAGAC 7/11	1
		2207	2809			Bcl I	T`GATC,A	-	Bcn I	CC,S`GG	7
Nae I	(1)	3205				Bfa I	C`TA,G	3	Bgl I	GCCN,NNN`NGGC	2
Nar I	(2)	284	2703			Bgl II	A`GATC,T	-	Blp I	GC`TNA,GC	-
Nci I	(7)	812	960	1266	1301	Bpm I	CTGGAG 22/20	2	Bsa I	GGTCTC 7/11	1
		2000	2707	2867		BsaA I	YAC GTR	3	BsaB I	GATNN NNATC	1
Nco I	(2)	87	3135			BsaH I	GR`CG,YC	4	BsaJ I	C`CNGG,G	7
Nde I	(2)	512	1443			BsaW I	W`CCGG,W	3	BseR I	GAGGAG 16/14	-
NgoM I	(1)	3203				Bsg I	GTGCAG 22/20	1	BsiC I	TT`CG,AA	2
Nla III	(19)	20	91	143	304	BsiE I	CG,RY`CG	5	BsiHKA I	G,WGCW`C	4
		435	679	747	967	BsiW I	C`GTAC,G	-	Bsm I	GAATG,C 7	1
		1031	1094	1259	1364	BsmA I	GTCTC`/9	3	BsmB I	CGTCTC 7/11	1
		1624	2344	2541	2922	BsmF I	GGGAC 15/19	4	BsoF I	GC`N,GC	32
Nla IV	(9)	3108	3139	3165		Bsp120 I	G`GGCC,C	1	Bsp1286 I	G,DGCH`C	1
		85	208	285	909	BspH I	T`CATG,A	2	BspM I	ACCTGC 10/14	3
		1652	1691	2463	2704	BspM II	T`CCGG,A	-	Bsr I	ACT,GG`	11
		2739				BsrB I	GAG CGG	4	BsrD I	GCAATG, 8	1
Not I	(1)	410				BsrG I	T`GTAC,A	-	Bssh II	G`CGCG,C	1
Nsp7524 I	(5)	300	963	1255	1620	Bsss I	C`TCGT,G	2	Bst1107 I	GTA TAC	1
		3104				BstB I	TT`CG,AA	2	BstE II	G`GTNAC,C	1
NspB II	(6)	415	1213	1332	1962	BstN I	CC`W,GG	5	BstU I	CG CG	13
		2207	2809			BstX I	CCAN,NNNN`NTGG	1	BstY I	R`GATC,Y	7
NspH I	(5)	304	967	1259	1624	Bsu36 I	CC`TNA,GG	1	Cac8 I	GCN NGC	22
		3108				Cfr10 I	R`CCGG,Y	2	ClA I	AT`CG,AT	7
Pal I	(18)	208	391	400	412	Csp6 I	G`TA,C	4	Dde I	C`TNA,G	7
		442	451	688	711	Dpn I	GA TC	14	DpnII	`GATC,	14
		799	1095	1635	1646	Dra I	TTT AAA	4	Dra III	CAC,NNN`GTG	1
		1664	2098	2611	2785	Drd I	GACNN,NN`NNGTC	3	Dsa I	C`CRYG,G	3
		3176	3203			Eae I	Y`GGCC,R	9	Eag I	C`GGCC,G	3
PflM I	(1)	106				Ear I	CTCTTC 7/10	4	Eco47 III	AGC GCT	1
Ple I	(2)	786	1999			Eco57 I	CTGAAG 21/19	3	Eco72 I	CAC GTG	-
Psp1406 I	(1)	947				EcoN I	CCTNN`N,NNAGG	-	EcoO109 I	RG`GNC,CY	1
Pvu II	(2)	1213	2809			EcoR I	G`AATT,C	1	EcoR II	`CCWGG,	5
Rsa I	(4)	69	592	1428	3009	EcoR V	GAT ATC	1	Ehe I	GGC GCC	2
Rsr II	(1)	3219				Esp I	GC`TNA,GC	-	Fnu4H I	GC`N,GC	32
Sac II	(1)	416				Fok I	GGATG 14/18	8	Fse I	GG,CCGG`CC	-
Sap I	(3)	1504	3047	3257		Fsp I	TGC GCA	1	Gdi II	`YGGC,CG	11
Sau3A I	(14)	385	814	2186	2261	Gsu I	CTGGAG 21/19	2	Hae I	WGG CCW	6
		2272	2280	2358	2370	Hae II	R,CGGC`Y	5	Hae III	GG CC	18
		2475	2874	2952	3033	Hga I	GACGC 9/14	5	HgiA I	G,WGCW`C	4
Sau96 I	(6)	3042	3120			HgiE II	ACCNNNNNNGGT -1/132	-	Hha I	G,CG`C	21
		206	207	687	907	Hinc II	GTY RAC	-	Hind II	GTY RAC	-
		1094	3219			Hind III	A`AGCT,T	1	Hinf I	G`ANT,C	11
ScrF I	(12)	249	812	960	1266	HinI I	GR`CG,YC	4	HinP I	G`CG,C	21
		1301	1648	1769	1782	Hpa I	GTT AAC	-	Hpa II	C`CG,G	16
		2000	2707	2867	3090	Hph I	GGTGA 12/11	6	Kas I	G`GCGC,C	2
Sec I	(7)	87	194	247	413	Kpn I	G,GTAC`C	-	Mae I	C`TA,G	3
		1780	2866	3135		Mae II	A`CG,T	11	Mae III	`GTNAC,	15
SfaN I	(16)	820	828	924	1002	Mbo I	`GATC,	14	Mbo II	GAAGA 12/11	9
		1064	1287	1420	1458	Mlu I	A`CGCG,T	-	Mme I	TCCRAC 25/23	2
		1496	1716	2662	2917	Mnl I	CCTC 10/10	20	Msc I	TGG CCA	1
		3001	3065	3133	3340	Mse I	T`TA,A	15	Msl I	CAYNN NNRTG	2
Sfc I	(3)	771	1885	2076		Msp I	C`CG,G	16	MspAl I	CMG CKG	6
Sfi I	(2)	397	448			Mun I	C`AATT,G	-	Nae I	GCC GGC	1
Sph I	(1)	3108				Nar I	GG`CG,CC	2	Nci I	CC`S,GG	7
Ssp I	(1)	295				Nco I	C`CATG,G	2	Nde I	CA`TA,TG	2
Stu I	(1)	711				NgoM I	G`CCGG,C	1	Nhe I	G`CTAG,C	-
Sty I	(3)	87	194	3135		Nla III	,CATG`	19	Nla IV	GGN NCC	9
Taq I	(11)	33	118	149	168	Not I	GC`GGCC,GC	1	Nru I	TCG CGA	-
		485	1720	2816	2972	Nsi I	A,TGCA`T	-	Nsp7524 I	R`CATG,Y	5
		2996	3032	3194		NspB II	CMG CKG	6	NspH I	R,CATG`Y	5
Tfi I	(7)	199	357	672	1176	PaeR7 I	C`TCGA,G	-	Pal I	GG CC	18
		1595	3188	3322		PflM I	CCAN,NNN`NTGG	1	Ple I	GAGTC 9/10	2
Tsp45 I	(8)	95	214	578	1061	Pme I	CTTT AAAC	-	Pml I	CAC GTG	-
		1274	1369	2823	3129	PpuM I	RG`GWC,CY	-	Psp1406 I	AA`CG,TT	1
Tth111 I	(3)	101	1367	2821		PspA I	C`CCGG,G	-	Pst I	C,TGCA`G	-
Tth111 II	(5)	1082	2209	2218	2248	Pvu I	CG,AT`CG	-	Pvu II	CAG CTG	2
		3139				Rsa I	GT AC	4	Rsr II	CG`GWC,CG	1
Vsp I	(2)	123	620			Sac I	G,AGCT`C	-	Sac II	CC,GC`GG	1
Xca I	(1)	1393				Sal I	G`TCGA,C	-	Sap I	GCTCTC 8/11	3
Xho II	(7)	814	2261	2272	2358	Sau3A I	`GATC,	14	Sau96 I	G`GNC,C	6
		2370	2874	3120		Sca I	AGT ACT	-	ScrF I	CC`N,GG	12
Xma III	(3)	398	410	2609		Sec I	C`CNGG,G	7	SfaN I	GCATC 9/13	16
Xmn I	(3)	114	124	1180		Sfc I	C`TRYA,G	3	Sfi I	GGCCN,NNN`NGGCC	2
						Sma I	CCC GGG	-	SnaB I	TAC GTA	-
						Spe I	A`CTAG,T	-	Sph I	G,CATG`C	1
						SpI I	C`GTAC,G	-	Srf I	GCCC GGGC	-
						Ssp I	AAT ATT	1	Stu I	AGG CCT	1
						Sty I	C`CWGG,G	3	Taq I	T`CG,A	11
						Tfi I	G`AWT,C	7	Tsp45 I	`GTSAC,	8
						Tth111 I	GACN`N,NGTC	3	Tth111 II	CAARCA 16/14	5
						Vsp I	AT`TA,AT	2	Xba I	T`CTAG,A	-
						Xca I	GTA TAC	1	Xcm I	CCANNNN,N`NNNNTGG-	-
						Xho I	C`TCGA,G	-	Xho II	R`GATC,Y	7
						Xma I	C`CCGG,G	-	Xma III	C`GGCC,G	3

Site usage in pMOS4:

Aat II	G,ACGT`C	2	Acc I	GT`MK,AC	1
Acc65 I	G`GTAC,C	-	Aci I	C`CG,C	-
Afl II	C`TTAA,G	-	Afl III	A`CRYG,T	1
Age I	A`CCGG,T	-	Aha II	GR`CG,YC	4
Ahd I	GACNN,N`NNGTC	2	Alu I	AG CT	-
Alw I	GGATC 8/9	9	AlwN I	CAG,NNN`CTG	2
Apa I	G,GGCC`C	1	Apal I	G`TGCA,C	2