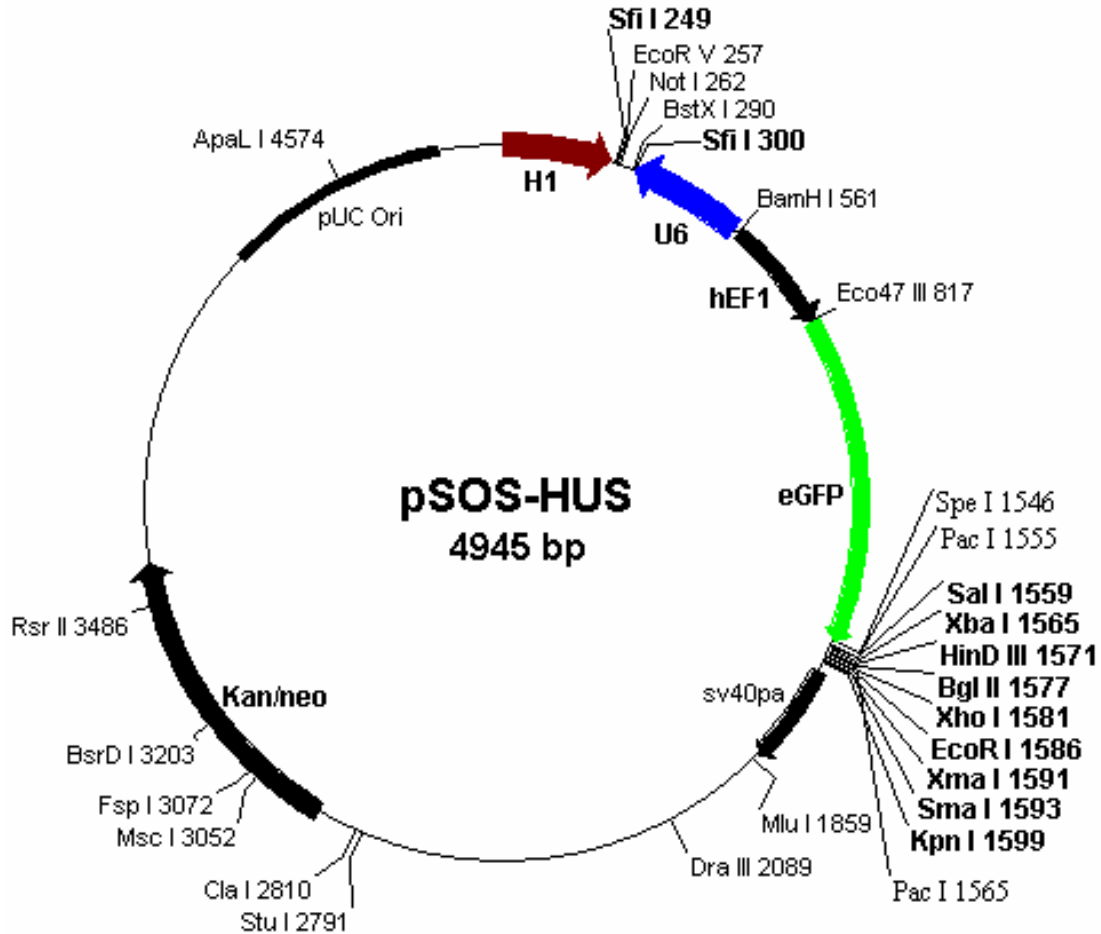


Vector: pSOS-HUS

Antibiotic Selection: Kan

Creator(s): Qing Luo, Molecular Oncology Lab of The University of Chicago Medical Center

Date of Construction: May and Nov, 2005



Sfi I Site Stuffer and siRNA Cassette Design:

H1>>> **ggccaaaacGGCC** EcoRV **gatatc** Not I **GCGGCCGC** M13Rev **cgaaacagctatga** BstX I **CCATgacgcTGGCCg** **ttttggcc** <<<U6
ccggttttgCCGG **ctatagcgcgcctttgtcgatactggtactgcga** ccggcaaaaccgg

Sfi I Digestion

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

pSOS-HUS Sequence (Sfi I sites, human EF1 α Promoter)
(By Qing Luo, 5/05 and 11/05)

TAGTTATTA**Cagctt**aattcgaacgctgacgtcatcaaccgctccaaggaatcgcgggcccagtgctactagggcgggaaca
cccagcgcgctgcccctggcaggaagatggctgtgagggacaggggagtgggcgcctgcaatatttgcatgctcgctatgt
gttctgggaaatcaccataaacgtgaaatgtctttggatttgggaaatcttataagttctgtatgagaccacagatc**ggccaa**
aacggcc gatatcGCGGCCGCggaacagctatgaCCAtgacgt
ggccggttttggcctcctttccacaagatatataaagccaagaaatcgaaatactttcaagttacggtaagcatatgatagtc
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gtgtttacagtcaaattaatttcaattatctcttaacagccttgtatcgtatgcaaatatgaaggaatcatgggaaata
ggcctcttctgcccacctt**ggatcc**gga tct gcat**CGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTC**
CCCGAGAAGTTGGGGGGAGGGTTCGGCAATTGAAACCGGTGCCCTAGAGAAGGTGGCGCGGGGTAACCTGGGAAAGTGTATGTCG
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ACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGTCTTTAAAAAACCTCCACACCTCC
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CGCGTAAATTTGTAAGCGTTAATATTTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTTAACCAATAGGCC
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CCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCC

		4838	4875			Hpa I	(1)	1736				
Cfr10 I	(7)	656	821	984	2190	Hpa II	(26)	565	582	657	822	
		3289	3470	3753				862	925	985	1516	
Cla I	(1)	2810						1592	1615	2191	2875	
Csp6 I	(6)	443	706	1262	1544			2952	2974	3002	3133	
		1596	3275					3223	3290	3471	3746	
Dde I	(9)	1437	1455	1574	2453			3754	3846	4320	4510	
		2752	3633	4082	4205			4536	4683			
		4614				Hph I	(10)	170	847	852	1180	
Dpn I	(27)	239	563	569	576			1204	1333	2091	3149	
		1335	1483	1521	1579			4040	4156			
		1610	1619	1632	2809	Kas I	(2)	135	2969			
		2813	2832	3143	3221	Kpn I	(1)	1599				
		3302	3311	3389	3765	Mae I	(12)	71	664	813	1547	
		3781	4140	4152	4230			1566	1621	1822	2240	
		4238	4249	4324				2793	3808	4142	4395	
DpnII	(27)	237	561	567	574	Mae II	(16)	30	186	429	434	
		1333	1481	1519	1577			771	898	1111	1282	
		1608	1617	1630	2807			2033	2045	2088	2198	
		2811	2830	3141	3219			3086	3273	4054	4187	
		3300	3309	3387	3763	Mae III	(14)	66	351	430	1016	
		3779	4138	4150	4228			1505	1761	2262	2274	
		4236	4247	4322				3090	3396	4086	4352	
Dra I	(5)	380	393	1675	4114			4468	4531			
		4133				Mbo I	(27)	237	561	567	574	
Dra III	(1)	2089						1333	1481	1519	1577	
Drd I	(3)	2044	2997	4786				1608	1617	1630	2807	
Dsa I	(5)	265	832	2702	2743			2811	2830	3141	3219	
		3402						3300	3309	3387	3763	
Eae I	(10)	241	250	262	292			3779	4138	4150	4228	
		905	1294	2876	3050			4236	4247	4322		
		3441	3468			Mbo II	(15)	119	537	1077	1122	
Eag I	(3)	250	262	2876				1125	1318	2228	2456	
Ear I	(4)	549	2440	3314	3524			3330	3540	3622	3789	
Eco47 III	(1)	817						3989	4159	4232		
Eco57 I	(6)	986	1032	1229	3115	Mlu I	(1)	1859				
		3547	4347			Mme I	(3)	2065	4496	4680		
EcoN I	(1)	667				Mnl I	(35)	113	313	551	632	
EcoO109 I	(2)	539	4068					721	842	923	929	
EcoR I	(1)	1586						1022	1160	1172	1223	
EcoR II	(15)	99	877	1002	1114			1343	1652	1691	1700	
		1189	1243	2507	2562			2117	2448	2726	2732	
		2579	3355	3934	4037			2758	2766	2769	2781	
		4727	4740	4861				2821	2885	3021	3378	
EcoR V	(1)	257						3570	3776	3808	4090	
Ehe I	(2)	137	2971					4460	4711	4784		
Fnu4H I	(40)	262	265	795	993	Msc I	(1)	3052				
		1052	1074	1358	1365	Mse I	(22)	15	379	392	472	
		1416	1419	1513	1750			1551	1555	1601	1605	
		2269	2291	2305	2743			1674	1735	1856	1877	
		2746	2879	2931	2942			1888	1900	1911	1928	
		3032	3037	3074	3115			2026	2297	4113	4127	
		3202	3205	3208	3444			4132	4184			
		3540	3581	3595	3709	Msl I	(3)	185	1320	3407		
		4048	4063	4274	4480	Msp I	(26)	565	582	657	822	
		4483	4548	4691	4846			862	925	985	1516	
Fok I	(7)	861	1227	2646	2837			1592	1615	2191	2875	
		3294	3319	3773				2952	2974	3002	3133	
Fsp I	(1)	3072						3223	3290	3471	3746	
Gdi II	(12)	242	249	251	261			3754	3846	4320	4510	
		263	291	906	1293			4536	4683			
		2875	2877	3440	3467	MspAl I	(7)	267	808	2471	2745	
Gsu I	(4)	1277	1517	3754	3809			3076	4305	4550		
Hae I	(6)	303	2791	3052	4414	Mun I	(2)	649	1723			
		4866	4877			Nae I	(3)	2192	3472	3755		
Hae II	(6)	139	819	2240	2248	Nar I	(2)	136	2970			
		2973	4648			Nci I	(8)	863	1517	1592	1593	
Hae III	(28)	60	243	252	264			2974	3134	3747	4511	
		294	303	540	907	Nco I	(3)	832	2702	3402		
		1007	1296	1408	1939	Nde I	(1)	364				
		2081	2736	2742	2748	NgoM I	(3)	2190	3470	3753		
		2791	2878	3052	3443	Nla III	(22)	156	287	531	836	
		3470	3979	4036	4069			1070	1100	1295	1490	
		4414	4848	4866	4877			1535	2403	2543	2615	
Hga I	(6)	295	2307	3679	3758			2706	2844	3189	3375	
		4199	4777					3406	3432	3788	4172	
HgiA I	(4)	1443	3083	3273	4578			4892	4943			
HgiE II	(1)	4299				Nla IV	(22)	60	137	563	586	
Hha I	(30)	90	92	98	138			661	713	871	1409	
		606	678	818	1123			1597	2128	2149	2161	
		1164	1480	2239	2247			2352	2513	2585	2971	
		2273	2295	2304	2317			3006	3854	3933	3978	
		2348	2964	2972	3036			4821	4860			
		3073	3339	3599	3775	Not I	(1)	262				
		3861	4264	4373	4547	Nsi I	(3)	2545	2617	4945		
		4647	4714			Nsp7524 I	(5)	152	2539	2611	3371	
Hinc II	(2)	1561	1736					4888				
Hind II	(2)	1561	1736			NspB II	(7)	267	808	2471	2745	
Hind III	(1)	1571						3076	4305	4550		
Hinf I	(14)	51	209	524	1562	NspH I	(5)	156	2543	2615	3375	
		2016	2038	2448	3455			4892				
		3589	3641	3699	3736	Pac I	(2)	1555	1605			
		4518	4914			PaeR7 I	(1)	1581				
HinI I	(5)	30	136	2970	3672	Pal I	(28)	60	243	252	264	
		3751						294	303	540	907	
HinP I	(30)	88	90	96	136			1007	1296	1408	1939	
		604	676	816	1121			2081	2736	2742	2748	
		1162	1478	2237	2245			2791	2878	3052	3443	
		2271	2293	2302	2315			3470	3979	4036	4069	
		2346	2962	2970	3034			4414	4848	4866	4877	
		3071	3337	3597	3773	Ple I	(2)	2024	2456			
		3859	4262	4371	4545	Psp1406 I	(1)	771				
		4645	4712			PspA I	(1)	1591				

Pvu I	(1)	577			Bgl II	A`GATC,T	1	Blp I	GC`TNA,GC	-	
Pvu II	(3)	808	2471	3076	Bpm I	CTGGAG 22/20	4	Bsa I	GGTCTC 7/11	2	
Rsa I	(6)	444	707	1263	1545	BsaA I	YAC GTR	3	BsaB I	GATNN NNATC	3
		1597	3276			BsaH I	GR`CG,YC	5	BsaJ I	C`CNNG,G	26
Rsr II	(1)	3486				BsaW I	W`CCGG,W	9	BseR I	GAGGAG 16/14	2
Sac II	(2)	268	2746			Bsg I	GTGCAG 22/20	4	BsiC I	TT`CG,AA	2
Sal I	(1)	1559				BsiE I	CG,RX`CG	6	BsiHKA I	G,WGCW`C	4
Sap I	(2)	3314	3524			BsiW I	C`GTAC,G	-	Bsm I	GAATG,C 7	2
Sau3A I	(27)	237	561	567	574	BsmA I	GTCTC`/9	5	BsmB I	CGTCTC 7/11	-
		1333	1481	1519	1577	BsmF I	GGGAC 15/19	9	BsoF I	GC`N,GC	40
		1608	1617	1630	2807	Bsp120 I	G`GGCC,C	1	Bsp1286 I	G,DGCH`C	2
		2811	2830	3141	3219	BspH I	T`CATG,A	2	BspM I	ACCTGC 10/14	3
		3300	3309	3387	3763	Bspm II	T`CCGG,A	1	Bsr I	ACT,GG`	11
		3779	4138	4150	4228	BsrB I	GAG CGG	5	BsrD I	GCAATG, 8	1
Sau96 I	(12)	58	59	539	1006	BsrG I	T`GTAC,A	-	Bssh II	G`CGCG,C	1
		1407	1490	2080	3486	BssS I	C`TCGT,G	4	Bst1107 I	GTA TAC	-
		3931	3977	4035	4068	BstB I	TT`CG,AA	2	BstE II	G`GTNAC,C	-
ScrF I	(23)	101	863	879	1004	BstN I	CC`W,GG	15	BstU I	CG CG	23
		1116	1191	1245	1517	BstX I	CCAN,NNNN`NTGG	1	BstY I	R`GATC,Y	13
		1592	1593	2509	2564	Bsu36 I	CC`TNA,GG	2	Cac8 I	GCN NGC	34
		2581	2974	3134	3357	Cfr10 I	R`CCGG,Y	7	Cla I	AT`CG,AT	1
		3747	3936	4039	4511	Csp6 I	G`TA,C	6	Dde I	C`TNA,G	9
		4729	4742	4863		Dpn I	GA TC	27	DpnII	`GATC,	27
Sec I	(26)	46	99	265	557	Dra I	TTT AAA	5	Dra III	CAC,NNN`GTG	1
		726	832	862	1002	Drd I	GACNN,NN`NNGTC	3	Dea I	C`CRYG,G	5
		1165	1189	1244	1591	Eae I	Y`GGCC,R	10	Eag I	C`GGCC,G	3
		2507	2579	2702	2737	Ear I	CTCTC 7/10	4	Eco47 III	AGC GCT	1
		2743	2792	3133	3402	Eco57 I	CTGAAG 21/19	6	Eco72 I	CAC GTG	-
		3807	3934	3935	4037	EcoN I	CCTNN`N,NNAGG	1	EcoO109 I	RG`GNC,CY	2
		4038	4728			EcoR I	G`AATT,C	1	EcoR II	`CCWGG,	15
SfaN I	(14)	933	1209	1224	1323	EcoR V	GAT ATC	1	Ehe I	GGC GCC	2
		1788	2551	2623	2929	Esp I	GC`TNA,GC	-	Fnu4H I	GC`N,GC	40
		3184	3268	3332	3400	Fok I	GGATG 14/18	7	Fse I	GG,CCGG`CC	-
		3607	4792			Fsp I	TGC GCA	1	Gdi II	`YGGC,CG	12
Sfc I	(3)	2308	4432	4623		Gsu I	CTGGAG 21/19	4	Hae I	WGG CCW	6
Sfi I	(2)	249	300			Hae II	R,GCCG`Y	6	Hae III	GG CC	28
Sma I	(1)	1593				Hga I	GACGC 9/14	6	HgiA I	G,WGCW`C	4
Spe I	(1)	1546				HgiE II	ACCNNNNNNGGT -1/131	1	Hha I	G,CG`C	30
Sph I	(3)	2543	2615	3375		Hinc II	GTY RAC	2	Hind II	GTY RAC	2
Ssp I	(3)	147	1881	2434		Hind III	A`AGCT,T	1	Hinf I	G`ANT,C	14
Stu I	(1)	2791				HinI I	GR`CG,YC	5	HinP I	G`CG,C	30
Sty I	(7)	46	557	832	2702	Hpa I	GTT AAC	1	Hpa II	C`CG,G	26
		2792	3402	3807		Hph I	GGTGA 12/11	10	Kas I	G`GCGC,C	2
Taq I	(20)	20	337	883	1177	Kpn I	G,GTAC`C	1	Mae I	C`TA,G	12
		1204	1219	1348	1560	Mae II	A`CG,T	16	Mae III	`GTNAC,	14
		1582	2122	2810	3083	Mbo I	`GATC,	27	Mbo II	GAAGA 12/11	15
		3239	3263	3299	3461	Mlu I	A`CCGG,T	1	Mme I	TCCRAC 25/23	3
		3652	3697	3952	4790	Mnl I	CCTC 10/10	35	Msc I	TGG CCA	1
Tfi I	(8)	51	209	524	3455	Mse I	T`TA,A	22	Msl I	CAYNN NNRTG	3
		3589	3699	3736	4914	Msp I	C`CG,G	26	MspAl I	CMG CRG	7
Tsp45 I	(7)	66	430	1016	1505	Mun I	C`AATT,G	2	Nae I	GCC GGC	3
		2262	3090	3396		Nar I	GG`CG,CC	2	Nci I	CC`S,GG	8
Tth111 I	(1)	3088				Nco I	C`CATG,C	3	Nde I	CA`TA,TG	1
Tth111 II	(5)	1316	3406	4266	4296	NgoM I	G`CCGG,C	3	Nhe I	G`CTAG,C	-
		4305				Nla III	,CATG`	22	Nla IV	GSN NCC	22
Vsp I	(1)	472				Not I	GC`GGCC,GC	1	Nru I	TCG CGA	-
Xba I	(2)	1565	1620			Nsi I	A,TGCA`T	3	Nsp7524 I	R`CATG,Y	5
Xho I	(1)	1581				NspB II	CMG CKG	7	NspH I	R,CATG`Y	5
Xho II	(13)	561	567	1333	1577	Pac I	TTA,AT`TAA	2	Paer7 I	C`TCGA,G	1
		1608	1617	3141	3387	Pal I	GG CC	28	Pf1M I	CCAN,NNN`NTGG	-
		3779	4138	4150	4236	Ple I	GAGTC 9/10	2	Pme I	CTTT AAAC	-
		4247				Pml I	CAC GTG	-	PpuM I	RG`GWC,CY	-
Xma I	(1)	1591				Psp1406 I	AA`CG,TT	1	PspA I	C`CCGG,G	1
Xma III	(3)	250	262	2876		Pst I	C,TGCA`G	-	Pvu I	CG,AT`CG	1

Site usage in pSOS-HUS:

Aat II	G,ACGT`C	1	Acc I	GT`MK,AC	1	Sau96 I	G`GNC,C	12	Sca I	AGT ACT	-
Acc65 I	G`GTAC,C	1	Aci I	C`CG,C	62	ScrF I	CC`N,GG	23	Sec I	C`CNNG,G	26
Afl II	C`TTAA,G	-	Afl III	A`CRYG,T	2	SfaN I	GCATC 9/13	14	Sfc I	C`TRYA,G	3
Age I	A`CCGG,T	2	Aha II	GR`CG,YC	5	Sfi I	GGCCN,NNN`NGGCC	2	Sma I	CCC GGG	1
Ahd I	GACNN,N`NNGTC	-	Alu I	AG CT	23	SnaB I	TAC GTA	-	Spe I	A`CTAG,T	1
Alw I	GGATC 8/9	18	AlwN I	CAG,NNN`CTG	2	Sph I	G,CATG`C	3	Sp1 I	C`GTAC,G	3
Apa I	G,GGCC`C	1	Apal I	G`TGCA,C	1	Srf I	GCCC GGGC	-	Ssp I	AAT ATT	-
Apo I	R`AATT,Y	4	Asc I	GG`CGCG,CC	-	Stu I	AGG CCT	1	Sty I	C`CWGG,G	7
Ase I	AT`TA,AT	1	Asp718	G`GTAC,C	1	Taq I	T`CG,A	20	Tfi I	G`AWT,C	8
Ava I	C`YCGR,G	4	Ava II	G`GWC,C	3	Tsp45 I	`GTSAC,	7	Tth111 I	GACN`N,NGTC	1
Avr II	C`CTAG,G	2	BamH I	G`GATC,C	1	Tth111 II	CAARCA 16/14	5	Vsp I	AT`TA,AT	1
Ban I	G`GYRC,C	8	Ban II	G,RCGY`C	4	Xba I	T`CTAG,A	2	Xca I	GTA TAC	-
Bbe I	G,CGCC`C	2	Bbs I	GAAGC 8/12	-	Xcm I	CCANNNN,N`NNNNTGG-	-	Xho I	C`TCGA,G	1
Bbv I	GCAGC 13/17	9	Bbv II	GAAGC 7/11	-	Xho II	R`GATC,Y	13	Xma I	C`CCGG,G	1
Bcl I	T`GATC,A	1	Bcn I	CC,S`GG	8	Xma III	C`GGCC,G	3	Xmn I	GAANN NNVTC	-
Bfa I	C`TA,G	12	Bgl I	GCCN,NNN`NGGC	2						