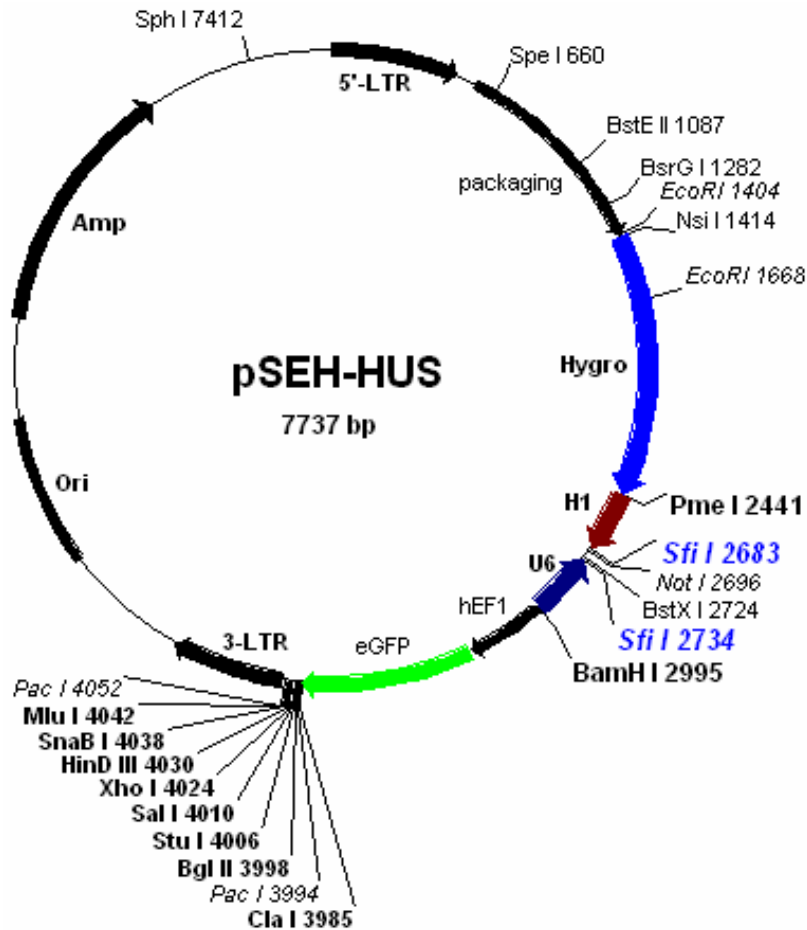


Vector: pSEH-HUS

Antibiotic Selection: Amp

Creator(s): Hong Yin, Molecular Oncology Lab of The University of Chicago

Date of Construction: July, 2007



Sfi I Site Stuffer and siRNA Cassette Design:

H1>>> **ggccaaaacGGCC**^{EcoRV}gatatc^{Not I}GCGGCCG^{M13Rev}Cggaaacagctatga^{BstX I}CCAtgacgc**TGGCCgttttggcc**
cgggttttgCCGGctatagcgccggcgctttgtcgatactggtactgcgaccggaacccgg <<<U6

Sfi I Digestion

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

pSEH-HUS Full-Length Sequence

TGAAAGACCC CACCTGTAGG TTTGGCAAGC TAGCTTAAGT AACGCCATT TGCAAGGCATGGAAAATACATAACTGAGAA TAGAGAAGTT AGATCAAGG
TTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTCCCGGCTCAGGGCCAAGAACAGATGGTCCCAGATGGGTCCTCCG
CCTCAGCAGTTTCTAGAGAACCATCAGATGTTTTCCAGGGTGCCTCAAGGACCTGAAATGACCTGTGCCTTATTTGAACTAACCAATCAGTTCGTTCTCGTCTCT
GTTTCGGCGCTTCTGCTCCCGAGCTCAATAAAGAGCCCAACCCCTCACTCGGCCCGCAGTCCCGCATAGACTGCGCTGCCCGGGTACCCGTTTCCCAAT
AAAGCCTCTTG CTGTTTGCAT CCGAATCGTG GACTCGCTGA TCCTGGGAG GGTCTCCTCAGATTTGATGACTGCCACCTCGGGGGTCTTATTGTTGGAG
GTTCCACCGA GATTTGGAGACCCCTGCCCA GGGACCACCGACCCCGCGC CGGGAGGTAA GCTGGCCAGC GGTCTGTTCTGTTCTGTCTC TGTCTTTGTTG
CTGTTTGTGTCGCGCATTAATGTTTGGCCTCGCTGTGATAGTGTAGTAACTAGCTCTGTATCTGGCCGACCCGTTGGTGGAACTGACGAGTCTGGAACCCCG
GCC CAACCTGGG AGACTCCAGGACTTTGGGGCCGTTTGTGGCCGACTGAGGAAGATCGATGGAAATCCGACCCTCAGGATCAGGATATGTTGTT
CTGGTAGGAGACGAGAACCT AAAACAGTTC CCGCTCCGT CTGAATTTTTGCTTTCCGTT TGGAACCGAAGCCGCGCTC TGTCTGCTG CAGCGCTGCA
GCATCGTTCT GTGTTGTTCT TGTCTGACTGTGTTCTGTA TTTGCTGAA AATTAGGGCC AGACTGTACT CACTCCCTAAGTTTACCTTACCTTACCTG
GAAAGATGTCGAGCGGATCG CTCACAACCA GTCGGTAGAT GTCAAGAAGAGACGTTGGT TACCTTCTGC TCTGCAGAAAT GGCCAACCTTAAACGTGCGA
TGGCCGAGAGACGGCACCTT TAACCGAGC CTCATCACCC AGGTAAAGAT CAAGGCTTTT TCACCTGGCCCGCATGGACA CCAGACAGGTTCCCTACA
TCGTGACCTG GGAAGCCTTG GCTTTTGACCCCTCCTCT GGTCAAGCCCTTTGACAC CTAAAGCCTC GCCTCTCTT CTCCATGGCCCGCTCTCT
CCCTTTGAACCTCTGTTTCGACCCCGCTCCCTTATCCAGCCCTCCTCTCTAGGCCGCGG**GAATTC atgcgat**ACCATGGGCGTGAAGAAAGC
TGAACCTACCCGACGCTGTCTGAGAAGTTTCTGATCGAAAAGTTTCGACAGCGTCTCCGACCTGATGCAGCTCTGGAGGGCGAAGAAATCTCGTGTCTTACGCTC
GATGTAGGAGCGGTGGATATGCTCGGGTAAATAGTATCGCCGATGGTTTCTACAAGATCGTTATGTTTATCGCACCTTGTGATCGGCCGCTCCCGATC
CGGAAGTCTTGACATTTGGGAATTCAGCGAGACCTGACCTATGCATCTCCCGGTCAGACGGGTTGTCAGTGTCAAGAGTCCGCTTGAACCGCACTCCGCG
TGTTCTGACGCGGTTCGGGAGGCGCATGGATGCGATCGCTGCGCCGATCTTAGCCAGACGAGCGGTTTCGGCCATTTCGACCGCAAGGAATCGTCAATACACT
ACATGGCTGATTTTCATATGCGCGATGCTGATCCCATGTTGATCACTGGCAAACTGTGATGAGACACACCGTCACTGCGCTCCGTCGCGCAGGCTCTGATGAGC
TGATGCTTTGGCCAGGACTCGCCGAGTCCGCGACCTCGTGACCGGATTTTCGGCTTCGCAAACTGTTCTGACCGCAAGTGGCCGCAATGACCGGCTATTGTA
CTGGAGCGAGGCGATGTTTCGGGATTCGAATACGAGGTCGCCAACATCTTCTTCGGAGGCCGTTGGTGTGATGGAGCAGCAGACGCGCTACTTCGAGCGG
AGGACTCCGGAGTTCGAGGATCGCCGCGCTCCGGCGTATATCTCCGCAACTTGGTCTTGACCAACTCTATCAGAGCTTGGTTGCGCAATTTCCGATGATGACG
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AGAAGTACTCGCCGATAGTGGAAACCGACGCCCGAGCCTCGTCCGTA **gtttaaacagctt**aattcgaaacgctgacgtcatcaacccg
ctccaaggaatcgcgggcccagtgctactaggcgggaaacacccagcgcgcggtgagccctggcaggaagatggctgtgaggggacag
gggagtgccgcccgtgcaatattgcatgtcgctatgtgtctggaatcaccataaacgtgaaatgtcttggatttggaatc
ttataagttctgatatgagaccacagat**ggccaaaaggcc gatatcGGGGCGCGgaaacagctatgaCCAtgacgct**
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acagtaaaatttaattctaatatctcttaacagccttctgatctgatatagaaatataaggaatcgaggaaataggcctctt
cctgcccagccttggatccgga tet gcat**CGTCCGGTGGCCGTCACTGGGCGAGAGCGCACATCGCCACAGTC**
CCCGAGAAGTTGGGGGAGGGGTTCGGCAATTGAACCCGTTGCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGT
ACTGGCTCCGCTTTTTCGGAGGTTGGGGGAGAACCGTATATAAGTGCAGTAGTTCGCGTGAACGTTCTTTTCGCAACGGGTT
TGCCGCCAGAACACAGCTGCTAGCGTACCGTTCGCCACCAatggt**gagcaagggcgaggagctgttaccgggggtggtgccat**

		4011	4025	4662	5224	Cfr10 I	R`CCGG,Y	8	Cla I	AT`CG,AT	1
		6668				Csp6 I	G`TA,C	13	Dde I	C`TNA,G	21
Tfi I	(12)	442	802	1521	1643	Dpn I	GA TC	36	DpnII	`GATC,	36
		1843	2094	2485	2643	Dra I	TTT AAA	6	Dra III	CAC,NNN`GTG	2
		2958	4702	4959	5099	Drd I	GACNN,NN`NNGTC	4	Dsa I	C`CRYG,G	8
Tsp45 I	(11)	1022	1231	1715	2500	Eae I	Y`GGCC,R	19	Eag I	C`GGCC,G	7
		2864	3450	3939	6273	Ear I	CTCTTC 7/10	7	Eco47 III	AGC GCT	3
		6484	7060	7371		Eco57 I	CTGAAG 21/19	6	Eco72 I	CAC GTG	-
Tth111 I	(6)	390	811	1217	1485	EcoN I	CCTNN`N,NNAGG	3	EcoO109 I	RG`GNC,CY	6
		1929	4491			EcoR I	G`AATT,C	2	EcoR II	`CCWGG,	22
Tth111 II	(10)	146	422	613	632	EcoR V	GAT ATC	4	Ehe I	GGC GCC	6
		1645	3750	4246	5713	Esp I	GC`TNA,GC	-	Fnu4H I	GC`N,GC	51
		5722	5752			Fok I	GGATG 14/18	13	Fse I	GG,CCGG`CC	-
Vsp I	(5)	2906	4767	4895	4954	Fsp I	TGC GCA	2	Gdi II	`YGGC,CG	24
		6189				Gsu I	CTGGAG 21/19	7	Hae I	WGG CCW	8
Xba I	(2)	218	4319			Hae II	R,GCGC`Y	11	Hae III	GG CC	46
Xho I	(1)	4024				Hga I	GACGC 9/14	16	HgiA I	G,WGCW`C	9
Xho II	(10)	2995	3001	3767	3998	HgiE II	ACCNNNNNNGGT -1/132	16	Hha I	G,CG`C	52
		5765	5776	5862	5874	Hinc II	GTY RAC	2	Hind II	GTY RAC	2
		6642	6659			Hind III	A`AGCT,T	1	Hinf I	G`ANT,C	20
Xma I	(2)	397	4498			HinI I	GR`CG,YC	12	HinP I	G`CG,C	52
Xma III	(7)	724	1630	1795	2365	Hpa I	GTT AAC	-	Hpa II	C`CG,G	39
		2684	2696	4017		Hph I	GGTGA 12/11	19	Kas I	G`GCGC,C	6
Xmn I	(3)	4730	4768	6616		Kpn I	G,GTAC`C	2	Mae I	C`TA,G	15
						Mae II	A`CG,T	19	Mae III	`GTNAC,	25
						Mbo I	`GATC,	36	Mbo II	GAAGA 12/11	20
						Mlu I	A`CGCG,T	1	Mme I	TCCRAC 25/23	8
						Mnl I	CCTC 10/10	66	Msc I	TGG CCA	2
						Mse I	T`TA,A	31	Msl I	CAYNN NNRTG	5
						Msp I	C`CG,C	39	MspAl I	CMG CKG	14
						Mun I	C`AATT,G	1	Nae I	GCC GGC	2
						Nar I	GG`CG,CC	6	Nci I	CC`S,GG	18
						Nco I	C`CATG,G	3	Nde I	CA`TA,TG	3
						Ngo I	G`CCGG,C	2	Nhe I	G`CTAG,C	2
						Nla III	,CATG`	31	Nla IV	GGN NCC	41
						Not I	GC`GGCC,GC	2	Nru I	TCG CGA	-
						Nsi I	A,TGCA`T	1	Nsp7524 I	R`CATG,Y	4
						NspB II	CMG CKG	14	NspH I	R,CATG`Y	4
						Pac I	TTA,AT`TAA	2	Paer7 I	C`TCGA,G	1
						Pal I	GG CC	46	Pf1M I	CCAN,NNN`NTGG	-
						Ple I	GAGTC 9/10	3	Pme I	CTTT AAAC	1
						Pml I	CAC GTG	-	PpuM I	RG`GWC,CY	3
						Pep1406 I	AA`CG,TT	3	PspA I	C`CCGG,G	2
						Pst I	C,TGCA`G	4	Pvu I	CG,AT`CG	4
						Pvu II	CAG CTG	4	Rsa I	GT AC	13
						Rsr II	CG`GWC,CG	1	Sac I	G,AGCT`C	2
						Sac II	CC,GC`GG	2	Sal I	G`TCGA,C	1
						Sap I	GCTCTTC 8/11	2	Sau3A I	`GATC,	36
						Sau96 I	G`GNC,C	35	Sca I	AGT ACT	2
						ScrF I	CC`N,GG	40	Sec I	C`CNNG,G	44
						SfaN I	GCATC 9/13	28	Sfc I	C`TRYA,G	10
						Sfi I	GGCCN,NNN`NGGCC	2	Sma I	CCC GGG	2
						SnaB I	TAC GTA	1	Spe I	A`CTAG,T	1
						Sph I	G,CATG`C	1	Spl I	C`GTAC,G	3
						Srf I	GCCC GGGC	-	Ssp I	AAT ATT	-
						Stu I	AGG CCT	1	Sty I	C`CWGG,G	10
						Taq I	T`CG,A	25	Tfi I	G`AWT,C	12
						Tsp45 I	`GTSAC,	11	Tth111 I	GACN`N,NGTC	6
						Tth111 II	CAARCA 16/14	10	Vsp I	AT`TA,AT	5
						Xba I	T`CTAG,A	2	Xca I	GTA TAC	-
						Xcm I	CCANNNN,N`NNNNTGG-	-	Xho I	C`TCGA,G	1
						Xho II	R`GATC,Y	10	Xma I	C`CCGG,G	2
						Xma III	C`GGCC,G	7	Xmn I	GAANN NNITTC	3

Site usage in pSEH-HUS:

Aat II	G,ACGT`C	4	Acc I	GT`MK,AC	1
Acc65 I	G`GTAC,C	2	Aci I	C`CG,C	89
Afl II	C`TTAA,G	3	Afl III	A`CRYG,T	2
Age I	A`CCGG,T	2	Aha II	GR`CG,YC	12
Ahd I	GACNN,N`NNGTC	4	Alu I	AG CT	44
Alw I	GGATC 8/9	19	AlwN I	CAG,NNN`CTG	2
Apa I	G,GGCC`C	1	ApaL I	G`TGCA,C	5
Apo I	R`AATT,Y	3	Asc I	GG`CGCG,CC	2
Ase I	AT`TA,AT	5	Asp718	G`GTAC,C	2
Ava I	C`YCGR,G	8	Ava II	G`GWC,C	15
Avr II	C`CTAG,G	-	BamH I	G`GATC,C	1
Ban I	G`GYRC,C	17	Ban II	G,RCGY`C	7
Bbe I	G,CGGC`C	6	Bbs I	GAAGAC 8/12	-
Bbv I	GCAGC 13/17	14	Bbv II	GAAGAC 7/11	-
Bcl I	T`GATC,A	-	Bcn I	CC,S`GG	18
Bfa I	C`TA,G	15	Bgl I	GCCN,NNN`NGCC	4
Bgl II	A`GATC,T	1	Blp I	GC`TNA,GC	-
Bpm I	CTGGAG 22/20	7	Bsa I	GGTCTC 7/11	7
BsaA I	YAC GTR	2	BsaB I	GATNN NNATC	1
BsaH I	GR`CG,YC	12	BsaJ I	C`CNNG,G	44
BsaW I	W`CCGG,W	10	BseR I	GAGGAG 16/14	5
Bsg I	GTGCAG 22/20	4	BsiC I	TT`CG,AA	1
BsiE I	CG,RY`CG	17	BsiHKA I	G,WGCW`C	9
BsiW I	C`GTAC,G	-	Bsm I	GAATG,C 7	-
BsmA I	GTCTC`/9	22	BsmB I	CGTCTC 7/11	8
BsmF I	GGGAC 15/19	12	BsoF I	GC`N,GC	51
Bsp120 I	G`GGCC,C	1	Bsp1286 I	G,DGCH`C	1
BspH I	T`CATG,A	4	BspM I	ACCTGC 10/14	1
BspM II	T`CCGG,A	4	Bsr I	ACT,GG`	20
BsrB I	GAG CGG	7	BsrD I	GCAATG, 8	2
BsrG I	T`GTAC,A	1	Bssh II	G`CGCG,C	5
BssS I	C`TCGT,G	6	Bst1107 I	GTA TAC	-
BstB I	TT`CG,AA	1	BstE II	G`GTNAC,C	1
BstN I	CC`W,GG	22	BstU I	CG CG	38
BstX I	CCAN,NNNN`NTGG	1	BstY I	R`GATC,Y	10
Bsu36 I	CC`TNA,GG	2	Cac8 I	GCN NGC	43