

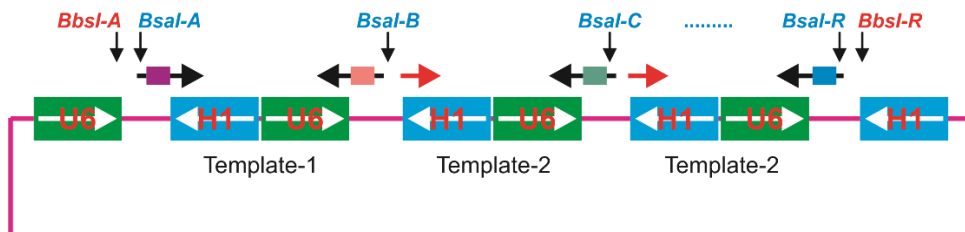
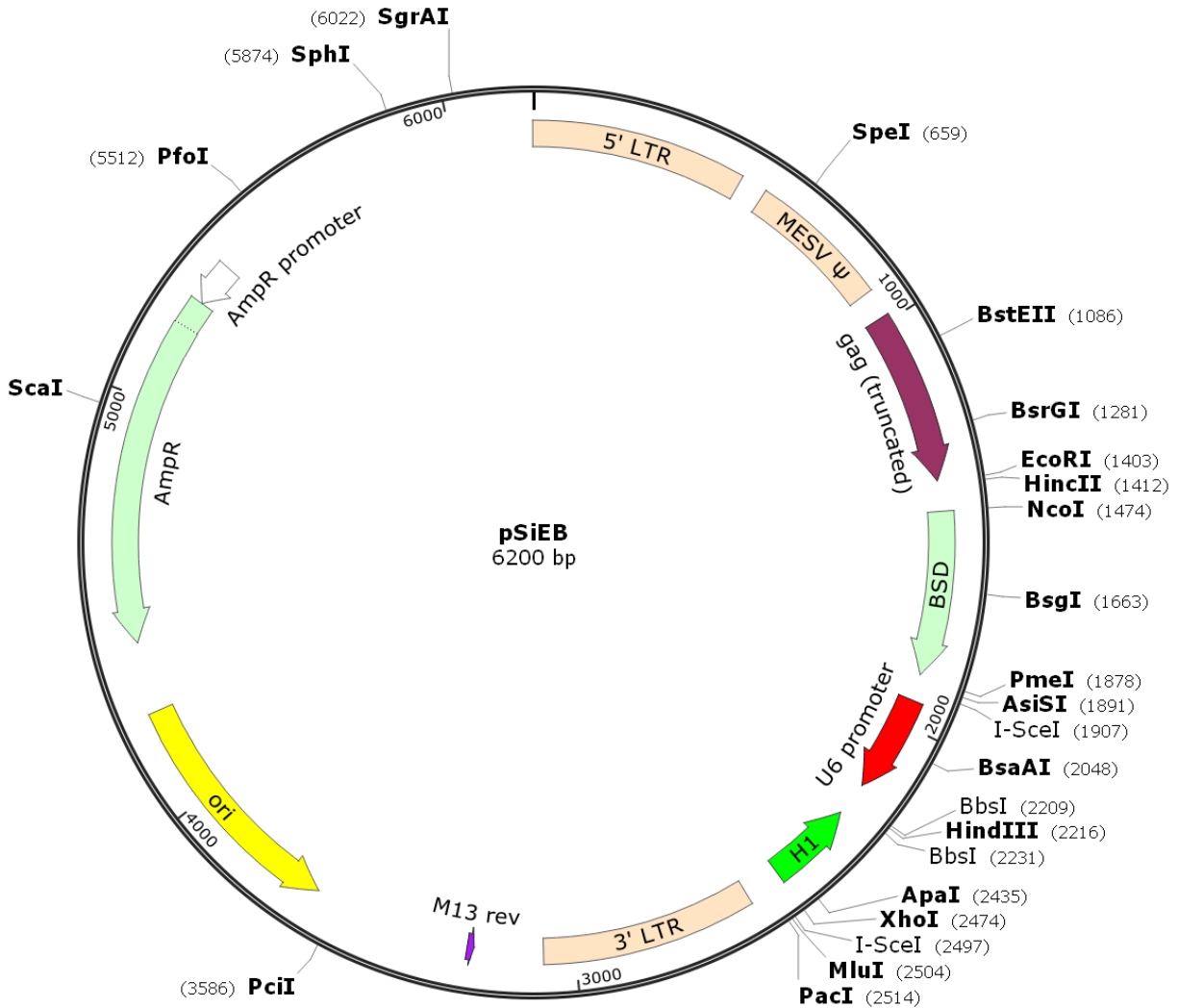
**Vector: pSiEB [Fast Assembly of Multiplex siRNAs (FAMSi) Destination Vector]**

**Antibiotic Selection:** Amp (*BSD* in mammalian cells)

**Creator(s):** Fang He, Zongyue Zeng, Alex J. Li, Ben Luu, Alissa Li & Na Ni @

Molecular Oncology Lab of The University of Chicago Medical Center

**Date of Construction:** February 2020



<b>BbsI-A</b> GAAGACagGGCA CTTCTGtcccgt-5'	<b>BsaI-B</b> GGTCTCGAACGA CCAGAGCTTGCT-5'	<b>BsaI-C</b> GGTCTCGTCCGA CCAGAGCAGGCT-5'	<b>BsaI-D</b> GGTCTCGGCGTA CCAGAGCCGCAT-5'	<b>BbsI-R</b> GAAGACgtTGGC CTTCTGcaaccg-5'
<b>BsaI-A</b> GGTCTCGGGCA CCAGAGCCCGT-5'				<b>BsaI-R</b> GGTCTCGTGGC CCAGAGCACCG-5'

## pSiEB Full-length Sequence

TGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGC AAGGCATGGAAAAATACATAACTGAGAATA  
GAGAAGTTAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCCTCCCCG  
GCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTG  
CCCCAAGGACCTGAAATGACCCGTGTCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCT  
CCCCGAGCTCAATAAAAAGAGCCACAACCCCTCACTCGGCGGCCAGTCCCTCCGATAGACTGCGTCGCCCGGGTACCCGTAT  
TCCCAATAAAGCCTCTTGTGTTTTGCATCCGAATCGTGGACTCGCTGATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGC  
CCACCTCGGGGGTCTTTTCATTTGGAGGTTCCACCGAGATTTGGAGACCCCTGCCCAGGGACCACCGACCCCCCGCCGGGAG  
GTAAGCTGGCCAGCGGTGCTTTCGTGTCTGTCTCTGTCTTTGTGCGTGTTCGTGCCGGCATCTAATGTTTGCCTGCGTCT  
GTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCTGAACACCCGGCCGAACCCCTGGG  
AGACGTCCCAGGGACTTTGGGGGCGCTTTTGTGGCCCCGACCTGAGGAAGGGAGTTCGATGTGGAATCCGACCCCGTCAGGAT  
ATGTGGTTCGGTAGGAGACGAGAACCATAAACAGTTCCCGCCTCCGCTGAAATTTTGCCTTTCGGTTTGGAAACCGAAGCCG  
CGCGTCTTGTCTGCTGCAGCGCTGCAGCATCGTTCGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAG  
GGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCAGTGGAAAGATGTCGAGCGGATCGCTCACAAACAGTCCGGTAG  
ATGTCAAGAAGAGACGTTGGTTACCCTTTCGTCTGCAGAAATGGCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTT  
TAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCCTTTTACCTGGCCCGCATGGACACCCAGACGTTCCCTTACAT  
GTGACTGGGAAGCCTTGGCTTTTGGACCCCTCCCTGGTCAAAGCCCTTGTACACCTAAGCCTCCGCTCCTCTTCCCTC  
CATCCGCCCGCTCTCCCTTGAACCTCCCTCGTTCGACCCCGCTCGATCCTCCCTTTATCCAGCCCTCACCTTCTCT  
AGGCGCCGGAATTCGTGACAAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACATAACCA  
TGGCCAAGCCTTGTCTCAAGAAGAATCCACCCCTCATGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAGGA  
CTACAGCGTCGCCAGCGCAGCTCTCTTAGCGACGGCCGATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCT  
TGTGCAGAACTCGTGGTGTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGA  
ACAGGGGCATCTTGAGCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGA  
CAGTGATGGACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAgttttaaacAAAC  
gcatcgacgcTAGGGATAACAGGGTAATAagatcCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATAT  
TTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGT  
GACGTAGAAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAAATTATGTTTTAAAAATGGACTATCATATGCTTACCCTAAC  
TTGAAAGTATTTGATTTCTTGGCTTTATATATCTTGTGGAAGGATTTCTTGGCTTTTATATATCTTGTGAAGACAGGGCAC  
CAAGCTTCCGAAGACGTTGGCTGGTCTCATAACAGAACTTATAAGATGTCCTCATACAGAACTTATAAGATTTCCCAAATCCAAA  
GACATTTACAGTTTATGGTGAATTTCCAGAACACATAGCGACATGCAAATATTGCAGGGCGCCACTCCCTGTCCCTCACAG  
CCATCTTCCTGCCAGGGCGCACGCGCGCTGGGTGTTCCCGCCTAGTGACACTGGGCCCCGGATTCCTTGGAGCGGGTTGATG  
ACGTCAGCGTTCGctcgagagctgATTACCCGTATTCCCTAaacgcgtttaattaaCGATAAAAATAAAGATTTTATTTAG  
TCTCCAGAAAAAGGGGGAAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGAAGGCATGG  
AAAAATACATAACTGAGAAATAGAGAAGTTTCAGATCAAGGTTAGGAACAGAGACAGAGACAGAAATATGGGCCAAAACAGGATATCT  
GTGGTAAGCAGTTCCTGCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCCTCCCTCAGCAGTTCTCTAGAGA  
ACCATCAGATGTTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCGTGTGCCCTTATTTGAACTAACCAATCAGTTTCGCTTCTCG  
CTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAAGAGCCACAACCCCTCACTCGGCGGCCAGTCCCTCCGATAGAC  
TGCGTCCGCCGGGTACCCGTGTATCCAATAAACCCCTTTCAGTGTGCATCCGACTTGTGGTCTCGCTGTTCCCTGGGAGGGT  
CTCCTCTGAGTGAATGACTACCCGTCAGCGGGGTCTTTTCATGGGTAACAGTTTCTTGAAGTTGGAGAACAACATTTCTGAGG  
GTAGGAGTCGAATATTAAGTAATCCTGACTCAATTAGCCACTGTTTTGAAATCCACATACTCCAATACTCCTGAAATAGTTCA  
TTATGGACAGCGCAGAAGAGCTGGGGAGAATTAATTCGTAATCATGGTCATAGCTGTTTCCCTGTGTGAAATTTGTTATCCGCT  
CACAAATCCACACAACATACGAGCCGGAAGCATAAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACACATTAATTT  
GCGTTGCGCTCACTGCCCGCTTTCCAGTCCGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAG  
GCGGTTTGCATATTGGGCGCTCTTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCTCGGCTGCGGCGAGCGGTATC  
AGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAA  
AAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGAC  
GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCC  
TGTTCGACCCCTGCCGCTTACCCGATACCTGTCCGCCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCTCATAGCTCACGCTGT  
AGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCCGCTGCGCT  
TATCCGGTAACTATCGTCTTGAATCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAG  
CAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCCTAACACGGCTACACTAGAAGGACAGTATTTGGT  
ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGTCTTTGATCCGGCAAACAAACCCAGCTGGTAGCG  
GTGTTTTTTTTGTTTTGCAAGCAGTACGATTACGGCGAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTTCTACGGGGTC  
TGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTTTTGGTCATGAGATTTATCAAAAAGGATCTTACCTTAGATCTTTTA  
AATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGG  
CACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG  
CTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCCACGCTCACCAGGCTCCAGATTTATCAGCAATAAACAGCCA  
GCCGGAAGGGCCGAGCGCAGAAGTGGTCCGCAACTTTATCCGCCCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCTAGAG  
TAAGTAGTTCCGACGTTAATAGTTTGCGCAACGTTGTTGCCATTTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTAT  
GGCTTCATTCAGTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTC  
GGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAATCTCTTACTG  
TCATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTTCTGAGAATAGTGTATGCGGCGACCGAG

TTGCTCTTGCCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCT  
TCGGGGCGAAAACCTCTCAAGGATCTTACCGTGTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACCTGATCTTCAG  
CATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAATGCCGCAAAAAAGGGAATAAGGGCGACACG  
GAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATA  
TTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCGCGGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAAACCA  
TTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTAAAA  
CCTCTGACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCG  
TCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGT  
GTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGG  
GCGATCGGTGCGGGCCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGGGATTAAGTTGGGTAAACGCCA  
GGTTTTTCCAGTCCACGACGTTGTAAAAACGACGGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCGGC  
CACGGGCGCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATG  
TCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGCGCCACGATGCGTCCGGCGTAGAGGCGATTAGTCC  
AATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTTACTCAACAATATCACCAGCTGAAGCCTATAGAGTACGA  
GCCATAGATAAAAATAAAGATTTTTATTTAGTCTCCAGAAAAAGGGGGGAA

## Zero-Cutters:

#	Enzyme	Specificity	14	BspEI	TCCGGA	28	PmlI	CACGTG
1	AccI	GTMKAC	15	BspMI	ACCTGCNNNNNNN	29	PshAI	GACNNNNGTCT
2	AgeI	ACCGGT	16	BstBI	TTCGAA	30	PspXI	VTCGAGB
3	AleI	CACNNNNGTG	17	BstXI	CCANNNNNTGG	31	RsrII	CGWCCG
4	AvrII	CCTAGG	18	BstZ17I	GTATAC	32	SacII	CCGCGG
5	BamHI	GGATCC	19	BtgZI	GCGATG(N)10NNNN	33	SalI	GTGCAC
6	BclI	TGATCA	20	ClaI	ATCGAT	34	SbfI	CCTGCAGG
7	BfuAI	ACCTGCNNNNNNN	21	DraIII	CACNNNNGTG	35	SfiI	GGCCNNNNNGGCC
8	BglII	AGATCT	22	FseI	GGCCGGCC	36	SnaBI	TACGTA
9	BlpI	GCTNAGC	23	HpaI	GTTAAC	37	SrfI	GCCCGGGC
10	BmgBI	CACGTC	24	MfeI	CAATTG	38	StuI	AGGCCT
11	BsiWI	CGTACG	25	NotI	GCGCCCGC	39	SwaI	ATTAAAT
12	BsmI	GAATGCN	26	NsiI	ATGCAT	40	XcmI	CCANNNNNNNTGG
13	BspDI	ATCGAT	27	PfIMI	CCANNNNNTGG			

## One-Cutters:

#	Enzyme	Specificity	Sites	&flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	ApaI	GGGCC	list	*2435/2431	
2	AsiSI	GCGATCGC	list	*1891/1889	
3	BsaAI	YACGTR	list	*2048	
4	BsaBI	GATNNNNATC	list	*#1774	
5	BsgI	GTGCAG(N)14NN	list	1663/1661	
6	BsrGI	TGTACA	list	1281/1285	
7	BstEII	GGTNACC	list	1086/1091	
8	CspCI	NN(N)11CAA(N)5GTGG(N)10NN	list	1493/1491+1528/1526	
9	EcoRI	GAATTC	list	1403/1407	
10	HincII	GTYRAC	list	1412	
11	HindIII	AAGCTT	list	2216/2220	
12	MluI	ACGCGT	list	*2504/2508	
13	NcoI	CCATGG	list	1474/1478	
14	NruI	TCGCGA	list	*#1707	
15	PacI	TTAATTAA	list	2514/2512	
16	PaeR7I	CTCGAG	list	*2474/2478	
17	PciI	ACATGT	list	3586/3590	
18	PmeI	GTTTAAAC	list	1878	
19	PspOMI	GGGCC	list	*2431/2435	
20	ScaI	AGTACT	list	4959	
21	SexAI	ACCWGGT	list	#1214/1219	
22	SgrAI	CRCCGGYG	list	*6022/6026	
23	SpeI	ACTAGT	list	659/663	
24	SphI	GCATGC	list	5874/5870	
25	XhoI	CTCGAG	list	*2474/2478	