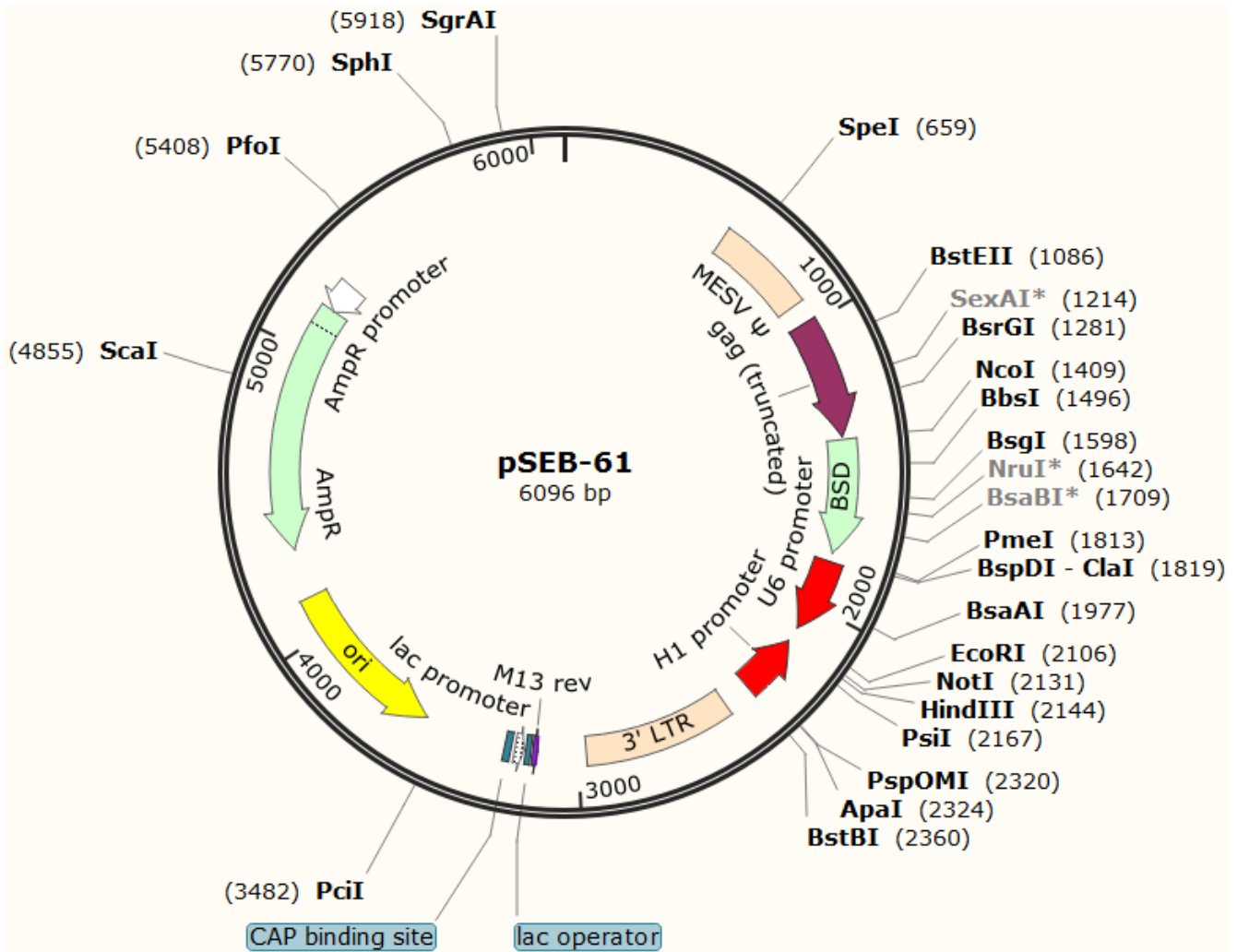


**Vector:** pSEB61 (U6-H1 driven siRNA or regRNA expression w/ EcoRI/HindIII sites)

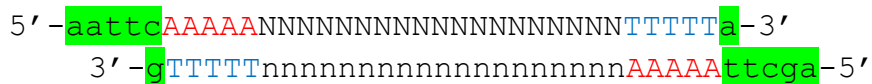
**Antibiotic Selection:** Amp

**Creator(s):** Li Li, Molecular Oncology Lab of The University of Chicago Medical Center

**Date of Construction:** September, 2017



### Design siRNA cassette (or PCR products cut w/ EcoRI/HindIII)



## pSEB61 Full Sequence

TGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAA  
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## Zero Cutters

#	Enzyme	Specificity	13	BsmI	GAATGCN	26	PmlI	CACGTG
1	AccI	GTMKAC	14	BspMI	ACCTGCNNNNNNNN	27	PshAI	GACNNNGTC
2	AgeI	ACCGGT	15	BstXI	CCANNNNNNTGG	28	PspXI	VCTCGAGB
3	AleI	CACNNNGTG	16	BstZ17I	GTATAC	29	RsrII	CGWCCG
4	AsiSI	GCGATCGC	17	BtgZI	GCGATG (N)10NNNN	30	SacII	CCGCGG
5	AvrII	CCTAGG	18	DraIII	CACNNNGTG	31	SalI	GTCGAC
6	BamHI	GGATCC	19	FseI	GGCCGGCC	32	SbfI	CCTGCAGG
7	BclI	TGATCA	20	HincII	GTYRAC	33	SfiI	GGCCNNNNNGGCC
8	BfuAI	ACCTGCNNNNNNNN	21	HpaI	GTTAAC	34	SnaBI	TACGTA
9	BglII	AGATCT	22	MfeI	CAATTG	35	SrfI	GCCCGGGC
10	BlpI	GCTNAGC	23	NsiI	ATGCAT	36	SwaI	ATTTAAAT
11	BmgBI	CACGTC	24	Paer7I	CTCGAG	37	XcmI	CCANNNNNNNNTGG
12	BsiWI	CGTACG	25	PflMI	CCANNNNNNTGG	38	XhoI	CTCGAG

## One-Cutters

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	ApaI	G <sup>↓</sup> GGCC <sup>↓</sup> C	<a href="#">list</a>	*2314/2310
2	BbsI	GAAGACNN <sup>↓</sup> NNNN <sup>↓</sup>	<a href="#">list</a>	1496/1500
3	BsaAI	YAC <sup>↓</sup> GTR	<a href="#">list</a>	*1967
4	BsgI	GTGCAG (N) <sub>14</sub> <sup>↓</sup> NN <sup>↓</sup>	<a href="#">list</a>	1598/1596
5	BspDI	AT <sup>↓</sup> CG <sup>↓</sup> AT	<a href="#">list</a>	*1819/1821
6	BspEI	T <sup>↓</sup> CCGG <sup>↓</sup> A	<a href="#">list</a>	*#1835/1839
7	BsrGI	T <sup>↓</sup> GTAC <sup>↓</sup> A	<a href="#">list</a>	1281/1285

8	BstBI	TT <sup>▼</sup> CG <sup>▲</sup> AA	<a href="#">list</a>	*2350/2352
9	BstEII	G <sup>▼</sup> GTNAC <sup>▲</sup> C	<a href="#">list</a>	1086/1091
10	ClaI	AT <sup>▼</sup> CG <sup>▲</sup> AT	<a href="#">list</a>	*1819/1821
11	CspCI	<sup>▲</sup> NN <sup>▼</sup> (N) <sub>11</sub> CAA (N) <sub>5</sub> GTGG (N) <sub>10</sub> <sup>▲</sup> NN <sup>▼</sup>	<a href="#">list</a>	1428/1426+1463/1461
12	EcoRI	G <sup>▼</sup> AATT <sup>▲</sup> C	<a href="#">list</a>	*2096/2100
13	HindIII	A <sup>▼</sup> AGCT <sup>▲</sup> T	<a href="#">list</a>	2134/2138
14	NcoI	C <sup>▼</sup> CATG <sup>▲</sup> G	<a href="#">list</a>	1409/1413
15	NotI	GC <sup>▼</sup> GGCC <sup>▲</sup> GC	<a href="#">list</a>	*2121/2125
16	NruI	TCG <sup>▼</sup> CGA	<a href="#">list</a>	*#1642
17	PciI	A <sup>▼</sup> CATG <sup>▲</sup> T	<a href="#">list</a>	3472/3476
18	PmeI	GTTT <sup>▼</sup> AAAC	<a href="#">list</a>	1813
19	PsiI	TTA <sup>▼</sup> TAA	<a href="#">list</a>	2157
20	PspOMI	G <sup>▼</sup> GGCC <sup>▲</sup> C	<a href="#">list</a>	*2310/2314
21	ScaI	AGT <sup>▼</sup> ACT	<a href="#">list</a>	4845
22	SexAI	A <sup>▼</sup> CCWGG <sup>▲</sup> T	<a href="#">list</a>	#1214/1219
23	SgrAI	CR <sup>▼</sup> CCGG <sup>▲</sup> YG	<a href="#">list</a>	*5908/5912
24	SpeI	A <sup>▼</sup> CTAG <sup>▲</sup> T	<a href="#">list</a>	659/663
25	SphI	G <sup>▼</sup> ATGC <sup>▲</sup>	<a href="#">list</a>	5760/5756
26	StuI	AGG <sup>▼</sup> CCT	<a href="#">list</a>	2116