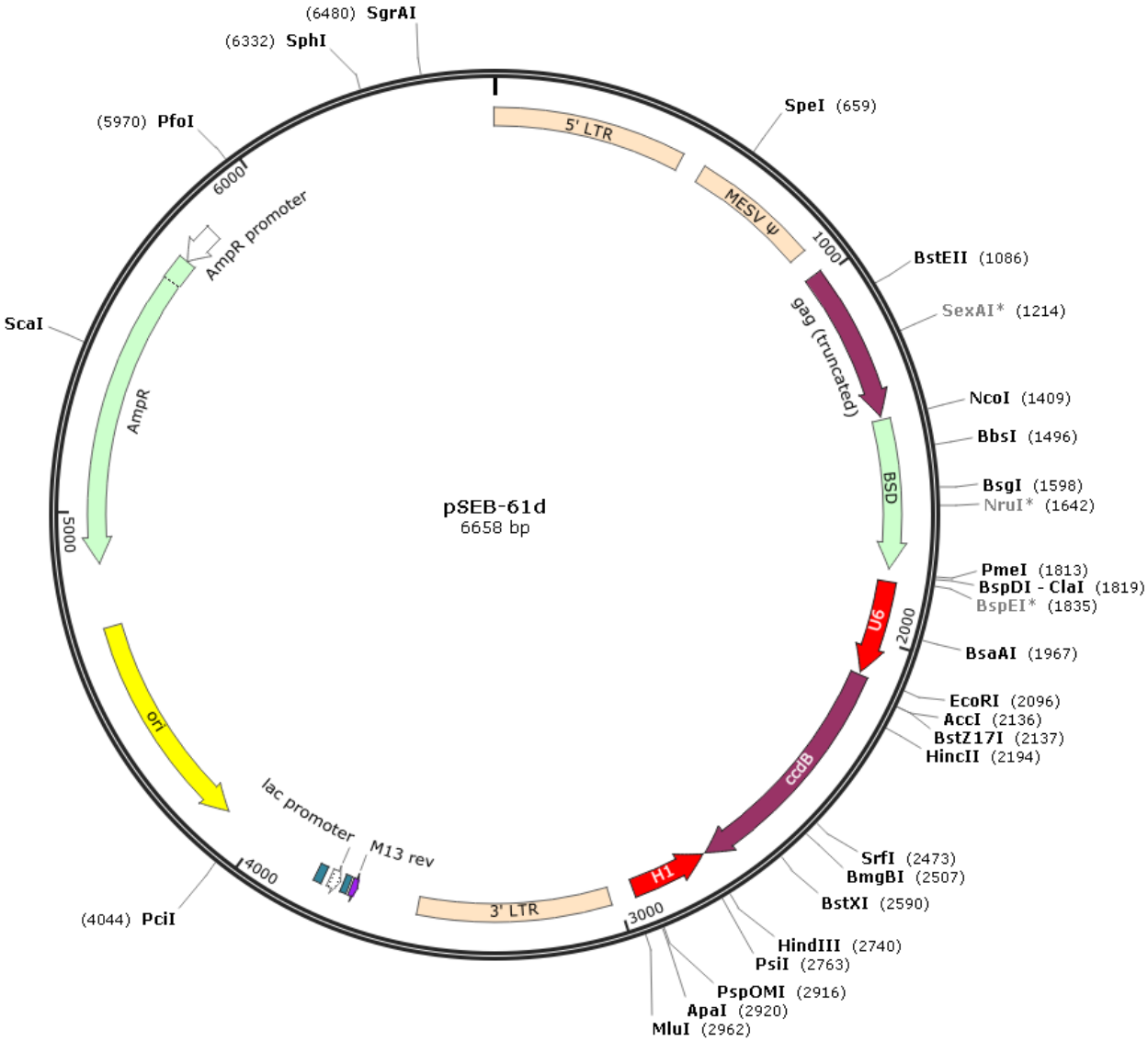
Vector: pSEB61D (U**6**-H**1** driven sRNA expression w/ EcoR*I*-*ccdB*-Hind*III* sites)

Antibiotic Selection: Amp

Creator(s): Bo Huang, Molecular Oncology Lab of The University of Chicago Medical Center

Date of Construction: October, 2017



Design sRNA cassette (cut w/ EcoRI/HindIII)

5′-aattcAAAAANNNNNNNNNNNNNNNNNNNTTTTTa-3′
3′-gTTTTTnnnnnnnnnnnnnnnnnnnAAAAAttcga-5′

pSEB61D Vector Sequence

TGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTAG
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AAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAA

Zero Cutters

#	Enzyme	Specificity
1	AgeI	ACCGGT
2	AleI	CACNNNNGTG
3	AsiSI	GCGATCGC
4	AvrII	CCTAGG
5	BamHI	GGATCC
6	BclI	TGATCA
7	BfuAI	ACCTGCNNNNNNNN
8	BglII	AGATCT
9	BlpI	GCTNAGC
10	BsiWI	CGTACG
11	BsmI	GAATGCN
12	BspMI	ACCTGCNNNNNNNN
13	BtgZI	GCGATG(N)10NNNN
14	DraIII	CACNNNGTG
15	FseI	GGCCGGCC
16	HpaI	GTTAAC
17	MfeI	CAATTG
18	NotI	GCGGCCGC
19	NsiI	ATGCAT
20	PaeR7I	CTCGAG
21	PflMI	CCANNNNNTGG
22	PmlI	CACGTG
23	PshAI	GACNNNNGTC
24	PspXI	VCTCGAGB
25	RsrII	CGGWCCG
26	SacII	CCGCGG
27	SalI	GTCGAC
28	SbfI	CCTGCAGG
29	SfiI	GGCCNNNNNGGCC
30	SnaBI	TACGTA
31	StuI	AGGCCT
32	SwaI	ATTTAAAT
33	XcmI	CCANNNNNNNNNTGG
34	XhoI	CTCGAG

One-Cutters

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	AccI	GT MK▲AC	list	2136/2138
2	ApaI	G▲GGCC C	list	*2920/2916
3	BbsI	GAAGACNN NNNN▲	list	1496/1500
4	BmgBI	CAC▲GTC	list	*2507
5	BsaAI	YAC▲GTR	list	*1967
6	BsgI	GTGCAG(N)$_{14}$▲NN	list	1598/1596
7	BspDI	AT CG▲AT	list	*1819/1821
8	BspEI	T CCGG▲A	list	*#1835/1839
9	BstBI	TT CG▲AA	list	*2956/2958
10	BstEII	G GTNAC▲C	list	1086/1091
11	BstXI	CCAN▲NNNN NTGG	list	2590/2586
12	BstZ17I	GTA▲TAC	list	2137
13	ClaI	AT CG▲AT	list	*1819/1821
14	CspCI	▲NN (N)$_{11}$CAA(N)$_5$GTGG(N)$_{10}$▲NN	list	1428/1426+1463/1461
15	EcoRI	G AATT▲C	list	2096/2100
16	HincII	GTY▲RAC	list	2194
17	HindIII	A AGCT▲T	list	2740/2744
18	MluI	A CGCG▲T	list	*2996/3000
19	NcoI	C CATG▲G	list	1409/1413
20	NruI	TCG▲CGA	list	*#1642
21	PciI	A CATG▲T	list	4078/4082
22	PmeI	GTTT▲AAAC	list	1813
23	PsiI	TTA▲TAA	list	2763
24	PspOMI	G GGCC▲C	list	*2916/2920
25	ScaI	AGT▲ACT	list	5451
26	SexAI	A CCWGG▲T	list	#1214/1219
27	SgrAI	CR CCGG▲YG	list	*6514/6518
28	SpeI	A CTAG▲T	list	659/663
29	SphI	G▲CATG C	list	6366/6362
30	SrfI	GCCC▲GGGC	list	*2473