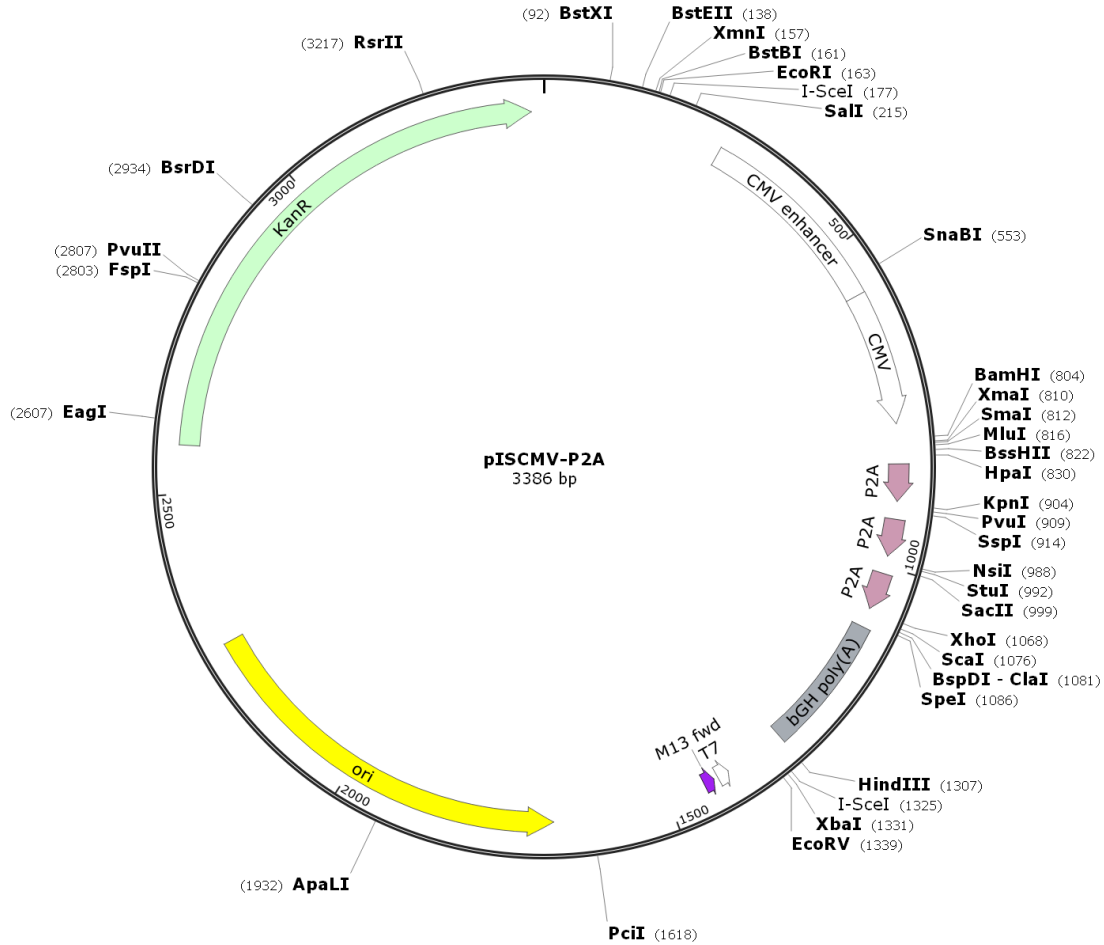


Vector: pISCMV-P2A (ISC flanking CMV-3xP2A expression vector)

Antibiotic Selection: Kan

Creator(s): Na Ni @ Molecular Oncology Lab of The University of Chicago Medical Center

Date of Construction: October 2020



3xP2A Coding Regions and Restriction Enzyme Sites

1	GGATCC AATTGCAATGATCATCATGAC AGATCT GTTAAC ggaagc	BamHI	BglII	HpaI
	G S N C N D H H D R S V N G S			
46	ggagctactaacttcagcctgctgaagcaggctggagacgtggag			
	G A T N F S L L K Q A G D V E			
91	gagaacctggacct GGTACC CGATCG AATATT ggatctggcgcc	KpnI	PvuI	SspI
	E N P G P G T R S N I G S G A			
136	accaacttttcctcctgaaacaggctggcgatgtcgaagaaaat			
	T N F S L L K Q A G D V E E N			
181	ccggccct ATGCAT AGGCCT CCGCGG ggctccggcgccaccaac	NsiI	StuI	SacII
	P G P M H R P P R G S G A T N			
226	ttctcctgctgaagcaggccggcgacgtggaggagaaccccggc			
	F S L L K Q A G D V E E N P G			
271	ccc CTCGAG atggtggagaaattcgtgggaacctggaaaatcgca	XhoI	BiFP	
	P L E M V E K F V G T W K I A			

pISCMV-P2A full-length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTACCCCTCACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTGG
 AGCCATGGGCATGGCTACTCAAGCTGATTTGATGGAGTTGGACATGGCCATGGCTGGTACCACGTCGTGGAATGCCTTCGA
 ATTC**TAGGGATAACAGGGTAAAT**GTCGAAATTCAGCACCTGCACATGGGAC**GTCGAC**TAATAGTAATCAATTACGGGGTCATT
 AGTTCATAGCCATATATGGAGTCCCGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGC
 CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTAC
 GGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
 CGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
 ATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGTTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTG
 ACGTCAATGGGAGTTTTGTTTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAAAT
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 TGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGG
 GCTGACCGCTTCTCGTGTCTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCT
 TCTGACCTTTCGTCTTCAAGaatt

Zero Cutters

#	Enzyme	Specificity			
1	AclI	AACGTT	10	AvrII	CCTAGG
2	AfeI	AGCGCT	11	BaeI	(N) 5 (N) 10ACN>NNNGTAYC (N) 7 (N) 5
3	AflII	CTTAAG	12	BbvCI	CCTCAGC
4	AgeI	ACCGGT	13	BcgI	NN (N) 10CGA (N) 6TGC (N) 10NN
5	AleI	CACNNNNGTG	14	BclI	TGATCA
6	ApaI	GGGCCC	15	BglII	AGATCT
7	AscI	GGCGCGCC	16	BlpI	GCTNAGC
8	AseI	ATTAAT	17	BmtI	GCTAGC
9	AsiSI	GCGATCGC	18	Bpu10I	CCTNAGC
			19	BsaI	GGTCTCN>NNN

20	BsaXI	NNN (N) 9AC (N) 5CTCC (N) 7NNN	35	PmeI	GTTTAAAC
21	BsiWI	CGTACG	36	PmlI	CACGTG
22	BsrGI	TGTACA	37	PpuMI	RGGWCCY
23	BstAPI	GCANNNNTGC	38	PshAI	GACNNNGTC
24	BstZ17I	GTATAC	39	PsiI	TTATAA
25	Bsu36I	CCTNAGG	40	PspOMI	GGGCCC
26	BtsI	GCAGTGNN	41	PspXI	VCTCGAGB
27	Eco53kI	GAGCTC	42	PstI	CTGCAG
28	EcoO109I	RGGNCCY	43	SacI	GAGCTC
29	FseI	GGCCGGCC	44	SbfI	CCTGCAGG
30	MfeI	CAATTG	45	SfiI	GGCCNNNNGGCC
31	NheI	GCTAGC	46	SgrAI	CRCCGGYG
32	NotI	GCGGCCGC	47	SrfI	GCCCCGGC
33	NruI	TCGCGA	48	SwaI	ATTTAAAT
34	PacI	TTAATTAA	49	XcmI	CCANNNNNNNNTGG

One-Cutters

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	Acc65I	G GTAC△C	list	#900/904
2	AccI	GT MK△AC	list	*216/218
3	ApaLI	G TGCA△C	list	*1932/1936
4	BamHI	G GATC△C	list	804/808
5	BsaBI	GATNN△NNATC	list	#803
6	BsgI	GTGCAG(N) ₁₄ △NN	list	187/185
7	BsmBI	CGTCTCN NNNN△	list	*868/872
8	BsmI	GAATG△CN	list	159/157
9	BspDI	AT CG△AT	list	*1081/1083
10	BspEI	T CCGG△A	list	*#801/805
11	BsrDI	GCAATG△NN	list	2934/2932
12	BssHII	G CGCG△C	list	*822/826
13	BstBI	TT CG△AA	list	*161/163
14	BstEII	G GTNAC△C	list	138/143
15	BstXI	CCAN△NNNN NTGG	list	92/88
16	Clal	AT CG△AT	list	*1081/1083
17	CspCI	△NN (N) ₁₁ CAA(N) ₅ GTGG(N) ₁₀ △NN	list	589/587+624/622
18	DraIII	CAC△NNN GTG	list	*149/146
19	EagI	C GGCC△G	list	*2607/2611
20	EcoNI	CCTNN N△NNAGG	list	948/949
21	EcoRI	G AATT△C	list	*163/167
22	EcoRV	GAT△ATC	list	1339
23	Esp3I	CGTCTCN NNNN△	list	*868/872
24	FspI	TGC△GCA	list	*2803
25	HindIII	A AGCT△T	list	1307/1311
26	HpaI	GTT△AAC	list	*830
27	KpnI	G△GTAC C	list	904/900
28	MluI	A CGCG△T	list	*816/820
29	NmeAIII	GCCGAG(N) ₁₉ △NN	list	2926/2924
30	NsiI	A△TGCA T	list	988/984
31	PaeR7I	C TCGA△G	list	*1068/1072

32	PciI	A CATG↓T	list	1618/1622
33	PfI	CCAN↓NNN NTGG	list	149/146
34	PvuI	CG↓AT CG	list	*909/907
35	PvuII	CAG↓CTG	list	2807
36	RsrII	CG↓GWC↓CG	list	*3217/3220
37	SacII	CC↓GC GG	list	*999/997
38	SalI	G TCGA↓C	list	*215/219
39	SalI	AGT↓ACT	list	1076
40	SexAI	A CCWGG↓T	list	#896/901
41	SmaI	CCC↓GGG	list	*812
42	SnaBI	TAC↓GTA	list	*553
43	SpeI	A CTAG↓T	list	1086/1090
44	SspI	AAT↓ATT	list	914
45	StuI	AGG↓CCT	list	992
46	TspMI	C CCGG↓G	list	*810/814
47	XbaI	T CTAG↓A	list	1331/1335
48	XhoI	C TCGA↓G	list	*1068/1072
49	XmaI	C CCGG↓G	list	*810/814
50	XmnI	GAANN↓NNTTC	list	157