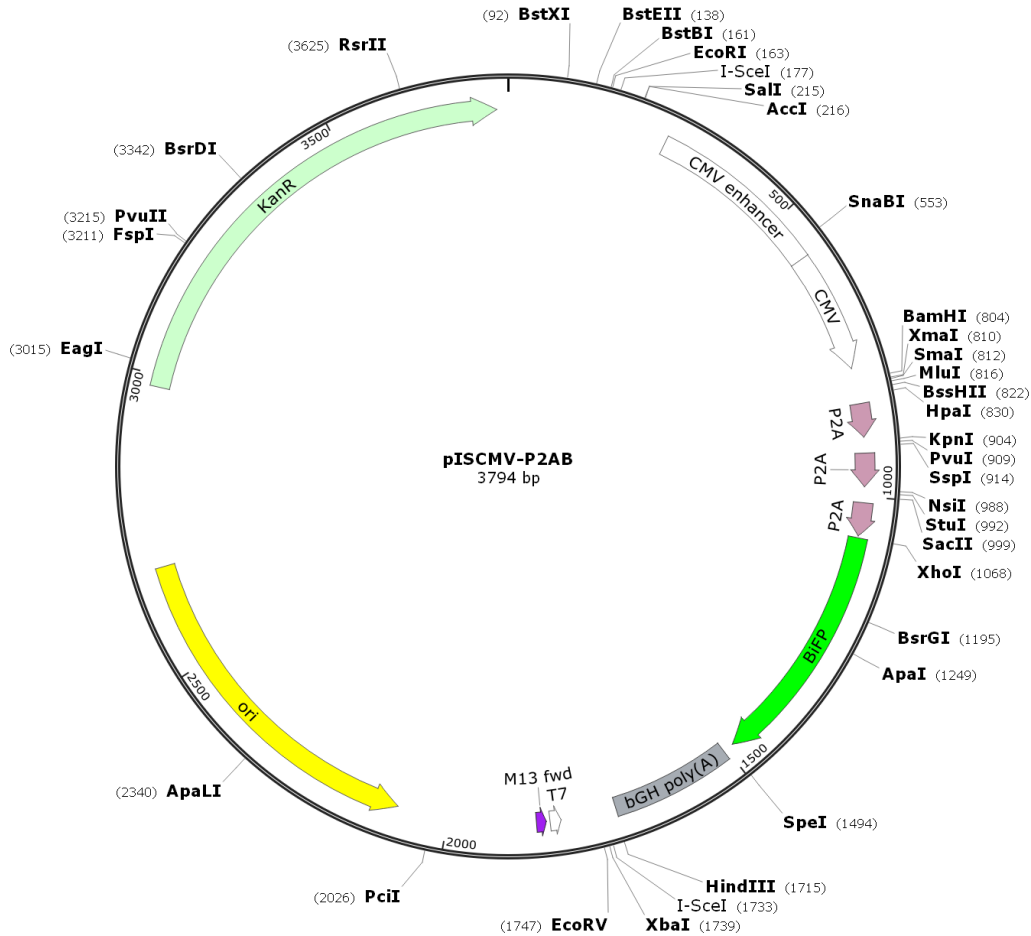


Vector: pISCMV-P2AB (ISC flanking CMV-3xP2A-BiFP expression vector)

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 AAT TGCAATGATCATCATGAC AGATCT GTAAC ggaagc	BamHI	BglII	HpaI
	G S N C N D H H D R S V N G S			
46	ggagctactaacttcagcctgctgaagcaggctggagaogtggag			
	G A T N F S L L K Q A G D V E			
91	gagaaccctggacct GGTACC CGATCG AATATT ggatctggcgcc	KpnI	PvuI	SspI
	E N P G P G T R S N I G S G A			
136	accaacttttcctcctgaaacaggctggcgatgtcgaagaaat			
	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	ttctcctgctgaagcaggccggcgacgtggaggagaaccccgcc			
	F S L L K Q A G D V E E N P G			
271	ccc CTCGAG atggtggagaaattcgtaggaaacctggaaaatcgca	XhoI	BiFP	
	P L E M V E K F V G T W K I A			

pISCMV-P2AB full-length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTACCCCTACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTGG
 AGCCATGGGCATGGCTACTCAAGCTGATTTGATGGAGTTGGACATGGCCATGGCTGGTACCACGTCGTGGAATGCCTTCGA
 ATTCTAGGGATAACAGGGTAAATGTCGAAATTCAGCACCTGCACATGGGACGTCGAC TAATAGTAATCAATTACGGGGTCATT
 AGTTCATAGCCCATATATGGAGTTCGCGCTTACATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCCGC
 CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTAC
 GGTAACCTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
 CGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
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 ACGTCAATGGGAGTTTTGTTTTGGCACCAAAATCAACGGGACTTTCAAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAT
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 TTA AAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA
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 TGACCTTTCGCTTCAAGaatt

Zero Cutters

#	Enzyme	Specificity			
1	AclI	AACGTT	3	AflII	CTTAAG
2	AfeI	AGCGCT	4	AgeI	ACCGGT
			5	AscI	GGCGGCC

6	AseI	ATTAAT	26	NheI	GCTAGC
7	AsiSI	GCGATCGC	27	NotI	GCGGCCGC
8	AvrII	CCTAGG	28	NruI	TCGCGA
9	BaeI	(N) 5 (N) 10ACNNNNGTAYC (N) 7 (N) 5	29	PacI	TTAATTAA
10	BbvCI	CCTCAGC	30	PmeI	GTTTAAAC
11	BcgI	NN (N) 10CGA (N) 6TGC (N) 10NN	31	PmlI	CACGTG
12	BclI	TGATCA	32	PpuMI	RGGWCCY
13	BglII	AGATCT	33	PshAI	GACNNNGTC
14	BlpI	GCTNAGC	34	PsiI	TTATAA
15	BmtI	GCTAGC	35	PspXI	VCTCGAGB
16	Bpu10I	CCTNAGC	36	PstI	CTGCAG
17	BsaI	GGTCTCNNNNN	37	SacI	GAGCTC
18	BsiWI	CGTACG	38	SbfI	CCTGCAGG
19	BspDI	ATCGAT	39	ScaI	AGTACT
20	BstAPI	GCANNNNNTGC	40	SfiI	GGCCNNNNNGGCC
21	BstZ17I	GTATAC	41	SgrAI	CRCCGGYG
22	ClaI	ATCGAT	42	SrfI	GCCCGGGC
23	Eco53kI	GAGCTC	43	SwaI	ATTTAAAT
24	FseI	GGCCGGCC	44	XcmI	CCANNNNNNNTGG
25	MfeI	CAATTG			

One-Cutters

#	Enzyme	Specificity	Sites &	
flanks	Cut positions			
(blunt - 5' ext. - 3' ext.)				
1	Acc65I	GGTACC	list	#900/904
2	AccI	GTMKAC	list	*216/218
3	AleI	CACNNNGTG	list	1269
4	Apal	GGGCCC	list	*1249/1245
5	ApaLI	GTGCAC	list	*2340/2344
6	BamHI	GGATCC	list	804/808
7	BsaBI	GATNNNNATC	list	#803
8	BsaXI	NNN(N)9AC(N)5CTCC(N)7NNN	list	1441/1438+1471/1468
9	BsgI	GTGCAG(N)14NN	list	187/185
10	BsmBI	CGTCTCNNNNN	list	*868/872
11	BsmI	GAATGCN	list	159/157
12	BspEI	TCCGGA	list	*#801/805
13	BsrDI	GCAATGNN	list	3342/3340
14	BsrGI	TGTACA	list	1195/1199
15	BssHII	GCGCGC	list	*822/826
16	BstBI	TTCGAA	list	*161/163
17	BstEII	GGTNACC	list	138/143
18	BstXI	CCANNNNNNTGG	list	92/88
19	Bsu36I	CCTNAGG	list	1153/1156
20	BtsI	GCAGTGNN	list	1468/1466
21	CspCI	NN(N)11CAA(N)5GTGG(N)10NN	list	589/587+624/622
22	DraIII	CACNNNGTG	list	*149/146
23	EagI	CGGCCG	list	*3015/3019
24	EcoNI	CCTNNNNNAGG	list	948/949
25	EcoO109I	RGGNCCY	list	1246/1249
26	EcoRI	GAATTC	list	*163/167
27	EcoRV	GATATC	list	1747
28	Esp3I	CGTCTCNNNNN	list	*868/872
29	FspI	TGCGCA	list	*3211
30	HindIII	AAGCTT	list	1715/1719
31	HpaI	GTTAAC	list	*830

32	KpnI	GGTACC	list	904/900
33	MluI	ACGCGT	list	*816/820
34	NmeAIII	GCCGAG(N)19NN	list	3334/3332
35	NsiI	ATGCAT	list	988/984
36	PaeR7I	CTCGAG	list	*1068/1072
37	PciI	ACATGT	list	2026/2030
38	PfI	CCANNNNTGG	list	149/146
39	PspOMI	GGGCC	list	*1245/1249
40	PvuI	CGATCG	list	*909/907
41	PvuII	CAGCTG	list	3215
42	RsrII	CGGWCCG	list	*3625/3628
43	SacII	CCGCGG	list	*999/997