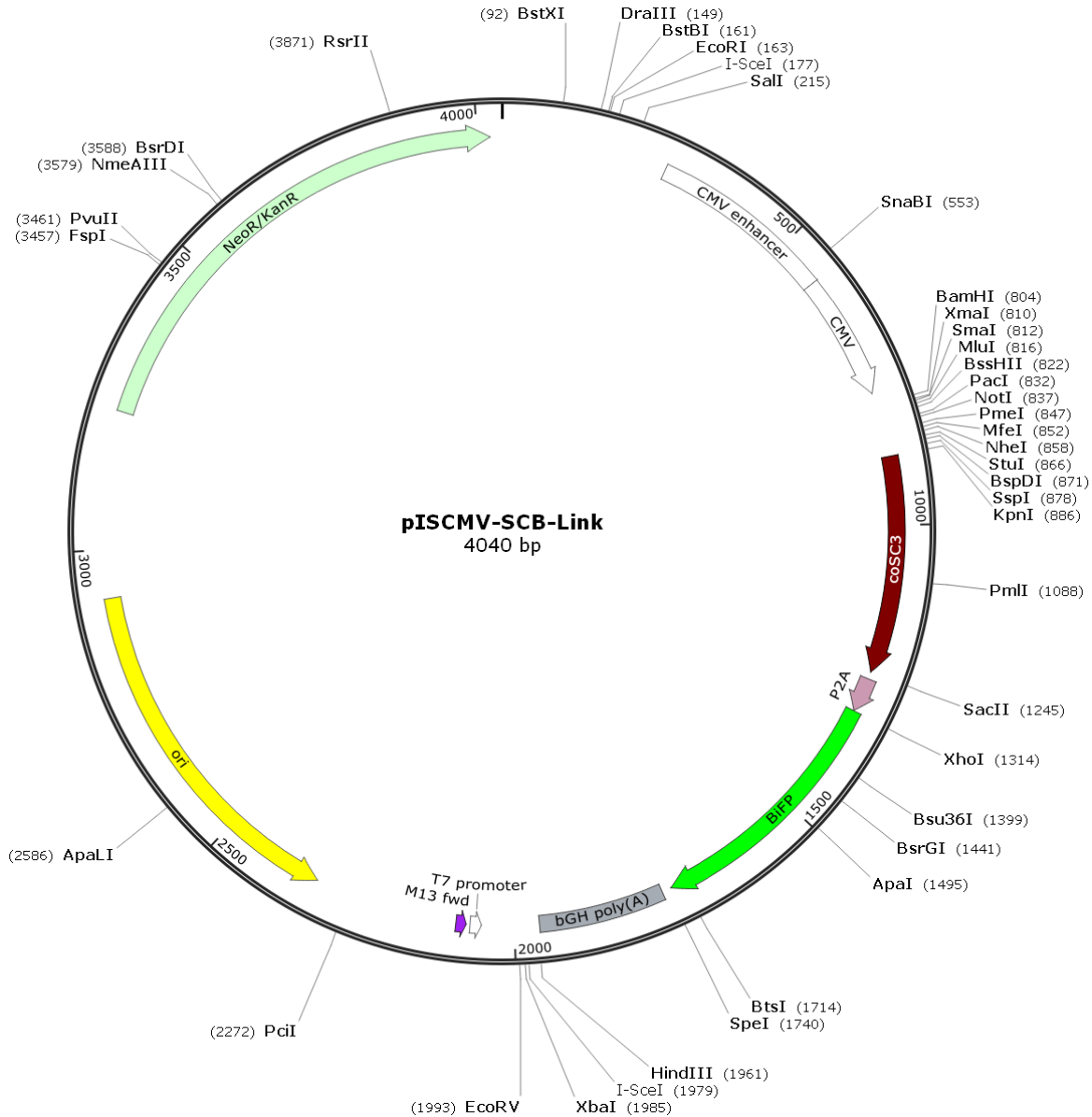


**Vector:** pISCMV-SCB-Link (ISC flanking CMV-Link-coSC3-P2A-BiFP expression vector)

**Antibiotic Selection:** Kan

**Creator(s):** Wei Zeng @ Molecular Oncology Lab of University of Chicago Medical Center

**Date of Construction:** November 2022



### Link-coSC3-P2A-BiFP Coding Regions and Restriction Enzyme Sites

1 xxxGGATCCCCCGGGACGCGTGCGCGCTTAATTAAGCGGCCGGT  
- G S P G T R A R L I K R P R

BamHI/XmaI/MluI/BssHII/PacI/NotI

46 TTAAACCAATTGGCTAGCAGGCCTATCGATAATATTGGTACCGGC  
L N Q L A S R P I D N I G T G

PmeI/MfeI/NheI/StuI/BspDI/SspI/KpnI

91 agcagcggatctGTGACAACCTGAGCGGCCTGTCTGGAGAACAG  
S S G S V T T L S G L S G E Q

Kinker --- coSC3...P2A...BiFP

## pISCMV-SCB-Link Vector full-length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTACCCCTCACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTGGAG  
CCATGGGCATGGCTACTCAAGCTGATTTGATGGAGTTGGACATGGCCATGGCTGGTGACCACGTCGTGGAATGCCTTCGAATTC  
TAGGGATAACAGGGTAATGTCGAAATTCAGCACCTGCACATGGGACGTTCGACTAATAGTAATCAATTACGGGGTCATTAGTTCA  
TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGAC  
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGC  
CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATT  
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CGTGCTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTCTTCTGACCTTTCGTCTT  
CAAGAatt

### Zero Cutters

#	Enzyme	Specificity	11	BclI	TGATCA	21	Esp3I	CGTCTCNNNNN
1	AclI	AACGTT	12	BglII	AGATCT	22	FseI	GGCCGGCC
2	AfeI	AGCGCT	13	BlpI	GCTNAGC	23	HpaI	GTTAAC
3	AflIII	CTTAAG	14	BsaI	GGTCTCNNNNN	24	NruI	TCGCGA
4	AgeI	ACCGGT	15	BsiWI	CGTACG	25	NsiI	ATGCAT
5	AscI	GGCGCGCC	16	BsmBI	CGTCTCNNNNN	26	PshAI	GACNNNNGTC
6	AseI	ATTAAT	17	BstAPI	GCANNNNNTGC	27	PsiI	TTATAA
7	AsiSI	CGCATCGC	18	BstZ17I	GTATAC	28	PspXI	VCTCGAGB
8	AvrII	CCTAGG	19	Eco53kI	GAGCTC	29	PstI	CTGCAG
10	BbvCI	CCTCAGC	20	EcoNI	CCTNNNNNAGG	30	PvuI	CGATCG

31	SacI	GAGCTC	34	SexAI	ACCWGGT	37	SrfI	GCCCCGGC
32	SbfI	CCTGCAGG	35	SfiI	GGCCNNNNNGGCC	38	SwaI	ATTTAAAT
33	ScaI	AGTACT	36	SgrAI	CRCCGGYG	39	XcmI	CCANNNNNNNTGG

## One-Cutters

#	Enzyme	Specificity	Sites & flanks	Cut positions
1	Acc65I	GGTACC	list *882/886	
2	AccI	GTMKAC	list *216/218	
3	ApaI	GGGCCC	list *1495/1491	
4	ApaLI	GTGCAC	list *2586/2590	
5	BamHI	GGATCC	list 804/808	
6	BcgI	NN (N) 10CGA (N) 6TGC (N) 10NN	list *851/849+885/883	
7	BmtI	GCTAGC	list 862/858	
8	Bpu10I	CCTNAGC	list 912/915	
9	BsaBI	GATNNNNATC	list #803	
10	BsgI	GTGCAG (N) 14NN	list 187/185	
11	BsmI	GAATGCN	list 159/157	
12	BspDI	ATCGAT	list *871/873	
13	BspEI	TCCGGA	list *#801/805	
14	BsrDI	GCAATGNN	list 3588/3586	
15	BsrGI	TGTACA	list 1441/1445	
16	BssHII	GCGCGC	list *822/826	
17	BstBI	TTCGAA	list *161/163	
18	BstXI	CCANNNNNTGG	list 92/88	
19	Bsu36I	CCTNAGG	list 1399/1402	
20	BtsI	GCAGTGNN	list 1714/1712	
21	ClaI	ATCGAT	list *871/873	
22	CspCI	NN (N) 11CAA (N) 5GTGG (N) 10NN	list 589/587+624/622	
23	DraIII	CACNNNGTG	list *149/146	
24	EcoRI	GAATTC	list *163/167	
25	EcoRV	GATATC	list 1993	
26	FspI	TGCGCA	list *3457	
27	HincII	GTYRAC	list *217	
28	HindIII	AAGCTT	list 1961/1965	
29	KpnI	GGTACC	list 886/882	
30	MfeI	CAATTG	list 852/856	
31	MluI	ACGCGT	list *816/820	
32	NheI	GCTAGC	list 858/862	
33	NmeAIII	GCCGAG (N) 19NN	list 3580/3578	
34	NotI	GCGGCCGC	list *837/841	
35	PacI	TTAATTAA	list 832/830	
36	PaeR7I	CTCGAG	list *1314/1318	
37	PaqCI	CACCTGCNNNNNNNN	list 209/213	
38	PciI	ACATGT	list 2272/2276	
39	PflMI	CCANNNNNTGG	list 149/146	
40	PmeI	GTTTAAAC	list 847	
41	PmlI	CACGTG	list *1088	
42	PpuMI	RGGWCCY	list 936/939	
43	PspOMI	GGGCCC	list *1491/1495	
44	PvuII	CAGCTG	list 3461	
45	RsrII	CGGWCCG	list *3871/3874	

46	SacII	CCGCGG	list	*1245/1243
47	SalI	GTCGAC	list	*215/219
48	SmaI	CCCGGG	list	*812
49	SnaBI	TACGTA	list	*553
50	SpeI	ACTAGT	list	1740/1744
51	SspI	AATATT	list	878
52	StuI	AGGCCT	list	866
53	TspMI	CCCGGG	list	*810/814
54	XbaI	TCTAGA	list	1985/1989
55	XhoI	CTCGAG	list	*1314/1318
56	XmaI	CCCGGG	list	*810/814