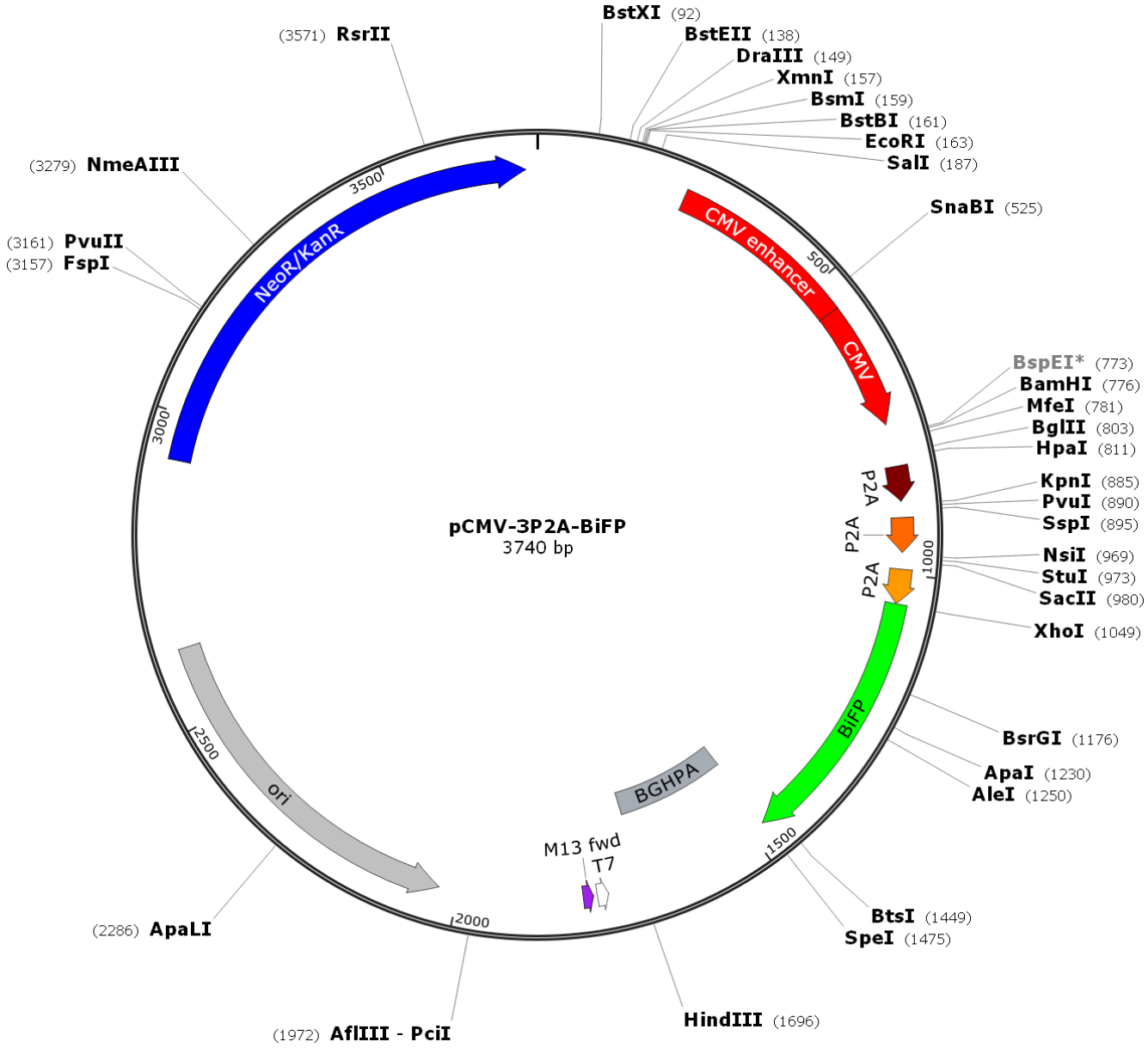
Vector: pCMV-3P2A-BiFP (based on pMOK Vector)

Antibiotic Selection: Kan

Creator(s): Hao Wang & Linjuan Huang @ Molecular Oncology Lab of The University of Chicago Medical Center

Date of Construction: April 2020



3xP2A Coding Regions and Restriction Enzyme Sites

```
   1 GGATCCAATTGCAATGATCATCATGACAGATCTGTTAACggaagc      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 gagaaccctggacctGGTACCCGATCGAATATTggatctggcgcc      KpnI   PvuI   SspI
     E  N  P  G  P  G  T  R  S  N  I  G  S  G  A

 136 accaacttttccctcctgaaacaggctggcgatgtcgaagaaaat
     T  N  F  S  L  L  K  Q  A  G  D  V  E  E  N

 181 cccggccctATGCATAGGCCTCCGCGGggctccggcgccaccaac      NsiI   StuI   SacII
     P  G  P  M  H  R  P  P  R  G  S  G  A  T  N

 226 ttctccctgctgaagcaggccggcgacgtggaggagaaccccggc
     F  S  L  L  K  Q  A  G  D  V  E  E  N  P  G

 271 cccCTCGAGatggtggagaaattcgtgggaacctggaaaatcgca      XhoI   BiFP
     P  L  E  M  V  E  K  F  V  G  T  W  K  I  A

 316 gacagtcataactttggagagtacctgaaggcaatcggagccccct
     D  S  H  N  F  G  E  Y  L  K  A  I  G  A  P

 361 aaggaactgtctgacggcggagatgccaccacaccaaccctgtac
     K  E  L  S  D  G  G  D  A  T  T  P  T  L  Y

 406 atcagccagaaggacggagataaaatgacagtgaagattgagaac
     I  S  Q  K  D  G  D  K  M  T  V  K  I  E  N

 451 gggccccctacattcctggacactcaggtgaagttcaaactgggc
     G  P  P  T  F  L  D  T  Q  V  K  F  K  L  G

 496 gaggagttcgacgagtttcccagcgataggagaaaggggggtgaaa
     E  E  F  D  E  F  P  S  D  R  R  K  G  V  K

 541 tccgtggtcaatctggtcggcgaaaagctggtgtacgtccagaaa
     S  V  V  N  L  V  G  E  K  L  V  Y  V  Q  K

 586 tgggacggcaaggagactacctatgtgcgggaaatcaaggatgga
     W  D  G  K  E  T  T  Y  V  R  E  I  K  D  G

 631 aaactggtcgtgaccctgacaatgggagatgtggtggcagtgcgg
     K  L  V  V  T  L  T  M  G  D  V  V  A  V  R

 676 agttacagacgggctacagaaTAA
     S  Y  R  R  A  T  E  *
```

pCMV-3P2A-BiFP Full-Length Sequence

GGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTACCCTCACTAAAGGGAACAAAAGCTGGTACGAGGACAGGCTGG
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Zero Cutters:

#	Enzyme	Specificity
1	AclI	AACGTT
2	AfeI	AGCGCT
3	AflII	CTTAAG
4	AgeI	ACCGGT
5	AscI	GGCGCGCC
6	AseI	ATTAAT
7	AsiSI	GCGATCGC
8	AvrII	CCTAGG
9	BaeI	(N)5(N)10ACNNNNGTAYC(N)7(N)5
10	BbvCI	CCTCAGC
11	BcgI	NN(N)10CGA(N)6TGC(N)10NN
12	BlpI	GCTNAGC
13	BmtI	GCTAGC
14	Bpu10I	CCTNAGC
15	BsaI	GGTCTCNNNNN
16	BsiWI	CGTACG
17	BspDI	ATCGAT
18	BssHII	GCGCGC
19	BstAPI	GCANNNNNTGC
20	BstZ17I	GTATAC
21	ClaI	ATCGAT
22	Eco53kI	GAGCTC
23	EcoRV	GATATC
24	FseI	GGCCGGCC
25	MluI	ACGCGT
26	NheI	GCTAGC

27	NotI	GCGGCCGC
28	NruI	TCGCGA
29	PacI	TTAATTAA
30	PmeI	GTTTAAAC
31	PmlI	CACGTG
32	PpuMI	RGGWCCY
33	PshAI	GACNNNNGTC
34	PsiI	TTATAA
35	PspXI	VCTCGAGB
36	PstI	CTGCAG
37	SacI	GAGCTC
38	SbfI	CCTGCAGG
39	ScaI	AGTACT
40	SfiI	GGCCNNNNNGGCC
41	SgrAI	CRCCGGYG
42	SmaI	CCCGGG
43	SrfI	GCCCGGGC
44	SwaI	ATTTAAAT
45	TspMI	CCCGGG
46	XbaI	TCTAGA
47	XcmI	CCANNNNNNNNNTGG
48	XmaI	CCCGGG

One-Cutters:

#	Enzyme	Specificity	Sites &
flanks	Cut positions		
(blunt - 5' ext. - 3' ext.)			
1	Acc65I	GGTACC	list
2	AccI	GTMKAC	list
3	AflIII	ACRYGT	list
4	AleI	CACNNNNGTG	list
5	ApaI	GGGCCC	list
6	ApaLI	GTGCAC	list
7	AvaI	CYCGRG	list
8	BamHI	GGATCC	list
9	BclI	TGATCA	list
10	BglII	AGATCT	list
11	BsaBI	GATNNNNATC	list
12	BsaXI	NNN(N)9AC(N)5CTCC(N)7NNN	list
13	BsgI	GTGCAG(N)14NN	list
14	BsmBI	CGTCTCNNNNN	list
15	BsmI	GAATGCN	list
16	BsoBI	CYCGRG	list
17	BspEI	TCCGGA	list
18	BsrGI	TGTACA	list
19	BstBI	TTCGAA	list
20	BstEII	GGTNACC	list
21	BstXI	CCANNNNNNTGG	list
22	Bsu36I	CCTNAGG	list
23	BtsI	GCAGTGNN	list
24	CspCI	NN(N)11CAA(N)5GTGG(N)10NN	list
25	DraIII	CACNNNGTG	list
26	EagI	CGGCCG	list
27	EcoNI	CCTNNNNNAGG	list
28	EcoO109I	RGGNCCY	list
29	EcoRI	GAATTC	list
30	Esp3I	CGTCTCNNNNN	list
31	FspI	TGCGCA	list
32	HindIII	AAGCTT	list
33	HpaI	GTTAAC	list
34	KpnI	GGTACC	list
35	MfeI	CAATTG	list
36	NmeAIII	GCCGAG(N)19NN	list
37	NsiI	ATGCAT	list
38	PaeR7I	CTCGAG	list
39	PciI	ACATGT	list
40	PflMI	CCANNNNNTGG	list
41	PspOMI	GGGCCC	list
42	PvuI	CGATCG	list
43	PvuII	CAGCTG	list
44	RsrII	CGGWCCG	list
45	SacII	CCGCGG	list
46	SalI	GTCGAC	list
47	SexAI	ACCWGGT	list
48	SnaBI	TACGTA	list
49	SpeI	ACTAGT	list