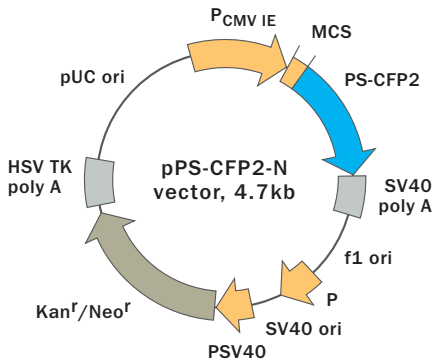


## Mammalian expression vector pPS-CFP2-N



For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

Product	Cat.#	Size
pPS-CFP2-N	<b>FP802</b>	20 µg

Please contact your local distributor for exact prices and delivery information.

Reporter	PS-CFP2
Reporter codon usage	mammalian
Promoter for PS-CFP2	P <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

### Multiple cloning site (MCS)

$\xrightarrow{\text{NheI}}$  G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. . . .  $\xrightarrow{\text{PS-CFP2}}$   
 $\xrightarrow{\text{AfeI}}$   $\xrightarrow{\text{XhoI}^*}$   $\xrightarrow{\text{PstI}}$   $\xrightarrow{\text{SacI}}$   $\xrightarrow{\text{SmaI/XmaI}}$

\* — not unique sites.

### Use

- PS-CFP2 expression in mammalian cells under the control of CMV promoter
- Generation of fusions to the PS-CFP2 N-terminus using vector MCS

### Vector description

pPS-CFP2-N vector is a mammalian expression vector encoding a photo-switchable cyan-to-green fluorescent protein, PS-CFP2. The vector allows generation of fusions to the PS-CFP2 N-terminus and expression PS-CFP2 fusions or PS-CFP2 alone in mammalian cells.

PS-CFP2 codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase PS-CFP2 translation, Kozak consensus translation initiation site is generated upstream of PS-CFP2 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between P<sub>CMV IE</sub> and PS-CFP2 coding sequence.

The vector backbone comprises immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the PS-CFP2 N-terminus when inserted in the same reading frame as PS-CFP2 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. PS-CFP2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization and tracking *in vivo*. Unmodified pPS-CFP2-N will express PS-CFP2, when transfected into eukaryotic (mammalian) cells.

**Note:** The plasmid DNA was isolated from dam<sup>+</sup>-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam<sup>-</sup> host and make fresh DNA.

## Expression in mammalian cells

pPS-CFP2-N vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

## Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

## Location of features

**P<sub>CMV IE</sub>:** 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

**MCS:** 591-671

### PS-CFP2

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681; Stop codon: 1393-1395

### SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1549-1554 & 1578-1583

mRNA 3' ends: 1587 & 1599

**f1 single-strand DNA origin:** 1646-2101

(Packages the noncoding strand of PS-CFP2)

### Bacterial promoter for expression of Kan<sup>r</sup> gene

-35 region: 2163-2168; -10 region: 2186-2191

Transcription start point: 2198

**SV40 origin of replication:** 2442-2577

### SV40 early promoter

Enhancer (72-bp tandem repeats): 2275-2346 & 2347-2418

21-bp repeats: 2422-2442, 2443-2463 & 2465-2485

Early promoter element: 2498-2504

Major transcription start points: 2494, 2532, 2538 & 2543

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2626-2628; stop codon: 3418-3420

G->A mutation to remove PstI site: 2808

C->A (Arg to Ser) mutation to remove BssHII site: 3154

### Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 3656-3661 & 3669-3674

**pUC plasmid replication origin:** 4005-4648

## References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II, Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al.* (1996) *Curr. Biol.* 6: 315-324.

Kozak M. (1987) *Nucleic Acids Res.* 15:8125-8148.

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