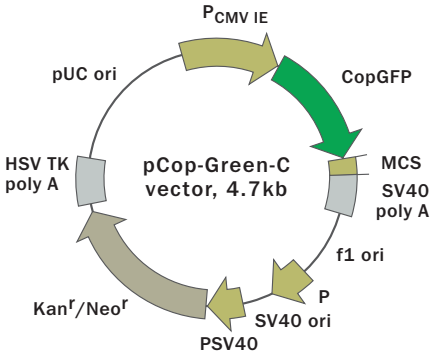
Mammalian expression vector pCop-Green-C



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pCop-Green-C	**FP501**	20 µg

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

Vector type	mammalian expression vector
Reporter	CopGFP
Reporter codon usage	mammalian
Promoter for CopGFP	P CMV IE
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

CopGFP → *BglII* *SacI* *EcoRI* *SalI* *KpnI* *ApaI* *BamHI* *STOPs*

TTC.GCC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A

XhoI *HindIII* *PstI** *SacII* *SmaI/XmaI* *XbaI#* *BclI#*

* - not unique sites. # - sites are blocked by methylation.

Use

- Generation of fusions to the CopGFP C-terminus

- Expression of CopGFP or its fusions in mammalian cells

Vector description

pCop-Green-C is an eukaryotic (mammalian) expression vector encoding green fluorescent protein CopGFP. The vector allows generation of fusions to the CopGFP C-terminus and expression CopGFP fusions or CopGFP alone in eukaryotic (mammalian) cells.

CopGFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase CopGFP translation, Kozak consensus translation initiation site is generated upstream of the CopGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between CopGFP coding sequence and SV40 polyadenylation signal (SV40 polyA).

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) 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 polyA 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^r/Neo^r 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 CopGFP C-terminus when inserted in the same reading frame as CopGFP and no intervening stop codons are present. CopGFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express CopGFP, when transfected into eukaryotic (mammalian) cells.

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

Expression in mammalian cells

pCop-Green-C 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

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
CopGFP
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615;
Stop codon: 1348-1350
Last amino acid in CopGFP: 1376-1378
MCS: 1279-1357
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1490-1495 & 1519-1524
mRNA 3' ends: 1528 & 1540
f1 single-strand DNA origin: 1587-2042
(packages the noncoding strand of CopGFP)
Bacterial promoter for expression of Kan^r gene
-35 region: 2160-2165;
-10 region: 2127-2132
Transcription start point: 2139
SV40 origin of replication: 2383-2518
SV40 early promoter
Enhancer (72-bp tandem repeats): 2216-2287 & 2288-2359
21-bp repeats: 2363-2383, 2384-2404, & 2406-2426
Early promoter element: 2439-2445
Major transcription start points: 2435, 2473, 2479 & 2484
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2567-2569; stop codon: 3359-3361
G->A mutation to remove PstI site: 2749
C->A (Arg to Ser) mutation to remove BssH II site: 3095
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3597-3602 & 3610-3615
pUC plasmid replication origin: 3946-4589

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.