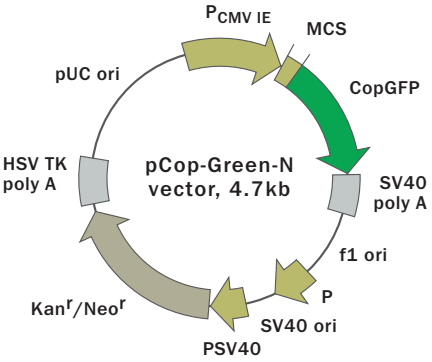
Mammalian expression vector pCop-Green-N



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

Product	Cat.#	Size
pCop-Green-N	**FP502**	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)

NheI BglII SacI HindIII EcoRI SalI KpnI ApaI BamHI AgeI CopGFP

GCTA.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.CCC.GCC*

Eco47III XhoI PstI* SacII SmaI/XmaI

* — not unique sites.

Use

- Generation of fusions to the CopGFP N-terminus

- Expression of CopGFP or its fusions in mammalian cells

Vector description

pCop-Green-N vector is an eukaryotic (mammalian) expression vector encoding green fluorescent protein CopGFP. The vector allows to generate fusions to the CopGFP N-terminus and to express CopGFP fusions or CopGFP alone in 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 CopGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and CopGFP coding sequence.

The vector backbone comprises 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 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^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 the CopGFP N-terminus when inserted in the same reading frame as CopGFP and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. CopGFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Notes: 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-N vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985). Unmodified pCop-Green-N will express CopGFP, when transfected into eukaryotic (mammalian) cells.

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

PCMV IE: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-671
CopGFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; Stop codon: 1496-1498
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1551-1556 & 1580-1585
mRNA 3' ends: 1589 & 1601
f1 single-strand DNA origin: 1648-2103
(Packages the noncoding strand of CopGFP)
Bacterial promoter for expression of Kan^r gene
-35 region: 2165-2170;
-10 region: 2188-2193
Transcription start point: 2200
SV40 origin of replication: 2444-2579
SV40 early promoter
Enhancer (72-bp tandem repeats): 2277-2348 & 2349-2420
21-bp repeats: 2424-2444, 2445-2465, & 2467-2487
Early promoter element: 2500-2506
Major transcription start points: 2496, 2534, 2540 & 2545
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2628-2630; stop codon: 3420-3422
G->A mutation to remove PstI site: 2810
C->A (Arg to Ser) mutation to remove BssHII site: 3156
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3658-3663 & 3671-3676
pUC plasmid replication origin: 4007-4650

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.