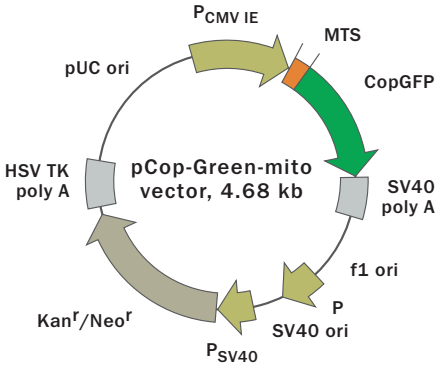
Mammalian expression vector pCop-Green-mito



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

Product	Cat.#	Size
pCop-Green-mito	**FP507**	20 µg

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

Vector type	mammalian expression vector
Reporter	CopGFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase
Reporter codon usage	mammalian
Promoter for CopGFP-MTS	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Use

- Expression of mitochondria-targeted CopGFP in mammalian cells under the control of CMV promoter

- Source of mitochondria-targeted CopGFP coding sequence

Vector description

pCop-Green-mito vector is a mammalian expression vector encoding mitochondria-targeted green fluorescent protein CopGFP. The vector can be used for green fluorescent labeling of mitochondria.

CopGFP codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). A mitochondrial targeting sequence (MTS) is fused to the CopGFP N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995).

The vector is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of the MTS-CopGFP hybrid sequence.

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.

The vector backbone also contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for reporter 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 (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pCop-Green-mito can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondrion-targeted CopGFP in many cell types resulting in green fluorescent labeling of mitochondria. 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-mito fusion
Start codon: 597-599
Mitochondrial targeting sequence (MTS): 597-683
Start of CopGFP coding sequence (ATG): 705-707
Stop codon: 1521-1523
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1577-1582 & 1606-1611
mRNA 3' ends: 1615 & 1627
f1 single-strand DNA origin: 1674-2129
(packages the noncoding strand of CopGFP)
Bacterial promoter expression of Kan^r gene:
-35 region: 2191-2196;
-10 region: 2214-2219
Transcription start point: 2226
SV40 origin of replication: 2470-2605
SV40 early promoter
Enhancer (72-bp tandem repeats): 2303-2374 & 2375-2446
21-bp repeats: 2450-2470, 2471-2491, & 2493-2513
Early promoter element: 2526-2532
Major transcription start points: 2522, 2560, 2566 & 2572
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2654-2656; stop codon: 3446-3448
G->A mutation to remove PstI site: 2836
C->A (Arg to Ser) mutation to remove BssHII site: 3172
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3684-3689 & 3697-3702
pUC plasmid replication origin: 4033-4676

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.

Rizzuto, R., *et al*. (1989) J. Biol. Chem. 264: 10595–10600.

Rizzuto, R., *et al*. (1995) Curr. Biol. 5: 635–642.