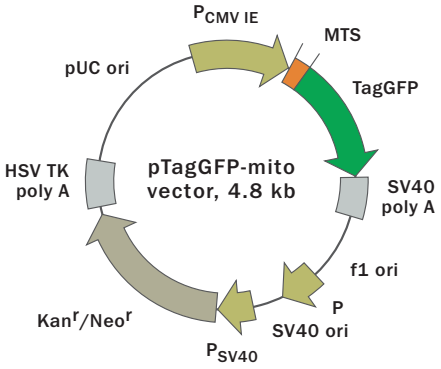
Mammalian expression vector pTagGFP-mito



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

Product	Cat.#	Size
pTagGFP-mito	**FP127**	20 µg

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

Vector type	mammalian expression vector
Reporter	TagGFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase
Reporter codon usage	mammalian
Promoter for TagGFP-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 TagGFP in mammalian cells under the control of CMV promoter

- Source of mitochondria-targeted TagGFP coding sequence

Vector description

pTagGFP-mito is a mammalian expression vector encoding mitochondria-targeted green fluorescent protein TagGFP. The vector can be used for cyan fluorescent labeling of mitochondria.

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

pTagGFP-mito is not intended as a cloning vector; however, the vector backbone contains unique restriction sites that permit excision of MTS-TagGFP 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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted TagGFP in many cell types resulting in green fluorescent labeling of mithochondria. 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
TagGFP-mito fusion
Start codon (ATG): 597-599
Mitochondrial targeting sequence (MTS): 597-704
Start of TagGFP coding sequence (ATG): 705-707
Stop codon: 1419-1421
SV40 early mRNA polyadenylation signals
Polyadenylation signals: 1575-1580 & 1604-1609
mRNA 3' ends: 1613 & 1625
f1 single-strand DNA origin: 1672-2127
Bacterial promoter for expression of Kan^r gene
-35 region: 2189-2194
-10 region: 2212-2217
Transcription start point: 2224
SV40 origin of replication: 2468-2603
SV40 early promoter
Enhancer (72-bp tandem repeats): 2301-2372 & 2373-2444
21-bp repeats: 2448-2468, 2469-2489 & 2491-2511
Early promoter element: 2524-2530
Major transcription start points: 2520, 2558, 2564 & 2569
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2652-2654
Stop codon: 3444-3446
G->A mutation to remove Pst I site: 2834
C->A (Arg to Ser) mutation to remove BssH II site: 3180
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals
Polyadenylation signals: 3682-3687 & 3695-3700
pUC plasmid replication origin: 4031-4674

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.