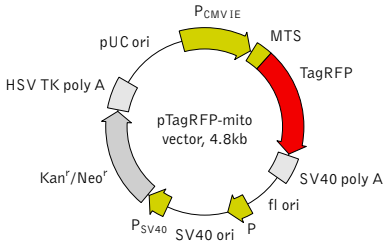
pTagRFP-mito vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
pTagRFP-mito vector	**FP147**	20 μg

Vector type	mammalian expression vector
Reporter	TagRFP
Reporter codon usage	mammalian
Promoter for TagRFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	red (orange) fluorescent labeling of mitochondria

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
TagRFP-mito fusion
Start codon (ATG): 597-599
Mitochondrial targeting sequence (MTS): 597-683
Start of TagRFP coding sequence (ATG): 705-707
Stop codon: 1416-1418
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1571-1576 & 1600-1605
mRNA 3' ends: 1609 & 1621
f1 single-strand DNA origin: 1668-2123
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2185-2190; -10 region: 2208-2213
Transcription start point: 2220
SV40 origin of replication: 2464-2599
SV40 early promoter
Enhancer (72-bp tandem repeats): 2297-2368 & 2369-2440
21-bp repeats: 2444-2464, 2465-2485 & 2487-2507
Early promoter element: 2520-2526
Major transcription start points: 2516, 2554, 2560 & 2565
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2648-2650; Stop codon: 3440-3442
G->A mutation to remove Pst I site: 2830
C->A (Arg to Ser) mutation to remove BssH II site: 3176
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3678-3683 & 3691-3696
pUC plasmid replication origin: 4027-4670

Vector description

pTagRFP-mito is a mammalian expression vector intended for red (orange) fluorescent labeling of mitochondria in living cells. The vector encodes red (orange) fluorescent protein TagRFP fused to mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989; Rizzuto et al. 1995]. MTS is fused to the TagRFP N-terminus.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

pTagRFP-mito vector can be used as a source of TagRFP-MTS hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

Note: The plasmid DNA was isolated from dam$^+$-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$^-$ host and make fresh DNA.

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 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

pTagRFP-mito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-MTS fusion in eukaryotic cells. 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.

References

Gorman, C. (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248

Rizzuto, R. et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." J Biol Chem, 264 (18): 10595–10600 / pmid: 2543673

Rizzuto, R. et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." Curr Biol, 5 (6): 635–642 / pmid: 7552174