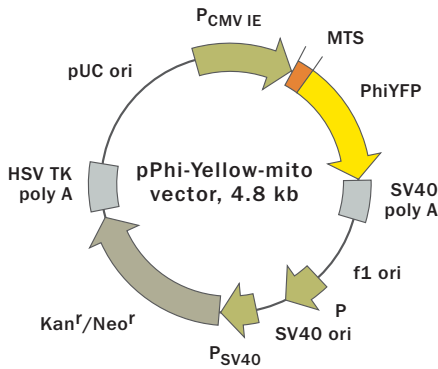


Mammalian expression vector pPhi-Yellow-mito



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

Use

- Expression of mitochondria-targeted PhiYFP in mammalian cells under the control of CMV promoter
- Source of mitochondria-targeted PhiYFP coding sequence

Product	Cat.#	Size
pPhi-Yellow-mito	FP607	20 µg
Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	PhiYFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase	
Reporter codon usage	mammalian	
Promoter for PhiYFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)	
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori	

Vector description

pPhi-Yellow-mito is an eukaryotic (mammalian) expression vector encoding mitochondria-targeted yellow fluorescent protein PhiYFP. The vector can be used for yellow fluorescent labeling of mitochondria.

PhiYFP codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). A mitochondrial targeting sequence (MTS) is fused to the PhiYFP 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-PhiYFP hybrid sequence.

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 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 PhiYFP in many cell types resulting in yellow 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

PhiYFP-mito fusion

Start codon (ATG): 597-599

Mitochondrial targetting sequence (MTS): 597-683

Start of Phi YFP coding sequence (ATG): 705-707

Stop codon: 1407-1409

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1623-1628 & 1652-1658

mRNA 3' ends: 1661 & 1673

f1 single-strand DNA origin: 1720-2175

(packages the noncoding strand of PhiYFP)

Bacterial promoter expression of Kan^r gene:

-35 region: 2237-2242;

-10 region: 2260-2265

Transcription start point: 2272

SV40 origin of replication: 2516-2651

SV40 early promoter

Enhancer (72-bp tandem repeats): 2349-2420 & 2421-2492

21-bp repeats: 2496-2516, 2517-2537, & 2539-2559

Early promoter element: 2572-2578

Major transcription start points: 2568, 2606, 2612 & 2617

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2700-2702;

Stop codon: 3492-3494

G->A mutation to remove PstI site: 2882

C->A (Arg to Ser) mutation to remove BssHII site: 3228

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 3730-3735 & 3743-3748

pUC plasmid replication origin: 4079-4722

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.

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MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.