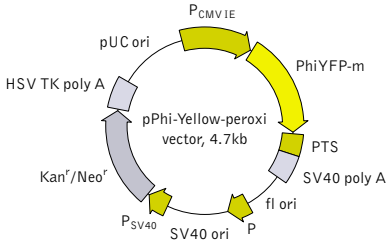
pPhi-Yellow-peroxi vector

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
pPhi-Yellow-peroxi vector	**FP606**	20 μg

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

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
PhiYFP-m
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615
A->T substitution (Gln to Leu): 989
Last amino acid in PhiYFP-m: 1312-1314
Peroxisomal targeting signal (PTS): 1315-1323
Stop codon: 1324-1326
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1534-1539 & 1563-1568
mRNA 3' ends: 1572 & 1584
f1 single-strand DNA origin: 1631-2086
Bacterial promoter for expression of Kan^r gene
-35 region: 2148-2153; -10 region: 2171-2176
Transcription start point: 2183
SV40 origin of replication: 2427-2562
SV40 early promoter
Enhancer (72-bp tandem repeats): 2260-2331 & 2332-2403
21-bp repeats: 2407-2427, 2428-2448 & 2450-2470
Early promoter element: 2483-2489
Major transcription start points: 2479, 2517, 2523 & 2528
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2611-2613; Stop codon: 3403-3405
G->A mutation to remove Pst I site: 2793
C->A (Arg to Ser) mutation to remove BssH II site: 3139
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3641-3646 & 3654-3659
pUC plasmid replication origin: 3990-4633

Vector description

pPhi-Yellow-peroxi is a mammalian expression vector intended for yellow fluorescent labeling of peroxisomes in living cells. The vector encodes yellow fluorescent protein PhiYFP-m targeted to the matrix of peroxisomes by tripeptide SKL (peroxisomal targeting signal, PTS) fused to the PhiYFP-m C-terminus.

Note: pPhi-Yellow-peroxi vector contains A->T substitution in position 989 (resulting in Gln to Leu substitution in amino acid position 126 of PhiYFP-m) that does not influence properties of the reporter.

PhiYFP-m codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the PhiYFP-m sequence[Kozak 1987].

pPhi-Yellow-peroxi can be used as a source of PhiYFP-m-PTS 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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of peroxisome-targeted PhiYFP-m in many cell types resulting in yellow fluorescent labeling of peroxisomes. 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

Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277