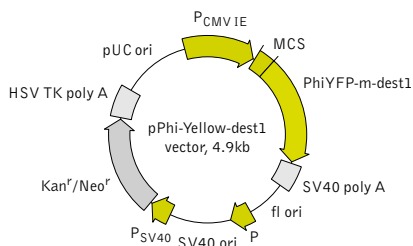


pPhi-Yellow-dest1 vector

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/support/vector-info.shtml>

Multiple cloning site (MCS)

... G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. A . . .

Restriction sites: *Afe I*, *Xho I*, *Hind III*, *Pst I*, *Kpn I*, *Apa I*, *BamH I*, *PhiYFP-m-dest1*, *Nhe I*, *Bgl II*, *Sac I*, *EcoR I*, *Sal I*, *Sac II*, *Sma I/Xma I*, *Age I*

* — not unique sites.

Location of features

pCMV IE: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 MCS: 591-671
 PhiYFP-m
 Kozak consensus translation initiation site: 672-682
 Start codon (ATG): 679-681
 Last amino acid in PhiYFP-m: 1378-1380
 Stop codon: 1516-1518
 MODC PEST sequence: 1396-1518
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1673-1678 & 1702-1707
 mRNA 3' ends: 1711 & 1723
 f1 single-strand DNA origin: 1770-2225
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 2287-2292; -10 region: 2310-2315
 Transcription start point: 2322
 SV40 origin of replication: 2566-2701
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2399-2470 & 2471-2542
 21-bp repeats: 2546-2566, 2567-2587 & 2589-2609
 Early promoter element: 2622-2628
 Major transcription start points: 2618, 2656, 2662 & 2667
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2750-2752; Stop codon: 3542-3544
 G->A mutation to remove Pst I site: 2932
 C->A (Arg to Ser) mutation to remove BssH II site: 3278
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3780-3785 & 3793-3798
 pUC plasmid replication origin: 4129-4772

References

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

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

Li et al. (1998) "Generation of destabilized green fluorescent protein as a transcription reporter." *J Biol Chem*, 273(52): 34970-34975 / pmid: 9857028

Product	Cat.#	Size
pPhi-Yellow-dest1 vector	FP608	20 µg
The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.		
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	PhiYFP-m expression in mammalian cells; generation of fusions to the PhiYFP-m-dest1 N-terminus	

Vector description

pPhi-Yellow-dest1 is a mammalian expression vector encoding destabilized yellow fluorescent protein PhiYFP-m. To generate PhiYFP-m-dest1 variant, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the PhiYFP-m C-terminus. This MODC region contains a PEST amino acid sequence that targets the protein for degradation and provides for rapid protein turnover [Li et al. 1998]. PhiYFP-m-dest1 retains fluorescent properties of the native protein and has a half-life of approximately 1-1.5 hours, as measured by fluorescence intensity of cells treated with the protein synthesis inhibitor, cycloheximide.

pPhi-Yellow-dest1 carries synthetic version of the PhiYFP-m-dest1 gene which 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 PhiYFP-m-dest1 coding sequence [Kozak 1987].

pPhi-Yellow-dest1 vector can be used to express PhiYFP-m-dest1 in eukaryotic (mammalian) cells. For example it can be used as a positive control with a pPhiYFP-m-PRL-dest1 promoterless vector (Cat.# FP605). The vector can be also used to generate destabilized PhiYFP-m-tagged fusion proteins. Multiple cloning site (MCS) is located upstream of PhiYFP-m-dest1 coding sequence.

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.

Generation of PhiYFP-m-dest1-tagged fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the PhiYFP-m-dest1 N-terminus when inserted in the same reading frame as PhiYFP-m and no in-frame stop codons are present. PhiYFP-m-dest1-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pPhi-Yellow-dest1 vector will express PhiYFP-m-dest1 when transfected into eukaryotic (mammalian) cells.

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.

Expression in mammalian cells

pPhi-Yellow-dest1 vector can be transfected into mammalian cells by any known transfection method. 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.

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