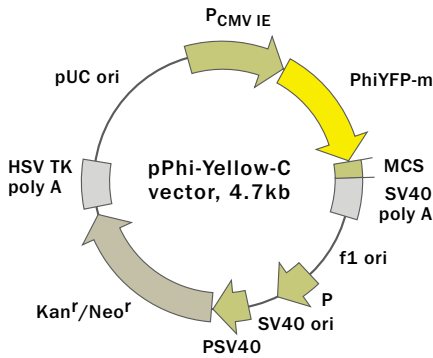


## Mammalian expression vector pPhi-Yellow-C



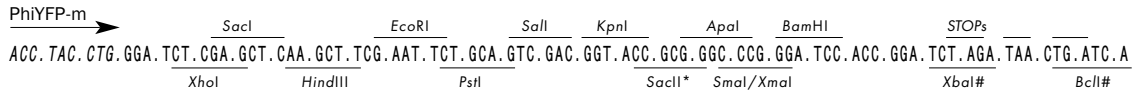
For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

Product	Cat.#	Size
pPhi-Yellow-C	<b>FP601</b>	20 µg

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 <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

### Multiple cloning site (MCS)



\* - not unique site. # - sites are blocked by methylation.

### Use

- Generation of fusions to the PhiYFP-m C-terminus
- Expression of PhiYFP-m or its fusions in mammalian cells

### Vector description

pPhi-Yellow-C vector is an eukaryotic (mammalian) expression vector encoding true yellow fluorescent protein, PhiYFP-m. The vector allows to generate fusions to the PhiYFP-m C-terminus and to express PhiYFP-m fusions or PhiYFP-m alone in mammalian cells.

PhiYFP-m codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase PhiYFP-m translation, Kozak consensus translation initiation site is generated upstream of the PhiYFP-m sequence (Kozak, 1987). Multiple cloning site (MCS) is located between PhiYFP-m coding sequence and SV40 polyadenylation signal (SV40 polyA).

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Generation of 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 C-terminus when inserted in the same reading frame as PhiYFP-m and no intervening stop codons are present. TurboYFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

**Notes:** The plasmid DNA was isolated from dam<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

PhiYFP-m gene is specially designed to generate C-fusions and was not tested in N-fusions. Despite its dimeric structure, TurboYFP is still suitable for generation of fusions with proteins of interest, however we recommend that you use TagYFP for these purposes.

## Expression in mammalian cells

The vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985). Unmodified vector will express PhiYFP-m, when transfected into eukaryotic (mammalian) cells.

## 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<sub>CMV IE</sub>**: 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; Stop codon: 1384-1386

Last amino acid in PhiYFP-m: 1312-1314

**MCS**: 1315-1400

### SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1526-1531 & 1555-1560

mRNA 3' ends: 1564 & 1576

**f1 single-strand DNA origin**: 1623-2078

(packages the noncoding strand of PhiYFP-m)

### Bacterial promoter for expression of Kan<sup>r</sup> gene

-35 region: 2140-2145; -10 region: 2163-2168

Transcription start point: 2175

**SV40 origin of replication**: 2419-2554

### SV40 early promoter

Enhancer (72-bp tandem repeats): 2252-2323 & 2324-2495

21-bp repeats: 2399-2419, 2420-2440, & 2442-2462

Early promoter element: 2475-2481

Major transcription start points: 2471, 2509, 2515 & 2520

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2603-2605; stop codon: 3395-3397

G->A mutation to remove PstI site: 2785

C->A (Arg to Ser) mutation to remove BssHII site: 3131

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

Polyadenylation signals: 3633-3638 & 3646-3651

**pUC plasmid replication origin**: 3982-4625

## 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.

Kozak M. (1987) *Nucleic Acids Res.* 15:8125-8148.

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