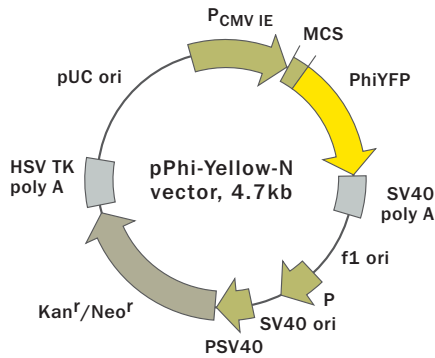
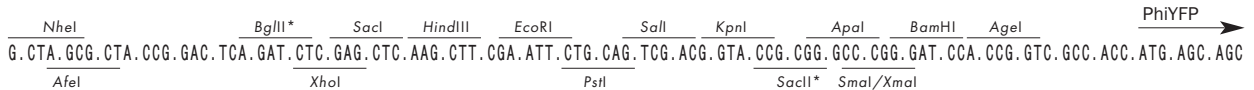


Mammalian expression vector pPhi-Yellow-N



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

Multiple cloning site (MCS)



* - not unique site.

Use

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

| Product | Cat.# | Size |
|---------------|-------|-------|
| pPhi-Yellow-N | FP602 | 20 µg |

Please contact your local distributor for exact prices and delivery information.

| | |
|----------------------|---|
| Vector type | mammalian expression vector |
| Reporter | PhiYFP |
| 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-N vector is an eukaryotic (mammalian) expression vector encoding true yellow fluorescent protein, PhiYFP. The vector allows to generate fusions to the PhiYFP N-terminus and to express PhiYFP fusions or PhiYFP alone in mammalian cells.

PhiYFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase PhiYFP translation, Kozak consensus translation initiation site is generated upstream of PhiYFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between P_{CMV IE} and PhiYFP coding sequence.

The vector backbone comprises 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 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^r/Neo^r 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 N-terminus when inserted in the same reading frame as PhiYFP and no intervening stop codons are present. The inserted sequence should contain an initiating ATG codon. PhiYFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Notes: 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.

PhiYFP gene is not suitable to generate fusions to its C-terminus. Please use pPhi-Yellow-C vector containing specially modified PhiYFP variant (PhiYFP-m) to generate such fusion proteins. As Phi-Yellow proteins capable of dimerise, we recommend that you use monomeric TagYFP for generation of fusions with oligomerizing proteins of interest.

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 pPhi-Yellow-N will express PhiYFP, 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_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

MCS: 591-671

PhiYFP

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681; Stop codon: 1381-1383

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1597-1602 & 1626-1631

mRNA 3' ends: 1635 & 1647

f1 single-strand DNA origin: 1694-2149

(packages the noncoding strand of PhiYFP)

Bacterial promoter for expression of Kan^r gene

-35 region: 2211-2216; -10 region: 2234-2239

Transcription start point: 2246

SV40 origin of replication: 2490-2625

SV40 early promoter

Enhancer (72-bp tandem repeats): 2323-2394 & 2395-2466

21-bp repeats: 2470-2490, 2491-2511 & 2513-2533

Early promoter element: 2546-2552

Major transcription start points: 2542, 2580, 2586 & 2591

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2674-2676; stop codon: 3466-3468

G->A mutation to remove PstI site: 2856

C->A (Arg to Ser) mutation to remove BssHII site: 3202

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

Polyadenylation signals: 3704-3709 & 3717-3722

pUC plasmid replication origin: 4053-4696

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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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.