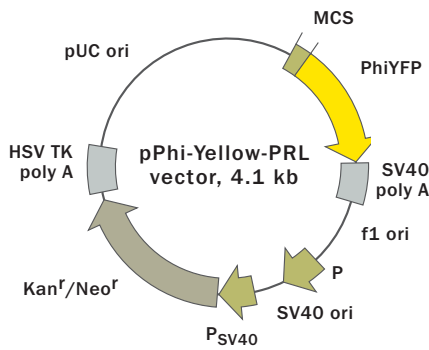


Promoterless vector pPhi-Yellow-PRL



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

Product	Cat.#	Size
pPhi-Yellow-PRL	FP604	20 µg

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

Vector type	promoterless vector
Reporter	PhiYFP
Reporter codon usage	mammalian
Promoter for PhiYFP	NO
Host cells	mammalian, bacterial
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

PhiYFP →
 A. 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. AGC. AGC
BglII*
SacI
HindIII
EcoRI
Sall
KpnI
ApaI
BamHI
AgeI
AfeI
XhoI
PstI
SacII*
SmaI/XmaI

* — not unique sites.

Use

- Monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS

Vector description

pPhi-Yellow-PRL vector is a promoterless vector encoding yellow fluorescent protein PhiYFP, which can be used as *in vivo* reporter of gene expression. PhiYFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase PhiYFP mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of PhiYFP coding sequence (Kozak, 1987).

Multiple cloning site (MCS) is located upstream of the Kozak consensus translation initiation site and can be used to clone a promoter or a promoter/enhancer combination of interest. Without the addition of a functional promoter, this vector will not express PhiYFP.

The vector backbone comprises 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) allows 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.

Note: This 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

The 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.

Location of features

MCS: 12-89

PhiYFP

Kozak consensus translation initiation site: 90-100

Start codon (ATG): 97-99;

Stop codon: 799-801

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1015-1020 & 1044-1049

mRNA 3' ends: 1053 & 1065

f1 single-strand DNA origin: 1112-1567

(packages the noncoding strand of PhiYFP)

Bacterial promoter expression of Kan^r gene:

-35 region: 1629-1634; -10 region: 1652-1657

Transcription start point: 1664

SV40 origin of replication: 1908-2043

SV40 early promoter

Enhancer (72-bp tandem repeats): 1741-1812 & 1813-1884

21-bp repeats: 1888-1908, 1909-1929, & 1931-1951

Early promoter element: 1964-1970

Major transcription start points: 1960, 1998, 2004 & 2009

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2092-2094; stop codon: 2884-2886

G->A mutation to remove PstI site: 2274

C->A (Arg to Ser) mutation to remove BssHII site: 2620

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

Polyadenylation signals: 3122-3127 & 3135-3140

pUC plasmid replication origin: 3471-4114

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.

Notice to Purchaser:

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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.