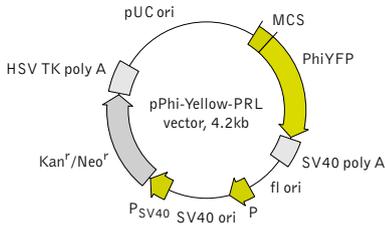


pPhi-Yellow-PRL vector

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)

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... A. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. A ...
      Bgl II*   Sac I   Hind III*   Pst I   Kpn I   Apa I   BamH I   PhiYFP
      EcoR I   Sal I   Sac II*   Sma I/Xma I   Age I
  
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* — not unique sites.

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
 Eukaryotic promoter for 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 Pst I site: 2274
 C->A (Arg to Ser) mutation to remove BssH II 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 (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

Product	Cat.#	Size
pPhi-Yellow-PRL vector	FP604	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	promoterless expression vector	
Reporter	PhiYFP	
Reporter codon usage	mammalian	
Promoter for PhiYFP	NO	
Host cells	mammalian, prokaryotic	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	Monitoring of activity of different promoters and promoter/enhancer combinations	

Vector description

pPhi-Yellow-PRL 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 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 contains 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.

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

The vector will express PhiYFP under the control of functional promoter cloned into the vector's MCS. pPhi-Yellow-PRL 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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