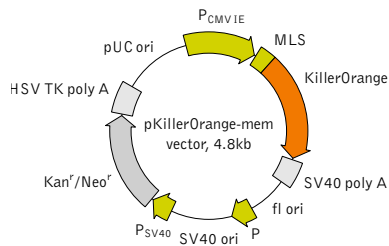


pKillerOrange-mem vector

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/products/vectors.shtml>

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 KillerOrange-mem fusion
 Start codon (ATG): 679-681
 Neuromodulin N-terminal sequence (mem): 679-738

Start of KillerOrange coding sequence (GAG): 742-744
 Stop codon: 1450-1452
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1605-1610 & 1634-1639
 mRNA 3' ends: 1643 & 1655
 f1 single-strand DNA origin: 1702-2157
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2219-2224; -10 region: 2242-2247
 Transcription start point: 2254
 SV40 origin of replication: 2498-2633
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2331-2402 & 2403-2474
 21-bp repeats: 2478-2498, 2499-2519 & 2521-2541
 Early promoter element: 2554-2560
 Major transcription start points: 2550, 2588, 2594 & 2599
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2682-2684; Stop codon: 3474-3476
 G->A mutation to remove Pst I site: 2864
 C->A (Arg to Ser) mutation to remove BssH II site: 3210
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3712-3717 & 3725-3730
 pUC plasmid replication origin: 4061-4704

References

- Gorman, C. (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, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
- Skene, J.H. and I. Virag (1989) "Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43." *J Cell Biol*, 108 (2): 613-624 / pmid: 2918027

Product	Cat.#	Size
pKillerOrange-mem vector	FP226	20 µg
Vector type	mammalian expression vector	
Reporter	KillerOrange	
Reporter codon usage	mammalian	
Promoter for KillerOrange	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	Expression of membrane-targeted KillerOrange in mammalian cells under the control of CMV promoter; source of membrane-targeted KillerOrange coding sequence	

Vector description

pKillerOrange-mem is a mammalian expression vector encoding membrane-targeted KillerOrange. KillerOrange localized on cellular membrane can be used for effective light-induced cell killing.

Note: Comparing to the mitochondrially targeted KillerOrange, irradiation of membrane-localized KillerOrange leads to even more effective and fast cell death (within 10-30 min). Moreover, membrane-targeted KillerOrange was shown to be suitable for the light induced cell killing within a developing zebrafish.

KillerOrange codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Membrane localization signal (MLS) of neuromodulin is linked to the KillerOrange N-terminus. The MLS (N-terminal 20 amino acid residues of neuromodulin) contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets KillerOrange to cellular membranes [Skene and Virag 1989].

pKillerOrange-mem vector can be used as a source of MLS-KillerOrange hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

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.

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.

Expression in mammalian cells

pKillerOrange-mem vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of membrane-targeted KillerOrange in eukaryotic cells. 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.

Notice to Purchaser:

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MSDS information is available at <http://www.evrogen.com/MSDS.shtml>