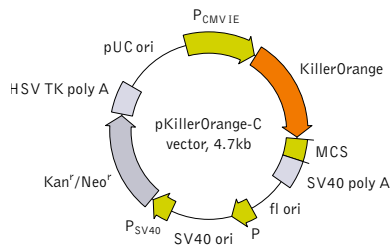


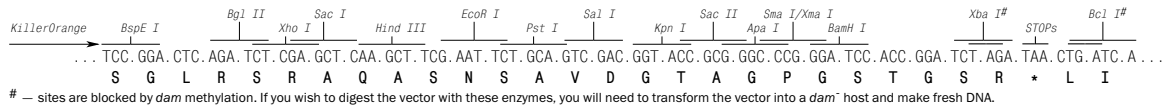
pKillerOrange-C vector

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>

Multiple cloning site (MCS)



Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 KillerOrange
 Kozak consensus translation initiation site: 606-616
 Start codon (ATG): 613-615
 Stop codon: 1402-1404
 Last amino acid in KillerOrange: 1321-1323
 MCS: 1324-1411
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1544-1549 & 1573-1578
 mRNA 3' ends: 1582 & 1594
 f1 single-strand DNA origin: 1641-2096
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2158-2163
 -10 region: 2181-2186
 Transcription start point: 2193
 SV40 origin of replication: 2437-2572
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2270-2341 & 2342-2413
 21-bp repeats: 2417-2437, 2438-2458 & 2460-2480
 Early promoter element: 2493-2499
 Major transcription start points: 2489, 2527, 2533 & 2538
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2621-2623
 Stop codon: 3413-3415
 G->A mutation to remove Pst I site: 2803
 C->A (Arg to Ser) mutation to remove BssH II site: 3149
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3651-3656 & 3664-3669
 pUC plasmid replication origin: 4000-4643

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
- Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125–8148 / pmid: 3313277

Product	Cat.#	Size
pKillerOrange-C vector	FP221	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	KillerOrange expression in mammalian cells; generation of fusions to the KillerOrange C-terminus	

Vector description

pKillerOrange-C is a mammalian expression vector encoding photosensitizer KillerOrange. The vector allows generation of fusions to the KillerOrange C-terminus and expression of KillerOrange fusions or KillerOrange alone in eukaryotic (mammalian) cells.

KillerOrange 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 the KillerOrange coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between KillerOrange coding sequence and SV40 polyadenylation signal (SV40 polyA).

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.

Generation of KillerOrange fusion proteins

A localization signal or a gene of interest can be cloned into MCS of the vector. It will be expressed as a fusion to the KillerOrange C-terminus when inserted in the same reading frame as KillerOrange and no in-frame stop codons are present. KillerOrange-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express KillerOrange when transfected into eukaryotic (mammalian) cells.

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

pKillerOrange-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of KillerOrange or its fusions 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>