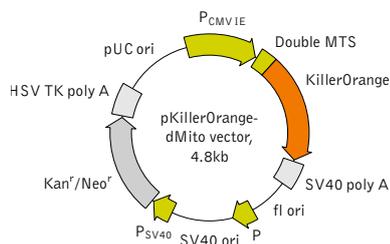


pKillerOrange-dMito vector

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-dMito fusion
 Start codon (ATG): 597-599
 Mitochondrial localization signal 1 (MLS-1): 597-689
 Mitochondrial localization signal 2 (MLS-2): 690-782
 Start of KillerOrange coding sequence(GAG): 801-803
 Stop codon: 1509-1511
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1664-1669 & 1693-1698
 mRNA 3' ends: 1702 & 1714
 f1 single-strand DNA origin: 1761-2216
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2278-2283; -10 region: 2301-2306
 Transcription start point: 2313
 SV40 origin of replication: 2557-2692
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2390-2461 & 2462-2533
 21-bp repeats: 2537-2557, 2558-2578 & 2580-2600
 Early promoter element: 2613-2619
 Major transcription start points: 2609, 2647, 2653 & 2658
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2741-2743; Stop codon: 3533-3535
 G->A mutation to remove Pst I site: 2923
 C->A (Arg to Ser) mutation to remove BssH II site: 3269
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3771-3776 & 3784-3789
 pUC plasmid replication origin: 4120-4763

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
- Rizzuto, R., M. Brini, et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." *Curr Biol*, 5 (6): 635-642 / pmid: 7552174
- Rizzuto, R., H. Nakase, et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." *J Biol Chem*, 264 (18): 10595-10600 / pmid: 2543673

Product	Cat.#	Size
pKillerOrange-dMito vector	FP224	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 mitochondria-targeted KillerOrange in mammalian cells under the control of CMV promoter; source of mitochondria-targeted KillerOrange coding sequence	

Vector description

pKillerOrange-dMito is a mammalian expression vector encoding mitochondria-targeted KillerOrange. KillerOrange codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Duplicated mitochondrial targeting sequence (MTS) is fused to the KillerOrange N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase [Rizzuto, Nakase, et al. 1989; Rizzuto, Brini, et al. 1995].

pKillerOrange-dMito vector can be used as a source of dMTS-KillerOrange hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice. Alternatively, dMTS-KillerOrange coding sequence can be amplified by PCR.

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-dMito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-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>