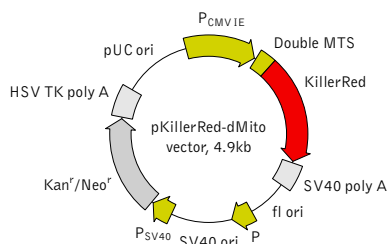


## pKillerRed-dMito vector

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<sub>CMV IE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 KillerRed-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 KillerRed coding sequence (ATG): 798-800  
 Stop codon: 1515-1517  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1671-1676 & 1700-1705  
 mRNA 3' ends: 1709 & 1721  
 f1 single-strand DNA origin: 1768-2223  
 Eukaryotic promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2285-2290; -10 region: 2308-2313  
 Transcription start point: 2320  
 SV40 origin of replication: 2564-2699  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2397-2468 & 2469-2540  
 21-bp repeats: 2544-2564, 2565-2585 & 2587-2607  
 Early promoter element: 2620-2626  
 Major transcription start points: 2616, 2654, 2660 & 2665  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2748-2750; Stop codon: 3540-3542  
 G->A mutation to remove Pst I site: 2930  
 C->A (Arg to Ser) mutation to remove BssH II site: 3276  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3778-3783 & 3791-3796  
 pUC plasmid replication origin: 4127-4770

### 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. 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

Rizzuto, R. 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

Product	Cat.#	Size
pKillerRed-dMito vector	<b>FP964</b>	20 µg
Vector type	mammalian expression vector	
Reporter	KillerRed	
Reporter codon usage	mammalian	
Promoter for KillerRed	P <sub>CMV IE</sub>	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	Expression of mitochondria-targeted KillerRed in mammalian cells under the control of CMV promoter; source of mitochondria-targeted KillerRed coding sequence	

### Vector description

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

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

**Note:** The plasmid DNA was isolated from dam<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Expression in mammalian cells

pKillerRed-dMito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted KillerRed in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

**Note:** KillerRed shows no cell toxic effects before light activation. Upon green light irradiation KillerRed generates reactive oxygen species (ROS) that damage the neighboring molecules.

### 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:

KillerRed-related materials (also referred to as "Products") are intended for research use only.

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>