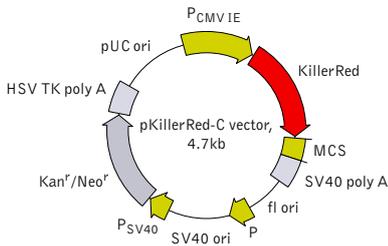


pKillerRed-C vector

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/support/vector-info.shtml>

pKillerRed-C vector MCS

$\xrightarrow{\text{KillerRed}}$
 $\xrightarrow{\text{BspE I}}$
 $\xrightarrow{\text{Xho I}}$
 $\xrightarrow{\text{Hind III}}$
 $\xrightarrow{\text{Pst I}}$
 $\xrightarrow{\text{Kpn I}}$
 $\xrightarrow{\text{Apa I}}$
 $\xrightarrow{\text{BamH I}}$
 $\xrightarrow{\text{STOPI}}$

 ... TCC. GGA. CTC. AGA. TCT. CGA. GCT. CAA. GGT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGA. TCT. AGA. TAA. CTG. ATC. A ...

— sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Location of features

P_{CMV IE}: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
KillerRed
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; **Stop codon:** 1408-1410
Last amino acid in KillerRed: 1327-1329
MCS: 1330-1417
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1550-1555 & 1579-1584
mRNA 3' ends: 1588 & 1600
f1 single-strand DNA origin: 1647-2102
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2164-2169; **-10 region:** 2187-2192
Transcription start point: 2199
SV40 origin of replication: 2443-2578
SV40 early promoter
Enhancer (72-bp tandem repeats): 2276-2347 & 2348-2419
21-bp repeats: 2423-2443, 2444-2464 & 2466-2486
Early promoter element: 2499-2505
Major transcription start points: 2495, 2533, 2539 & 2544
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2627-2629; **Stop codon:** 3419-3421
G->A mutation to remove Pst I site: 2809
C->A (Arg to Ser) mutation to remove BssH II site: 3155
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3657-3662 & 3670-3675
pUC plasmid replication origin: 4006-4649

References

Bulina et al. (2006) "Chromophore-assisted light inactivation (CALI) using the phototoxic fluorescent protein KillerRed." *Nat Protoc*, 1 (2): 947-53 / pmid: 17406328
 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. 143-90.
 Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-24 / pmid: 8805248
 Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-48 / pmid: 3313277

Product	Cat.#	Size
pKillerRed-C vector	FP961	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	mammalian expression vector	
Reporter	KillerRed	
Reporter codon usage	mammalian	
Promoter for KillerRed	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	KillerRed expression in mammalian cells; generation of fusions to the KillerRed C-terminus	

Vector description

pKillerRed-C is a mammalian expression vector encoding red fluorescent protein KillerRed. The vector allows generation of fusions to the KillerRed C-terminus and expression of KillerRed fusions or KillerRed alone in eukaryotic (mammalian) cells.

KillerRed 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 KillerRed sequence [Kozak 1987]. Multiple cloning site (MCS) is located between KillerRed 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 KillerRed-fusion proteins

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

pKillerRed-C 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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