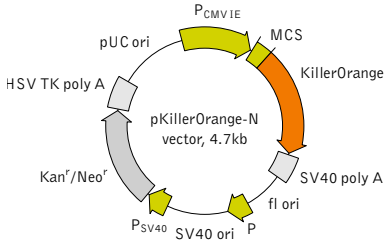
pKillerOrange-N vector

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.

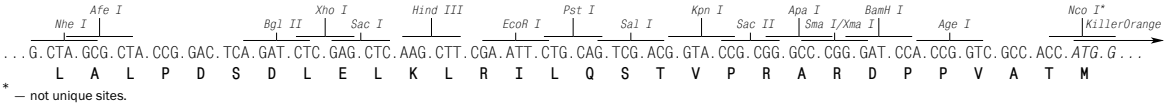


For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
pKillerOrange-N vector	**FP222**	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 N-terminus

Multiple cloning site (MCS)



* — not unique sites.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-678
KillerOrange
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
Stop codon: 1390-1392
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1545-1550 & 1574-1579
mRNA 3' ends: 1583 & 1595
f1 single-strand DNA origin: 1642-2097
Bacterial promoter for expression of Kan^r gene
-35 region: 2159-2164; -10 region: 2182-2187
Transcription start point: 2194
SV40 origin of replication: 2438-2573
SV40 early promoter
Enhancer (72-bp tandem repeats): 2271-2342 & 2343-2414
21-bp repeats: 2418-2438, 2439-2459 & 2461-2481
Early promoter element: 2494-2500
Major transcription start points: 2490, 2528, 2534 & 2539
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2622-2624; Stop codon: 3414-3416
G->A mutation to remove Pst I site: 2804
C->A (Arg to Ser) mutation to remove BssH II site: 3150
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3652-3657 & 3665-3670
pUC plasmid replication origin: 4001-4644

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

Vector description

pKillerOrange-N is a mammalian expression vector encoding photosensitizer KillerOrange. The vector allows generation of fusions to the KillerOrange N-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 $P_{CMV\ IE}$ and KillerOrange coding sequence.

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 N-terminus when inserted in the same reading frame as KillerOrange and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. 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-N 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.