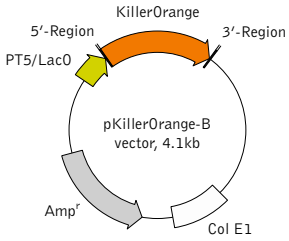
pKillerOrange-B vector

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-B vector	**FP223**	20 μg

Vector type	bacterial expression vector
Reporter	KillerOrange
Reporter codon usage	mammalian
Promoter for KillerOrange	T5 promoter/lac operator
Host cells	prokaryotic
Selection	ampicillin
Replication	ColE1 ori
Use	Source of the KillerOrange coding sequence; KillerOrange expression in bacterial cells

5' Region

RBS ATG.AGA.GGA.TCG.GGA.TCC.GAG.T . . .
BamH I

3' Region

STOP
. . . TGA.AAG.CTT . . .
Hind III

Location of features

T5 promoter/lac operator element: 7-87
T5 transcription start: 61
KillerOrange coding sequence: 115-843
Lambda t0 transcriptional termination region: 865-959
rrnB T1 transcriptional termination region: 1721-1819
ColE1 origin of replication: 2295
beta-lactamase coding sequence: 3913-3053

Vector description

pKillerOrange-B is a prokaryotic expression vector encoding photosensitizer KillerOrange. Reporter codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

The vector is primarily intended as a source of KillerOrange coding sequence. Flanking restriction sites are convenient for excision of KillerOrange sequence and its further insertion into other expression vectors of choice. Alternatively, 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 can be also used for KillerOrange expression in prokaryotes under the control of T5 promoter/lac operator. The vector backbone contains ColE1 origin of replication and ampicillin resistance gene for propagation and selection in *E. coli*.

References

Haas, J. et al. (1996) “Codon usage limitation in the expression of HIV-1 envelope glycoprotein.” Curr Biol, 6 (3): 315–324 / pmid: 8805248