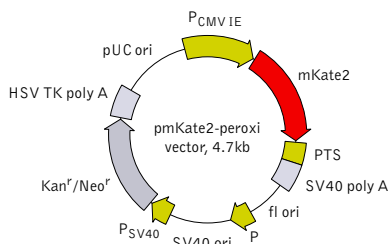


pmKate2-peroxi vector

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>

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 mKate2-PTS1 fusion
 Start codon (ATG): 679-681
 Start of mKate2 coding sequence (ATG): 679-681
 Peroxisomal Targeting Signal 1 (PTS1): 1387-1395
 Stop codon: 1396-1398
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1550-1555 & 1579-1584
 mRNA 3' ends: 1588 & 1600
 f1 single-strand DNA origin: 1647-2102
 Bacterial 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

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. 143-190.

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

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

Product	Cat.#	Size
pmKate2-peroxi vector	FP313	20 µg
Vector type	mammalian expression vector	
Reporter	mKate2	
Reporter codon usage	mammalian	
Promoter for mKate2	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	far-red fluorescent labeling of peroxisomes	

Vector description

pmKate2-peroxi is a mammalian expression vector intended for far-red fluorescent labeling of peroxisomes in living cells. The vector encodes far-red fluorescent protein mKate2 targeted to the matrix of peroxisomes by tripeptide SKL (peroxisomal targeting signal, PTS) fused to the mKate2 C-terminus.

mKate2 codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the mKate2 sequence [Kozak 1987].

pmKate2-peroxi can be used as a source of mKate2-PTS hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of peroxisome-targeted mKate2 in many cell types resulting in far-red fluorescent labeling of peroxisomes. 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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