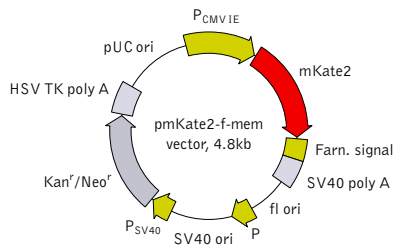


pmKate2-f-mem vector

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_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 Kozak consensus translation initiation site: 606-616
 mKate2
 Start codon (ATG): 613-615
 Last amino acid in mKate2: 1312-1314
 Farnesylation signal: 1330-1389
 Stop codon: 1390-1392
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1584-1589 & 1613-1618
 mRNA 3' ends: 1622 & 1634
 f1 single-strand DNA origin: 1681-2136
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2198-2203; -10 region: 2221-2226
 Transcription start point: 2233
 SV40 origin of replication: 2477-2612
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2310-2381 & 2382-2453
 21-bp repeats: 2457-2477, 2478-2498 & 2500-2520
 Early promoter element: 2533-2539
 Major transcription start points: 2529, 2567, 2573 & 2578
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2661-2663; Stop codon: 3453-3455
 G->A mutation to remove Pst I site: 2843
 C->A (Arg to Ser) mutation to remove BssH II site: 3189
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3691-3696 & 3704-3709
 pUC plasmid replication origin: 4040-4683

References

Aronheim, A. et al. (1994) "Membrane targeting of the nucleotide exchange factor Sos is sufficient for activating the Ras signaling pathway." *Cell*, 78 (6): 949-961 / pmid: 7923364

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

Hancock, JF et al. (1991) "Methylation and proteolysis are essential for efficient membrane binding of prenylated p21K-ras(B)." *EMBO J*, 10 (3): 641-646 / pmid: 2001678

Kozak, M. (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-f-mem vector	FP186	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 plasma membrane	

Vector description

pmKate2-f-mem is a mammalian expression vector intended for far-red fluorescent labeling of plasma membrane in living cells. The vector encodes far-red fluorescent protein mKate2 targeted to plasma membrane by 20 amino acid farnesylation signal from c-Ha-Ras [Aronheim et al. 1994; Hancock et al. 1991]. The farnesylation signal is fused to the mKate2 C-terminus.

mKate2 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 mKate2-f-mem coding sequence [Kozak 1987].

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

pmKate2-f-mem vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-f-mem 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.

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