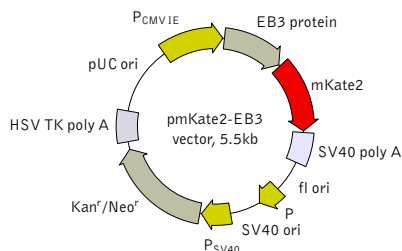


pmKate2-EB3 vector

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
 EB3: 652-1494
 mKate2: 1516-2015
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 2367-2372 & 2396-2401
 mRNA 3' ends: 2405 & 2417
 f1 single-strand DNA origin: 2464-2919
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2981-2986; -10 region: 3004-3009
 Transcription start point: 3016
 SV40 origin of replication: 3260-3395
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 3093-3164 & 3165-3236
 21-bp repeats: 3240-3260, 3261-3281 & 3283-3303
 Early promoter element: 3316-3322
 Major transcription start points: 3312, 3350, 3356 & 3361
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3444-3446; Stop codon: 4236-4238
 G->A mutation to remove Pst I site: 3626
 C->A (Arg to Ser) mutation to remove BssH II site: 3972
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4474-4479 & 4487-4492
 pUC plasmid replication origin: 4823-5466

Product	Cat.#	Size
pmKate2-EB3 vector	FP316	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 EB3 protein	

Vector description

pmKate2-EB3 is a mammalian expression vector encoding mKate2-EB3 fusion protein. The vector can be used for fluorescent labeling of EB3 protein in living cells.

mKate2 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Human microtubule-associated EB3 protein is fused to the mKate2 N-terminus.

pmKate2-EB3 vector can be used as a source of mKate2-EB3 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

pmKate2-EB3 vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-EB3 fusion 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.

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

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MSDS information is available at <http://www.evrogen.com/MSDS.shtml>