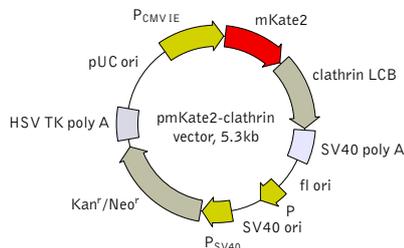


## pmKate2-clathrin vector

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<sub>CMV IE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 Kozak consensus translation initiation site: 606-616  
 mKate2-clathrin fusion: 613-1992  
 mKate2: 613-1314  
 Start codon (ATG): 613-615  
 Last amino acid in mKate2: 1312-1314  
 Clathrin LCB: 1360-1992  
 Stop codon: 1993-1995  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 2156-2161 & 2185-2190  
 mRNA 3' ends: 2194 & 2206  
 f1 single-strand DNA origin: 2253-2708  
 Bacterial promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2770-2775; -10 region: 2793-2798  
 Transcription start point: 2805  
 SV40 origin of replication: 3049-3184  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2882-2953 & 2954-3025  
 21-bp repeats: 3029-3049, 3050-3070 & 3072-3092  
 Early promoter element: 3105-3111  
 Major transcription start points: 3101, 3139, 3145 & 3150  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 3233-3235; Stop codon: 4025-4027  
 G->A mutation to remove Pst I site: 3415  
 C->A (Arg to Ser) mutation to remove BssH II site: 3761  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 4263-4268 & 4276-4281  
 pUC plasmid replication origin: 4612-5255

Product	Cat.#	Size
pmKate2-clathrin vector	<b>FP322</b>	20 µg
Vector type	mammalian expression vector	
Reporter	mKate2	
Reporter codon usage	mammalian	
Promoter for mKate2	P <sub>CMV IE</sub>	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	far-red fluorescent labeling of clathrin LCB	

### Vector description

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

mKate2 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Human clathrin LCB is fused to the mKate2 C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the mKate2-clathrin coding sequence [Kozak 1987].

pmKate2-clathrin vector can be used as a source of mKate2-clathrin 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<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Expression in mammalian cells

pmKate2-clathrin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-clathrin 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
- Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

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