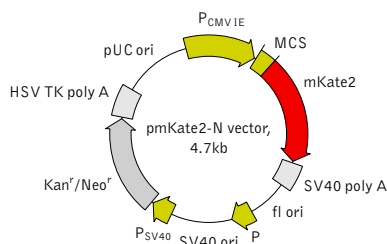


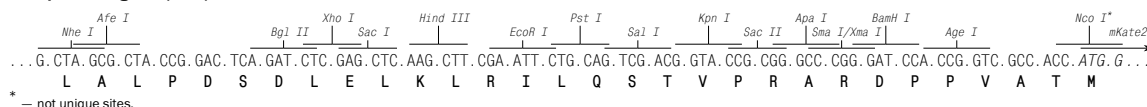
## pmKate2-N vector

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>

### Multiple cloning site (MCS)



### Location of features

P<sub>CMV IE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 MCS: 591-671  
 mKate2  
 Kozak consensus translation initiation site: 672-682  
 Start codon (ATG): 679-681; Stop codon: 1375-1377  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1530-1535 & 1559-1564  
 mRNA 3' ends: 1568 & 1580  
 f1 single-strand DNA origin: 1627-2082  
 Bacterial promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2144-2149; -10 region: 2167-2172  
 Transcription start point: 2179  
 SV40 origin of replication: 2423-2558  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2256-2327 & 2328-2399  
 21-bp repeats: 2403-2423, 2424-2444 & 2446-2466  
 Early promoter element: 2479-2485  
 Major transcription start points: 2475, 2513, 2519 & 2524  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2607-2609; Stop codon: 3399-3401  
 G->A mutation to remove Pst I site: 2789  
 C->A (Arg to Ser) mutation to remove BssH II site: 3135  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3637-3642 & 3650-3655  
 pUC plasmid replication origin: 3986-4629

### 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

Product	Cat.#	Size
pmKate2-N vector	<b>FP182</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	mKate2 expression in mammalian cells; generation of fusions to the mKate2 N-terminus	

### Vector description

pmKate2-N is a mammalian expression vector encoding far-red fluorescent protein mKate2. The vector allows generation of fusions to the mKate2 N-terminus and expression of mKate2 fusions or mKate2 alone in eukaryotic (mammalian) cells.

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 coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between P<sub>CMV IE</sub> and mKate2 coding sequence.

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.

### Generation of mKate2 fusion proteins

A localization signal or a gene of interest can be cloned into MCS of the vector. It will be expressed as a fusion to the mKate2 N-terminus when inserted in the same reading frame as mKate2 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. mKate2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express mKate2 when transfected into eukaryotic (mammalian) cells.

**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.

### Expression in mammalian cells

pmKate2-N vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mKate2 or its fusions 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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mKate2-related materials (also referred to as "Products") are intended for research use only. The Products are covered by U.S. Pat. 7,638,615; European Pat. 1994149; and other Evrogen Patents and/or Patent applications pending. By use of these Products, you accept the terms and conditions of the applicable Limited Use Label License #001: <http://www.evrogen.com/products/Evrogen-FP-license.shtml>.

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