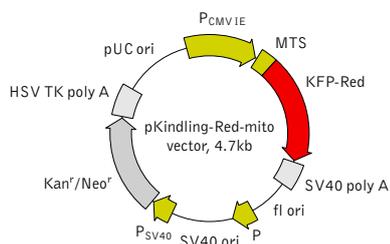


## pKindling-Red-mito vector

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  
 KFP-Red-mito fusion  
 Start codon (ATG): 597-599  
 Mitochondrial targeting sequence (MTS): 597-683  
 Start of KFP-Red coding sequence (ATG): 705-707  
 Stop codon: 1401-1403  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1556-1561 & 1585-1590  
 mRNA 3' ends: 1594 & 1606  
 f1 single-strand DNA origin: 1653-2108  
 Bacterial promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2170-2175; -10 region: 2193-2198  
 Transcription start point: 2205  
 SV40 origin of replication: 2449-2584  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2282-2353 & 2354-2425  
 21-bp repeats: 2429-2449, 2450-2470 & 2472-2492  
 Early promoter element: 2505-2511  
 Major transcription start points: 2501, 2539, 2545 & 2550  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2633-2635; Stop codon: 3425-3427  
 G->A mutation to remove Pst I site: 2815  
 C->A (Arg to Ser) mutation to remove BssH II site: 3161  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3663-3668 & 3676-3681  
 pUC plasmid replication origin: 4012-4655

Product	Cat.#	Size
pKindling-Red-mito vector	<b>FP401</b>	20 µg
Vector type	mammalian expression vector	
Reporter	KFP-Red	
Reporter codon usage	mammalian	
Promoter for KFP-Red	P <sub>CMV IE</sub>	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	monitoring the movements of individual mitochondria	

### Vector description

pKindling-Red-mito is a mammalian expression vector intended for monitoring the movements of individual mitochondria in living cells. The vector encodes kindling red fluorescent protein KFP-Red fused to mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989; Rizzuto et al. 1995]. MTS is fused to the KFP-Red N-terminus.

KFP-Red codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

pKindling-Red-mito vector can be used as a source of KFP-Red-MTS 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

pKindling-Red-mito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the KFP-Red-MTS 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
- Rizzuto, R. et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." *J Biol Chem*, 264 (18): 10595-10600 / pmid: 2543673
- Rizzuto, R. et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." *Curr Biol*, 5 (6): 635-642 / pmid: 7552174

### Notice to Purchaser:

KFP-Red-related materials (also referred to as "Products") are intended to be used by academic (non-commercial) entities and for research purposes only. Any use of the proprietary nucleic acid or protein other than for research use or by a commercial entity is strictly prohibited. Transfer of this product by purchaser to any other party is specifically prohibited.

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

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