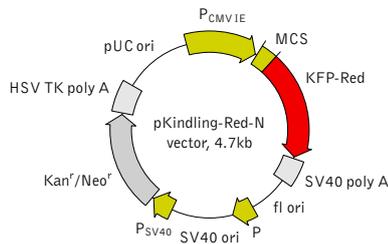


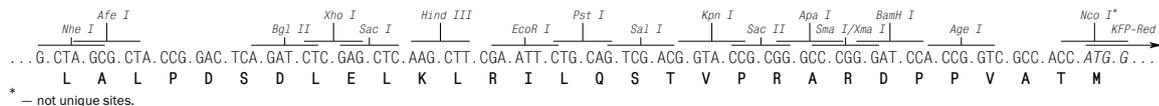
pKindling-Red-N vector

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_{CMVIE} : 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 MCS: 591-671
 KFP-Red
 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^r 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
pKindling-Red-N vector	FP301	20 μ g
Vector type	mammalian expression vector	
Reporter	KFP-Red	
Reporter codon usage	mammalian	
Promoter for KFP-Red	P_{CMVIE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	KFP-Red expression in mammalian cells; generation of fusions to the KFP-Red N-terminus	

Vector description

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

KFP-Red 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 KFP-Red coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between P_{CMVIE} and KFP-Red coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P_{CMVIE}) 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.

Generation of KFP-Red 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 KFP-Red N-terminus when inserted in the same reading frame as KFP-Red and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. KFP-Red-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express KFP-Red when transfected into eukaryotic (mammalian) cells.

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.

Expression in mammalian cells

pKindling-Red-N vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of KFP-Red 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 μ g/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

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>