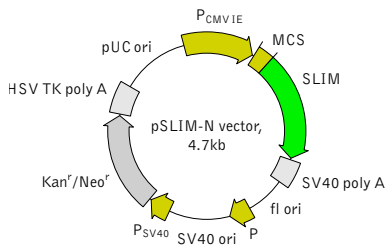


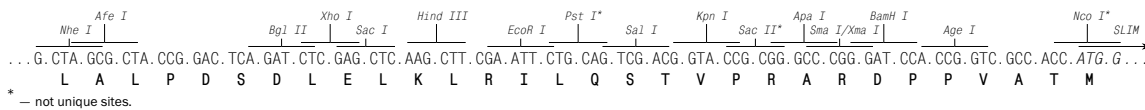
## pSLIM-N vector

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: 592-678  
 SLIM  
 Kozak consensus translation initiation site: 672-682  
 Start codon (ATG): 679-681; Stop codon: 1396-1398  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1551-1556 & 1580-1585  
 mRNA 3' ends: 1589 & 1601  
 f1 single-strand DNA origin: 1648-2103  
 Eukaryotic promoter for expression of  $Kan^r$  gene  
 -35 region: 2165-2170; -10 region: 2188-2193  
 Transcription start point: 2200  
 SV40 origin of replication: 2444-2579  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2277-2348 & 2349-2420  
 21-bp repeats: 2424-2444, 2445-2465 & 2467-2487  
 Early promoter element: 2500-2506  
 Major transcription start points: 2496, 2534, 2540 & 2545  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2628-2630; stop codon: 3420-3422  
 G->A mutation to remove Pst I site: 2810  
 C->A (Arg to Ser) mutation to remove BssH II site: 3156  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3658-3663 & 3671-3676  
 pUC plasmid replication origin: 4007-4650

### 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
pSLIM-N vector	FP212	20 $\mu$ g
Vector type	mammalian expression vector	
Reporter	SLIM	
Reporter codon usage	mammalian	
Promoter for SLIM	$P_{CMVIE}$	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	SLIM expression in mammalian cells; generation of fusions to the SLIM N-terminus	

### Vector description

pSLIM-N is a mammalian expression vector encoding green fluorescent protein SLIM. The vector allows generation of fusions to the SLIM N-terminus and expression of SLIM fusions or SLIM alone in eukaryotic (mammalian) cells.

SLIM 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 SLIM coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between  $P_{CMVIE}$  and SLIM 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 SLIM 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 SLIM N-terminus when inserted in the same reading frame as SLIM and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. SLIM-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express SLIM 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

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

### Notice to Purchaser:

SLIM-related materials (also referred to as "Products") are intended for research use only.

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