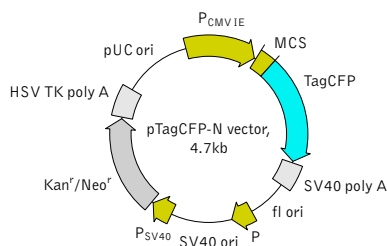


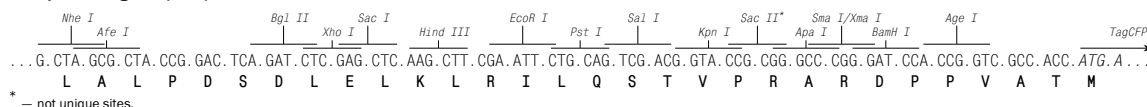
pTagCFP-N vector

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

$P_{CMV IE}$: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 MCS: 589-678
 TagCFP
 Kozak consensus translation initiation site: 672-682
 Start codon (ATG): 679-681; Stop codon: 1393-1395
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1549-1554 & 1578-1583
 mRNA 3' ends: 1587 & 1599
 f1 single-strand DNA origin: 1646-2101
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 2163-2168; -10 region: 2186-2191
 Transcription start point: 2198
 SV40 origin of replication: 2442-2577
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2275-2346 & 2347-2418
 21-bp repeats: 2422-2442, 2443-2463 & 2465-2485
 Early promoter element: 2498-2504
 Major transcription start points: 2494, 2532, 2538 & 2543
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2626-2628; Stop codon: 3418-3420
 G->A mutation to remove Pst I site: 2808
 C->A (Arg to Ser) mutation to remove BssH II site: 3154
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3656-3661 & 3669-3674
 pUC plasmid replication origin: 4005-4648

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
pTagCFP-N vector	FP112	20 μ g
Vector type	mammalian expression vector	
Reporter	TagCFP	
Reporter codon usage	mammalian	
Promoter for TagCFP	$P_{CMV IE}$	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	TagCFP expression in mammalian cells; generation of fusions to the TagCFP N-terminus	

Vector description

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

TagCFP 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 TagCFP coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between $P_{CMV IE}$ and TagCFP coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV IE}$) 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 TagCFP 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 TagCFP N-terminus when inserted in the same reading frame as TagCFP and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TagCFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagCFP 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

pTagCFP-N vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TagCFP 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.

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