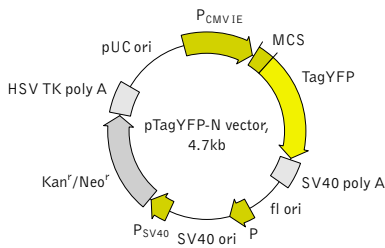


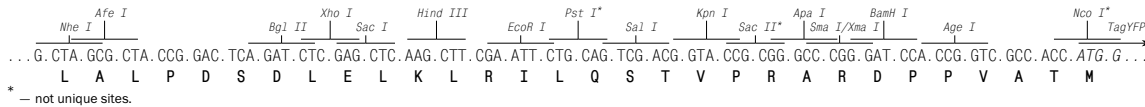
pTagYFP-N vector

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: 592-678
TagYFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; **Stop codon:** 1396-1398
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1552-1557 & 1581-1586
mRNA 3' ends: 1590 & 1602
f1 single-strand DNA origin: 1649-2104
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2166-2171; **-10 region:** 2189-2194
Transcription start point: 2201
SV40 origin of replication: 2445-2580
SV40 early promoter
Enhancer (72-bp tandem repeats): 2278-2349 & 2350-2421
21-bp repeats: 2425-2445, 2446-2466 & 2468-2488
Early promoter element: 2501-2507
Major transcription start points: 2497, 2535, 2541 & 2546
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2629-2631; **Stop codon:** 3421-3423
G->A mutation to remove Pst I site: 2811
C->A (Arg to Ser) mutation to remove BssH II site: 3157
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3659-3664 & 3672-3677
pUC plasmid replication origin: 4008-4651

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
pTagYFP-N vector	FP132	20 µg
Vector type	mammalian expression vector	
Reporter	TagYFP	
Reporter codon usage	mammalian	
Promoter for TagYFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	TagYFP expression in mammalian cells; generation of fusions to the TagYFP N-terminus	

Vector description

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

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

pTagYFP-N vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TagYFP 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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MSDS information is available at <http://www.evrogen.com/MSDS.shtml>