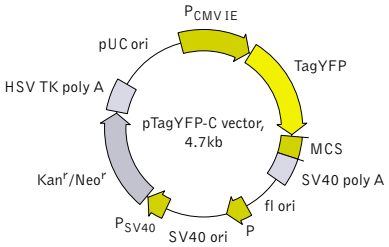
pTagYFP-C vector

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/support/vector-info.shtml

Product	Cat.#	Size
pTagYFP-C vector	**FP131**	20 μg

The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.

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 C-terminus

pTagYFP-C vector MCS

TagYFP → BspE I | Xho I | Hind III | Pst I* | Kpn I | Apa I | BamH I | STOPs

. . . TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A . . .

Bgl II | Sac I | EcoR I | Sal I | Sac II* | Sma I/Xma I | Xba I# | Bcl I#

* — not unique sites.
\# — sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
TagYFP
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; Stop codon: 1408-1410
Last amino acid in TagYFP: 1327-1329
MCS: 1330-1407
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1550-1555 & 1579-1584
mRNA 3' ends: 1588 & 1600
f1 single-strand DNA origin: 1647-2102
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2164-2169; -10 region: 2187-2192
Transcription start point: 2199
SV40 origin of replication: 2443-2578
SV40 early promoter
Enhancer (72-bp tandem repeats): 2276-2347 & 2348-2419
21-bp repeats: 2423-2443, 2444-2464 & 2466-2486
Early promoter element: 2499-2505
Major transcription start points: 2495, 2533, 2539 & 2544
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2627-2629; Stop codon: 3419-3421
G->A mutation to remove Pst I site: 2809
C->A (Arg to Ser) mutation to remove BssH II site: 3155
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3657-3662 & 3670-3675
pUC plasmid replication origin: 4006-4649

References

Gorman (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. 143–90.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–24 / pmid: 8805248

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–48 / pmid: 3313277

Vector description

pTagYFP-C is a mammalian expression vector encoding yellow fluorescent protein TagYFP. The vector allows generation of fusions to the TagYFP C-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 sequence [Kozak 1987]. Multiple cloning site (MCS) is located between TagYFP coding sequence and SV40 polyadenylation signal (SV40 polyA).

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) should be cloned into MCS of the vector. It will be expressed as a fusion to the TagYFP C-terminus when inserted in the same reading frame as TagYFP and no intervening stop codons are present. 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-C vector can be transfected into mammalian cells by any known transfection method. 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.