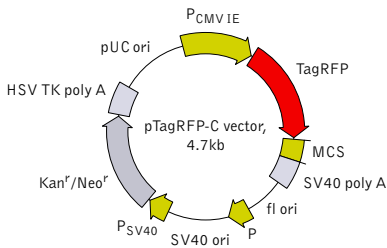


## pTagRFP-C vector

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>

### pTagRFP-C vector MCS

TagRFP → BspE I → Xho I → Hind III → Pst I → Kpn I → Apa I → BamH I → STOPS → Xba I# → Bcl I#

... 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 . . .

# — 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*<sup>-</sup> host and make fresh DNA.

### Location of features

P<sub>CMV IE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 TagRFP  
 Kozak consensus translation initiation site: 606-616  
 Start codon (ATG): 613-615; Stop codon: 1402-1404  
 Last amino acid in TagRFP: 1321-1323  
 MCS: 1324-1401  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1544-1549 & 1573-1578  
 mRNA 3' ends: 1582 & 1594  
 f1 single-strand DNA origin: 1641-2096  
 Eukaryotic promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2158-2163; -10 region: 2181-2186  
 Transcription start point: 2193  
 SV40 origin of replication: 2437-2572  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2270-2341 & 2342-2413  
 21-bp repeats: 2417-2437, 2438-2458 & 2460-2480  
 Early promoter element: 2493-2499  
 Major transcription start points: 2489, 2527, 2533 & 2538  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2621-2623; Stop codon: 3413-3415  
 C->A mutation to remove Pst I site: 2803  
 C->A (Arg to Ser) mutation to remove BssH II site: 3149  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3651-3656 & 3664-3669  
 pUC plasmid replication origin: 4000-4643

### 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

Product	Cat.#	Size
pTagRFP-C vector	FP141	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	TagRFP
Reporter codon usage	mammalian
Promoter for TagRFP	P <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	TagRFP expression in mammalian cells; generation of fusions to the TagRFP C-terminus

### Vector description

pTagRFP-C is a mammalian expression vector encoding red (orange) fluorescent protein TagRFP. The vector allows generation of fusions to the TagRFP C-terminus and expression of TagRFP fusions or TagRFP alone in eukaryotic (mammalian) cells.

TagRFP 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 TagRFP sequence [Kozak 1987]. Multiple cloning site (MCS) is located between TagRFP coding sequence and SV40 polyadenylation signal (SV40 polyA).

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Generation of TagRFP-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 TagRFP C-terminus when inserted in the same reading frame as TagRFP and no intervening stop codons are present. TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagRFP, when transfected into eukaryotic (mammalian) cells.

**Note:** The plasmid DNA was isolated from *dam*<sup>+</sup>-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*<sup>-</sup> host and make fresh DNA.

### Expression in mammalian cells

pTagRFP-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.

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