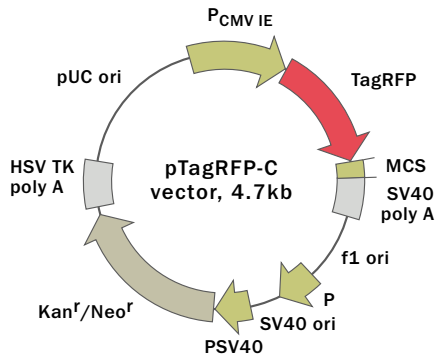
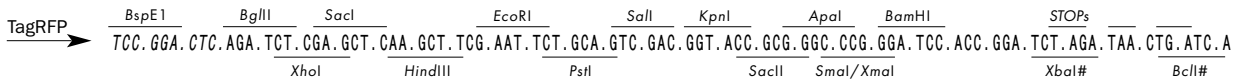


## Mammalian expression vector pTagRFP-C



For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

### Multiple cloning site (MCS)



# - sites are blocked by methylation. If you wish to digest the vector using these sites, you will need to transform the vector into a dam<sup>-</sup> host and make fresh DNA.

### Use

- Generation of fusions to the TagRFP C-terminus
- Expression of TagRFP or its fusions in mammalian cells

Product	Cat.#	Size
pTagRFP-C	FP141	20 µg

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

### Vector description

pTagRFP-C is an eukaryotic (mammalian) expression vector encoding monomeric red fluorescent protein TagRFP. The vector allows to generate fusions to the TagRFP C-terminus and to express TagRFP fusions or TagRFP alone in mammalian cells.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TagRFP translation, 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 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression 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 fusions

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 in-frame stop codons are present. TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTagRFP-C 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 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.

## 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

### Bacterial 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

G->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 C. (1985) In DNA cloning: A Practical Approach, Vol. II, Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. et al. (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.

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To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.