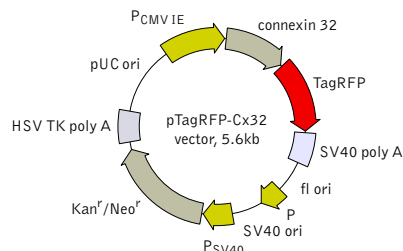


## pTagRFP-Cx32 vector

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>

### Location of features

PCMV IE: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 Connexin 32: 697-1545  
 TagRFP: 1567-2280  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 2433-2438 & 2462-2467  
 mRNA 3' ends: 2471 & 2483  
 f1 single-strand DNA origin: 2530-2985  
 Bacterial promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 3047-3052; -10 region: 3070-3075  
 Transcription start point: 3082  
 SV40 origin of replication: 3326-3461  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 3159-3230 & 3231-3302  
 21-bp repeats: 3306-3326, 3327-3347 & 3349-3369  
 Early promoter element: 3382-3388  
 Major transcription start points: 3378, 3416, 3422 & 3427  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 3510-3512; Stop codon: 4302-4304  
 G->A mutation to remove Pst I site: 3692  
 C->A (Arg to Ser) mutation to remove BssH II site: 4038  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 4540-4545 & 4553-4558  
 pUC plasmid replication origin: 4889-5532

Product	Cat.#	Size
pTagRFP-Cx32 vector	<b>FP363</b>	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	red (orange) fluorescent labeling of connexin 32

### Vector description

pTagRFP-Cx32 is a mammalian expression vector encoding TagRFP-Cx32 fusion protein. The vector can be used for fluorescent labeling of connexin 32 in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al. 1996). Human connexin 32 is fused to the TagRFP N-terminus.

pTagRFP-Cx32 can be used as a source of TagRFP-Cx32 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

**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.

The vector backbone also 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.

### Expression in mammalian cells

pTagRFP-Cx32 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-Cx32 fusion 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.

### 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. 143-190.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248

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