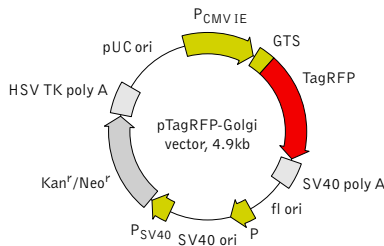


## pTagRFP-Golgi vector

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

**P<sub>CMV IE</sub>**: 1-589  
**Enhancer region**: 59-465  
**TATA box**: 554-560  
**Transcription start point**: 583  
**Golgi targeting sequence (GTS), fragment of human beta 1,4- galactosyltransferase**: 597-842  
**TagRFP**: 864-1577  
**SV40 early mRNA polyadenylation signal**  
**Polyadenylation signals**: 1730-1735 & 1759-1764  
**mRNA 3' ends**: 1768 & 1780  
**f1 single-strand DNA origin**: 1827-2282  
**Bacterial promoter for expression of Kan<sup>r</sup> gene**  
**-35 region**: 2344-2349; **-10 region**: 2367-2372  
**Transcription start point**: 2379  
**SV40 origin of replication**: 2623-2758  
**SV40 early promoter**  
**Enhancer (72-bp tandem repeats)**: 2456-2527 & 2528-2599  
**21-bp repeats**: 2603-2623, 2624-2644 & 2646-2666  
**Early promoter element**: 2679-2685  
**Major transcription start points**: 2675, 2713, 2719 & 2724  
**Kanamycin/neomycin resistance gene**  
**Neomycin phosphotransferase coding sequences**:  
**Start codon (ATG)**: 2807-2809; **Stop codon**: 3599-3601  
**G->A mutation to remove Pst I site**: 2989  
**C->A (Arg to Ser) mutation to remove BssH II site**: 3335  
**Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal**  
**Polyadenylation signals**: 3837-3842 & 3850-3855  
**pUC plasmid replication origin**: 4186-4829

### 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

Product	Cat.#	Size
pTagRFP-Golgi vector	FP367	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 Golgi apparatus	

### Vector description

pTagRFP-Golgi is a mammalian expression vector intended for red (orange) fluorescent labeling of Golgi apparatus in living cells. The vector encodes red (orange) fluorescent protein TagRFP fused to Golgi targeting sequence (GTS). GTS is fused to the TagRFP N-terminus.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al. 1996).

pTagRFP-Golgi can be used as a source of TagRFP-GTS 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 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-Golgi can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-GTS 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.

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