

pTagRFP-Golgi vector

Cat# FP367

Vector description

pTagRFP-Golgi is a mammalian expression vector encoding TagRFP protein fused to Golgi targeting sequence (GTS). The vector can be used for fluorescent labeling of Golgi apparatus in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas *et al.*, 1996). Golgi targeting sequence (fragment of human β -1,4-galactosyltransferase) is fused to the TagRFP N-terminus.

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.

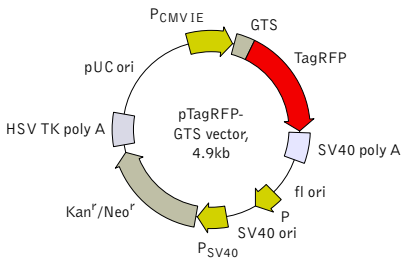
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus ($P_{\text{CMV IE}}$) 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.

Vector map

For vector sequence, please visit our Web site at <http://www.evrogen.com/support/vector-info.shtml>



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].

Location of features

PCMV IE: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

Golgi targeting sequence (GTS), fragment of human beta 1,4- galactosyl-transferase: 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^r 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

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 C. High efficiency gene transfer into mammalian cells. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.). 1985; 143-90.

Haas J, Park EC, Seed B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr Biol.* 1996; 6 (3):315-24. / pmid: 8805248

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

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