

pTagRFP-vinculin vector

Cat# FP372

Vector description

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

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas *et al.*, 1996]. Human vinculin is fused to the TagRFP C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagRFP-vinculin coding sequence [Kozak, 1987].

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

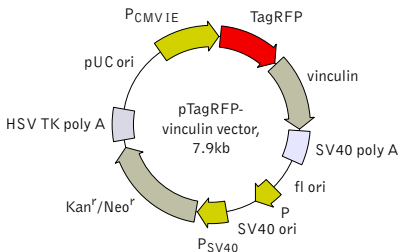
Note: The plasmid DNA was isolated from *dam*⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus (P_{CMVIE}) 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-vinculin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-vinculin 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
Kozak consensus translation initiation site: 606-616
TagRFP
Start codon (ATG): 613-615
Last amino acid in TagRFP: 1321-1323
Vinculin: 1393-4593
Stop codon: 4591-4593
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 4746-4751 4775-4780
mRNA 3' ends: 4784 4796
f1 single-strand DNA origin: 4843-5298
Bacterial promoter for expression of Kan^r gene
-35 region: 5360-5365
-10 region: 5383-5388
Transcription start point: 5395
SV40 origin of replication: 5639-5774
SV40 early promoter
Enhancer (72-bp tandem repeats): 5472-5543 5544-5615
21-bp repeats: 5619-5639, 5640-5660 5662-5682
Early promoter element: 5695-5701
Major transcription start points: 5691, 5729, 5735 5740
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 5823-5825
Stop codon: 6615-6617
G->A mutation to remove Pst I site: 6005
C->A (Arg to Ser) mutation to remove BssH II site: 6351
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 6853-6858 6866-6871
pUC plasmid replication origin: 7202-7845

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

Notice to Purchaser:

Evrogen FP-related products are intended for research use only and covered by Evrogen Patents and/or Patent applications pending. By use of these products, you accept the terms and conditions of the applicable Limited Use Label License (enclosed).

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION: 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.