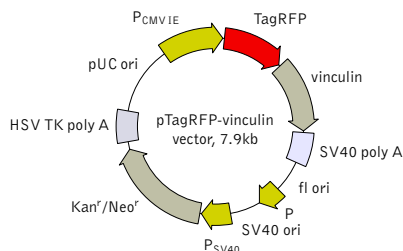


pTagRFP-vinculin vector

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/products/vectors.shtml>

Location of features

P_{CMV 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

Product	Cat.#	Size
pTagRFP-vinculin vector	FP372	20 µg
Vector type	mammalian expression vector	
Reporter	TagRFP	
Reporter codon usage	mammalian	
Promoter for TagRFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	red (orange) fluorescent labeling of vinculin	

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 vector 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_{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.

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].

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. (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, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
- Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

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