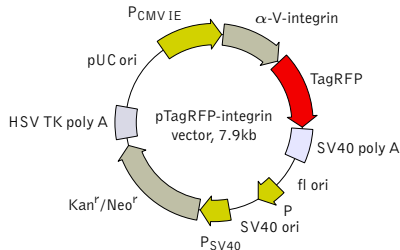


pTagRFP-integrin vector

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
 Alpha-V-Integrin: 613-3756
 TagRFP: 3832-4545
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 4698-4703 & 4727-4732
 mRNA 3' ends: 4736 & 4748
 f1 single-strand DNA origin: 4795-5250
 Bacterial promoter for expression of Kan^r gene
 -35 region: 5312-5317; -10 region: 5335-5340
 Transcription start point: 5347
 SV40 origin of replication: 5591-5726
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 5424-5495 & 5496-5567
 21-bp repeats: 5571-5591, 5592-5612 & 5614-5634
 Early promoter element: 5647-5653
 Major transcription start points: 5643, 5681, 5687 & 5692
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 5775-5777; Stop codon: 6567-6569
 G->A mutation to remove Pst I site: 5957
 C->A (Arg to Ser) mutation to remove BssH II site: 6303
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 6805-6810 & 6818-6823
 pUC plasmid replication origin: 7154-7797

Product	Cat.#	Size
pTagRFP-integrin vector	FP361	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 α-V-integrin	

Vector description

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

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

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

Notice to Purchaser:

Evrogen Fluorescent Protein Products (the Products) are intended for research use only. The Products are covered by U.S. Pat. 7,417,131 and other 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.

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.