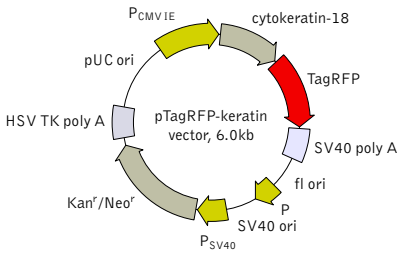
pTagRFP-keratin vector

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

Product	Cat.#	Size
pTagRFP-keratin vector	**FP369**	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 cytokeratin-18

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Keratin: 600-1889
TagRFP: 1941-2654
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2807-2812 & 2836-2841
mRNA 3' ends: 2845 & 2857
f1 single-strand DNA origin: 2904-3359
Bacterial promoter for expression of Kan^r gene
-35 region: 3421-3426; -10 region: 3444-3449
Transcription start point: 3456
SV40 origin of replication: 3700-3835
SV40 early promoter
Enhancer (72-bp tandem repeats): 3533-3604 & 3605-3676
21-bp repeats: 3680-3700, 3701-3721 & 3723-3743
Early promoter element: 3756-3762
Major transcription start points: 3752, 3790, 3796 & 3801
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3884-3886; Stop codon: 4676-4678
G->A mutation to remove Pst I site: 4066
C->A (Arg to Ser) mutation to remove BssH II site: 4412
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4914-4919 & 4927-4932
pUC plasmid replication origin: 5263-5906

Vector description

pTagRFP-keratin is a mammalian expression vector encoding TagRFP-keratin fusion protein. The vector can be used for fluorescent labeling of cytokeratin-18 in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Human cytokeratin-18 is fused to the TagRFP N-terminus.

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