

pTagRFP-keratin vector

Cat# FP369

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, i.e. humanized (Haas *et al.*, 1996). Human cytokeratin-18 is fused to the TagRFP N-terminus.

pTagRFP-keratin 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 an expression vector of choice.

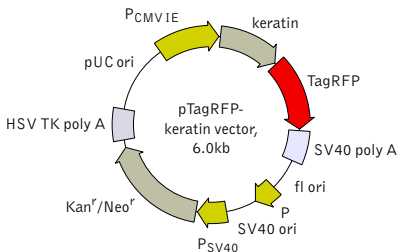
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

The vector backbone also contains an 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-keratin 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].

Location of features

PCMV 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

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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