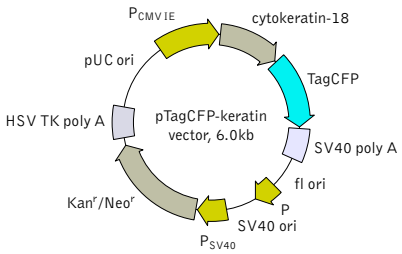
pTagCFP-keratin vector

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
pTagCFP-keratin vector	**FP119**	20 μg

Vector type	mammalian expression vector
Reporter	TagCFP
Reporter codon usage	mammalian
Promoter for TagCFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	cyan 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-TagCFP fusion: 600-2657
Keratin: 600-1889
Start codon (ATG): 600-602
Last amino acid in Keratin: 1887-1889
TagCFP: 1941-2657
Stop codon: 2655-2657
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2811-2816 & 2840-2845
mRNA 3' ends: 2849 & 2861
f1 single-strand DNA origin: 2908-3363
Bacterial promoter for expression of Kan^r gene
-35 region: 3425-3430; -10 region: 3448-3453
Transcription start point: 3460
SV40 origin of replication: 3704-3839
SV40 early promoter
Enhancer (72-bp tandem repeats): 3537-3608 & 3609-3680
21-bp repeats: 3684-3704, 3705-3725 & 3727-3747
Early promoter element: 3760-3766
Major transcription start points: 3756, 3794, 3800 & 3805
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3888-3890; Stop codon: 4680-4682
G->A mutation to remove Pst I site: 4070
C->A (Arg to Ser) mutation to remove BssH II site: 4416
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4918-4923 & 4931-4936
pUC plasmid replication origin: 5267-5910

Vector description

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

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

pTagCFP-keratin vector can be used as a source of TagCFP-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

pTagCFP-keratin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagCFP-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