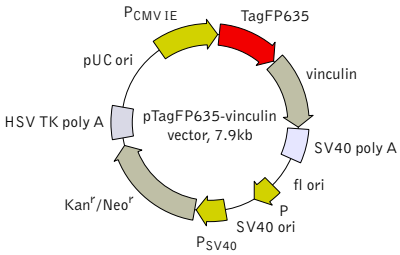
# pTagFP635-vinculin vector

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/support/vector-info.shtml

| Product | Cat.# | Size |
|---|---|---|
| pTagFP635-vinculin vector | **FP388** | 20 μg |

The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.

| | |
|---|---|
| Vector type | mammalian expression vector |
| Reporter | TagFP635 |
| Reporter codon usage | mammalian |
| Promoter for TagFP635 | $P_{CMV\ IE}$ |
| Host cells | mammalian |
| Selection | prokaryotic - kanamycin<br>eukaryotic - neomycin (G418) |
| Replication | prokaryotic - pUC ori<br>eukaryotic - SV40 ori |
| Use | far-red fluorescent labeling of vinculin |

## 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
TagFP635
Start codon (ATG): 613-615
Last amino acid in TagFP635: 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

## Vector description

pTagFP635-vinculin is a mammalian expression vector encoding TagFP635-vinculin fusion protein. The vector can be used for fluorescent labeling of vinculin in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Human vinculin is fused to the TagFP635 C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagFP635-vinculin coding sequence [Kozak 1987].

pTagFP635-vinculin can be used as a source of TagFP635-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

pTagFP635-vinculin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-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 (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

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277