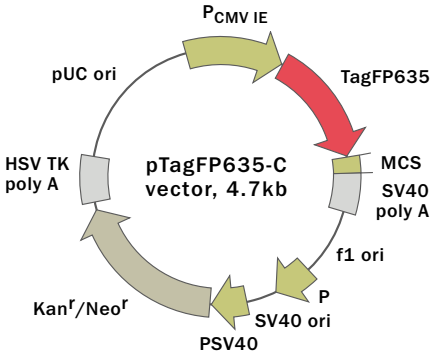
Mammalian expression vector pTagFP635-C



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pTagFP635-C	**FP161**	20 µg

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 eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

TagFP635 → BspE1 BglII SacI EcoRI SalI KpnI ApaI BamHI STOPs

TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A

XhoI HindIII PstI SacII SmaI/XmaI XbaI# BclI#

- sites are blocked by methylation.

Use

- Generation of fusions to the TagFP635 C-terminus

- Expression of TagFP635 or its fusions in mammalian cells

Vector description

pTagFP635-C is an eukaryotic (mammalian) expression vector encoding monomeric far-red fluorescent protein TagFP635. The vector allows to generate fusions to the TagFP635 C-terminus and to express TagFP635 fusions or TagFP635 alone in mammalian cells.

TagFP635 codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TagFP635 translation, Kozak consensus translation initiation site is generated upstream of the TagFP635 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between TagFP635 coding sequence and SV40 polyadenylation signal (SV40 polyA).

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 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to TagFP635 C-terminus when inserted in the same reading frame as TagFP635 and no in-frame stop codons are present. TagFP635-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTagFP635-C vector will express TagFP635, when transfected into eukaryotic (mammalian) cells.

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.

Expression in mammalian cells

pTagFP635-C can be transfected into mammalian cells by any known transfection method. 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.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
TagFP635
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615
Stop codon: 1401-1403
Last amino acid in TagFP635: 1321-1323
MCS: 1324-1403
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1543-1548 & 1572-1577
mRNA 3' ends: 1581 & 1593
f1 single-strand DNA origin: 1640-2095
Bacterial promoter for expression of Kan^r gene
-35 region: 2157-2162
-10 region: 2180-2185
Transcription start point: 2192
SV40 origin of replication: 2436-2571
SV40 early promoter
Enhancer (72-bp tandem repeats): 2269-2340 & 2341-2412
21-bp repeats: 2416-2436, 2437-2457 & 2459-2479
Early promoter element: 2492-2498
Major transcription start points: 2488, 2526, 2532 & 2537
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2620-2622
Stop codon: 3412-3414
G->A mutation to remove Pst I site: 2802
C->A (Arg to Ser) mutation to remove BssH II site: 3148
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3650-3655 & 3663-3668
pUC plasmid replication origin: 3999-4642

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al*. (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.