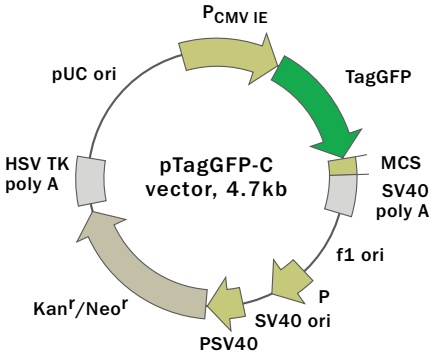
Mammalian expression vector pTagGFP-C



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

Product	Cat.#	Size
pTagGFP-C	**FP121**	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	TagGFP
Reporter codon usage	mammalian
Promoter for TagGFP	P CMV IE
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

TagGFP →

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									
BspEI	XhoI	HindIII	PstI		SacII*	SmaI/XmaI		XbaI#	BclI#

* - not unique sites. # - sites are blocked by methylation.

Use

- Generation of fusions to the TagGFP C-terminus

- Expression of TagGFP or its fusions in mammalian cells

Vector description

pTagGFP-C is an eukaryotic (mammalian) expression vector encoding green fluorescent protein TagGFP. The vector allows generation of fusions to the TagGFP C-terminus and expression TagGFP fusions or TagGFP alone in eukaryotic (mammalian) cells.

TagGFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TagGFP translation, Kozak consensus translation initiation site is generated upstream of the TagGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between TagGFP 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 TagGFP C-terminus when inserted in the same reading frame as TagGFP and no intervening stop codons are present. TagGFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagGFP, 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

pTagGFP-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
TagGFP
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; stop codon: 1405-1407
Last amino acid in TagGFP: 1324-1326
MCS: 1327-1404
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1547-1552 & 1576-1581
mRNA 3' ends: 1585 & 1597
f1 single-strand DNA origin: 1644-2099
Bacterial promoter for expression of Kan^r gene
-35 region: 2161-2166; -10 region: 2184-2189
Transcription start point: 2196
SV40 origin of replication: 2440-2575
SV40 early promoter
Enhancer (72-bp tandem repeats): 2273-2344 & 2345-2416
21-bp repeats: 2420-2440, 2441-2461 & 2463-2483
Early promoter element: 2496-2502
Major transcription start points: 2492, 2530, 2536 & 2541
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2624-2626; stop codon: 3416-3418
G->A mutation to remove Pst I site: 2806
C->A (Arg to Ser) mutation to remove BssH II site: 3152
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3654-3659 & 3667-3672
pUC plasmid replication origin: 4003-4646

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.