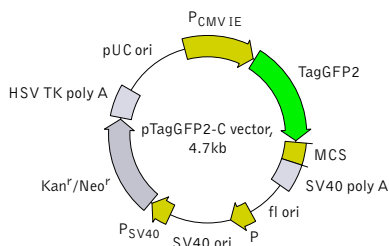


pTagGFP2-C vector

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>

pTagGFP2-C vector MCS

pTagGFP2 → BspE I ... 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 . . .

Xho I
Hind III
Pst I
Kpn I
Apa I
BamH I
STOPs

Bgl II
Sac I
EcoR I
Sal I
Sac II*
Sma I/Xma I
Xba I#
Bcl I#

* — not unique site.

— sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 TagGFP2
 Kozak consensus translation initiation site: 600-610
 Start codon (ATG): 607-609; Stop codon: 1399-1401
 Last amino acid in TagGFP2: 1318-1320
 MCS: 1321-1398
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1541-1546 & 1570-1575
 mRNA 3' ends: 1579 & 1591
 f1 single-strand DNA origin: 1638-2093
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2155-2160; -10 region: 2178-2183
 Transcription start point: 2190
 SV40 origin of replication: 2434-2569
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2267-2338 & 2339-2410
 21-bp repeats: 2414-2434, 2435-2455 & 2457-2477
 Early promoter element: 2490-2496
 Major transcription start points: 2486, 2524, 2530 & 2535
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2618-2620; Stop codon: 3410-3412
 G->A mutation to remove Pst I site: 2800
 C->A (Arg to Ser) mutation to remove BssH II site: 3146
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3648-3653 & 3661-3666
 pUC plasmid replication origin: 3997-4640

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. 143-90.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-24 / PMID: 8805248

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-48 / PMID: 3313277

| Product | Cat.# | Size |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|-------|
| pTagGFP2-C vector | FP191 | 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 | TagGFP2 | |
| Reporter codon usage | mammalian | |
| Promoter for TagGFP2 | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic - kanamycin eukaryotic - neomycin (G418) | |
| Replication | prokaryotic - pUC ori eukaryotic - SV40 ori | |
| Use | TagGFP2 expression in mammalian cells; generation of fusions to the TagGFP2 C-terminus | |

Vector description

pTagGFP2-C is a mammalian expression vector encoding green fluorescent protein TagGFP2. The vector allows generation of fusions to the TagGFP2 C-terminus and expression of TagGFP2 fusions or TagGFP2 alone in eukaryotic (mammalian) cells.

TagGFP2 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the TagGFP2 sequence [Kozak 1987]. Multiple cloning site (MCS) is located between TagGFP2 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 polyadenylation signals (SV40 polyA) 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.

Generation of TagGFP2-fusion proteins

A localization signal (or a gene of interest) should be cloned into MCS of the vector. It will be expressed as a fusion to the TagGFP2 C-terminus when inserted in the same reading frame as TagGFP2 and no intervening stop codons are present. TagGFP2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagGFP2, 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

pTagGFP2-C vector 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.

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