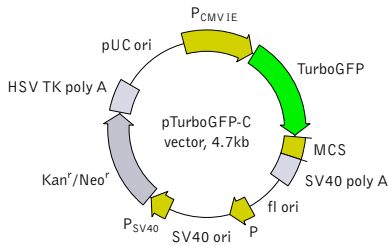


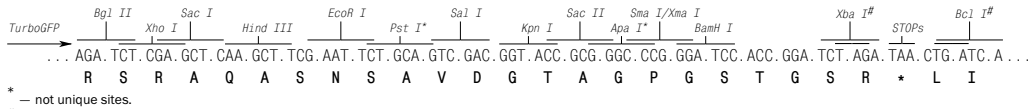
pTurboGFP-C vector

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/products/vectors.shtml>

Multiple cloning site (MCS)



* — not unique sites.

— 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
 TurboGFP
 Kozak consensus translation initiation site: 606-616
 Start codon (ATG): 613-615; Stop codon: 1378-1380
 Last amino acid in TurboGFP: 1306-1308
 MCS: 1309-1394
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1520-1525 & 1549-1554
 mRNA 3' ends: 1558 & 1570
 f1 single-strand DNA origin: 1617-2072
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 2134-2139; -10 region: 2157-2162
 Transcription start point: 2169
 SV40 origin of replication: 2413-2548
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2246-2317 & 2318-2389
 21-bp repeats: 2393-2413, 2414-2434 & 2436-2456
 Early promoter element: 2469-2475
 Major transcription start points: 2465, 2503, 2509 & 2514
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2597-2599; Stop codon: 3389-3391
 G->A mutation to remove Pst I site: 2779
 C->A (Arg to Ser) mutation to remove BssH II site: 3125
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3627-3632 & 3640-3645
 pUC plasmid replication origin: 3976-4619

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

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

| Product | Cat.# | Size |
|-----------------------|--|-------|
| pTurboGFP-C vector | FP511 | 20 µg |
| Vector type | mammalian expression vector | |
| Reporter | TurboGFP | |
| Reporter codon usage | mammalian | |
| Promoter for TurboGFP | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic - kanamycin eukaryotic - neomycin (G418) | |
| Replication | prokaryotic - pUC ori eukaryotic - SV40 ori | |
| Use | TurboGFP expression in mammalian cells; generation of fusions to the TurboGFP C-terminus | |

Vector description

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

TurboGFP 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 TurboGFP coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between TurboGFP 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 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.

Generation of TurboGFP fusion proteins

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

pTurboGFP-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TurboGFP or its fusions 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.

Notice to Purchaser:

TurboGFP-related materials (also referred to as "Products") are intended for research use only. The Products are covered by U.S. Pat. 7,678,893; European Pat. 1576157; and other Evrogen Patents and/or Patent applications pending. By use of these Products, you accept the terms and conditions of the applicable Limited Use Label License #001: <http://www.evrogen.com/products/Evrogen-FP-license.shtml>. The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MSDS information is available at <http://www.evrogen.com/MSDS.shtml>