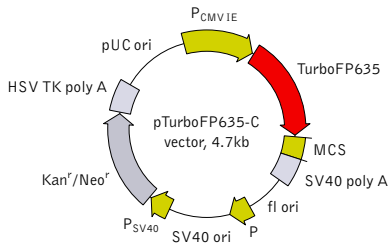


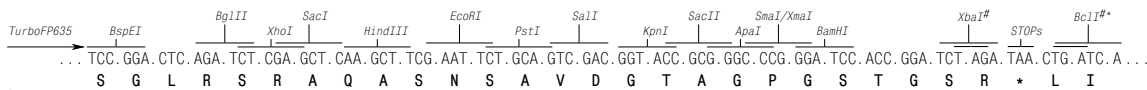
pTurboFP635-C vector

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
 TurboFP635
 Kozak consensus translation initiation site: 606-616
 Start codon (ATG): 613-615; Stop codon: 1396-1398
 Last amino acid in TurboFP635: 1315-1317
 MCS: 1318-1398
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1538-1543 & 1567-1572
 mRNA 3' ends: 1576 & 1588
 f1 single-strand DNA origin: 1635-2090
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 2152-2157; -10 region: 2175-2180
 Transcription start point: 2187
 SV40 origin of replication: 2431-2566
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2264-2335 & 2336-2407
 21-bp repeats: 2411-2431, 2432-2452 & 2454-2474
 Early promoter element: 2487-2493
 Major transcription start points: 2483, 2521, 2527 & 2532
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2615-2617; Stop codon: 3407-3409
 G->A mutation to remove Pst I site: 2797
 C->A (Arg to Ser) mutation to remove BssH II site: 3143
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3645-3650 & 3658-3663
 pUC plasmid replication origin: 3994-4637

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

Notice to Purchaser:

TurboFP635-related materials (also referred to as "Products") are intended for research use only. The Products are covered by U.S. Pat. 8,138,320; European Pat. 1994149; 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>

Product	Cat.#	Size
pTurboFP635-C vector	FP721	20 µg
Vector type	mammalian expression vector	
Reporter	TurboFP635	
Reporter codon usage	mammalian	
Promoter for TurboFP635	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	TurboFP635 expression in mammalian cells; generation of fusions to the TurboFP635 C-terminus	

Vector description

pTurboFP635-C is a mammalian expression vector encoding far-red fluorescent protein TurboFP635. The vector allows generation of fusions to the TurboFP635 C-terminus and expression of TurboFP635 fusions or TurboFP635 alone in eukaryotic (mammalian) cells.

TurboFP635 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 TurboFP635 sequence [Kozak 1987]. Multiple cloning site (MCS) is located between TurboFP635 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 TurboFP635-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 TurboFP635 C-terminus when inserted in the same reading frame as TurboFP635 and no intervening stop codons are present. TurboFP635-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TurboFP635, 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.

Despite its dimeric structure, TurboFP635 is still suitable for generation of fusions with some proteins of interest. However, we recommend to use monomeric TagFPs for this purpose.

Expression in mammalian cells

pTurboFP635-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.