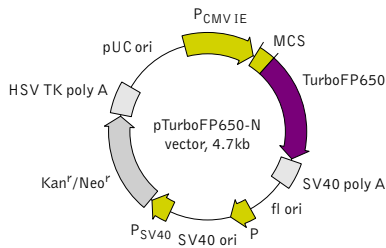


pTurboFP650-N vector

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>

Multiple cloning site (MCS)

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... G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. G ...
      Afe I           Xho I           Hind III           Pst I           Kpn I           Apa I           BamH I           TurboFP650
      Nhe I           Bgl II           Sac I           EcoR I           Sal I           Sma I/Xma I           Age I
  
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* — not unique site.

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 MCS: 591-671
 TurboFP650
 Kozak consensus translation initiation site: 672-682
 Start codon (ATG): 679-681; Stop codon: 1381-1383
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 1536-1541 & 1565-1570
 mRNA 3' ends: 1574 & 1586
 f1 single-strand DNA origin: 1633-2088
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2150-2155; -10 region: 2173-2178
 Transcription start point: 2185
 SV40 origin of replication: 2429-2564
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 2262-2333 & 2334-2405
 21-bp repeats: 2409-2429, 2430-2450 & 2452-2472
 Early promoter element: 2485-2491
 Major transcription start points: 2481, 2519, 2525 & 2530
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 2613-2615; Stop codon: 3405-3407
 G->A mutation to remove Pst I site: 2795
 C->A (Arg to Ser) mutation to remove BssH II site: 3141
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3643-3648 & 3656-3661
 pUC plasmid replication origin: 3992-4635

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. 143-190.

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248

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

Notice to Purchaser:

Evrogen Fluorescent Protein Products (the Products) are intended for research use only. The Products are covered by U.S. Pat. 7,417,131 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.

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.

MATERIAL SAFETY DATA SHEET INFORMATION: To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

Product	Cat.#	Size
pTurboFP650-N vector	FP732	20 µg
Vector type	mammalian expression vector	
Reporter	TurboFP650	
Reporter codon usage	mammalian	
Promoter for TurboFP650	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	TurboFP650 expression in mammalian cells; generation of fusions to the TurboFP650 N-terminus	

Vector description

pTurboFP650-N is a mammalian expression vector encoding near-infrared fluorescent protein TurboFP650. The vector allows generation of fusions to the TurboFP650 N-terminus and expression of TurboFP650 fusions or TurboFP650 alone in eukaryotic (mammalian) cells.

TurboFP650 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 TurboFP650 sequence [Kozak 1987]. Multiple cloning site (MCS) is located between P_{CMV IE} and TurboFP650 coding sequence.

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 TurboFP650-tagged 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 the TurboFP650 N-terminus when inserted in the same reading frame as TurboFP650 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TurboFP650-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TurboFP650, 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, TurboFP650 is still suitable for generation of fusions with proteins of interest, however we recommend to use TagFPs for these purposes.

Expression in mammalian cells

pTurboFP650-N 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.