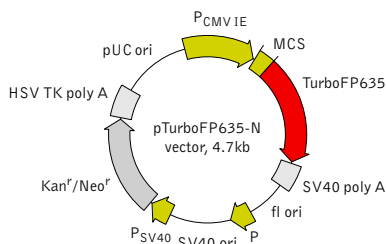


## pTurboFP635-N vector

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)

... 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 . . .

\* — not unique site.

### Location of features

P<sub>CMV IE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 MCS: 591-671  
 TurboFP635  
 Kozak consensus translation initiation site: 672-682  
 Start codon (ATG): 679-681; Stop codon: 1384-1386  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1540-1545 & 1569-1574  
 mRNA 3' ends: 1578 & 1590  
 f1 single-strand DNA origin: 1637-2092  
 Eukaryotic promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2154-2159; -10 region: 2177-2182  
 Transcription start point: 2189  
 SV40 origin of replication: 2433-2568  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2266-2337 & 2338-2409  
 21-bp repeats: 2413-2433, 2434-2454 & 2456-2476  
 Early promoter element: 2489-2495  
 Major transcription start points: 2485, 2523, 2529 & 2534  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2617-2619; Stop codon: 3409-3411  
 G->A mutation to remove Pst I site: 2799  
 C->A (Arg to Ser) mutation to remove BssH II site: 3145  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3647-3652 & 3660-3665  
 pUC plasmid replication origin: 3996-4639

### 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

Product	Cat.#	Size
pTurboFP635-N vector	FP722	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	TurboFP635	
Reporter codon usage	mammalian	
Promoter for TurboFP635	P <sub>CMV IE</sub>	
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 N-terminus	

### Vector description

pTurboFP635-N is a mammalian expression vector encoding far-red fluorescent protein TurboFP635. The vector allows generation of fusions to the TurboFP635 N-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 TurboFP635 sequence [Kozak 1987]. Multiple cloning site (MCS) is located between P<sub>CMV IE</sub> and TurboFP635 coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Generation of TurboFP635-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 TurboFP635 N-terminus when inserted in the same reading frame as TurboFP635 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. 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<sup>+</sup>-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<sup>-</sup> host and make fresh DNA. Despite its dimeric structure, TurboFP635 is still suitable for generation of fusions with proteins of interest, however we recommend to use TagFPs for these purposes.

### Expression in mammalian cells

pTurboFP635-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.

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