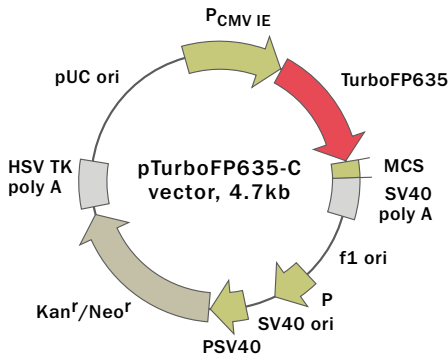
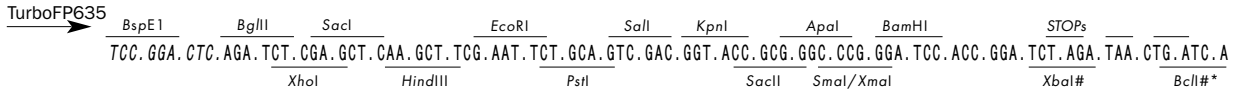


Mammalian expression vector pTurboFP635-C



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Multiple cloning site (MCS)



* - not unique site. # - sites are blocked by methylation.

Use

- Generation of fusions to the TurboFP635 C-terminus
- Expression of TurboFP635 or its fusions in mammalian cells

Product	Cat.#	Size
pTurboFP635-C	FP721	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	TurboFP635
Reporter codon usage	mammalian
Promoter for TurboFP602	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pTurboFP635-C is an eukaryotic (mammalian) expression vector encoding red fluorescent protein TurboFP635 from sea anemone *Entacmaea quadricolor*. The vector allows to generate fusions to the TurboFP635 C-terminus and to express 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 TurboFP635 translation, 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 polyA direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of 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 C-terminus when inserted in the same reading frame as TurboFP635 and no in-frame stop codons are present. TurboFP635-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTurboFP635-C vector will express TurboFP635, when transfected into eukaryotic (mammalian) cells.

Notes: 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 proteins of interest, however we recommend to use TagFP635 for these purposes.

Expression in mammalian cells

The 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.

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 TurboGFP: 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

Bacterial 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) In DNA cloning: A Practical Approach, Vol. II, Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al.* (1996) *Curr. Biol.* 6: 315-324.

Kozak M. (1987) *Nucleic Acids Res.* 15:8125-8148.

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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.