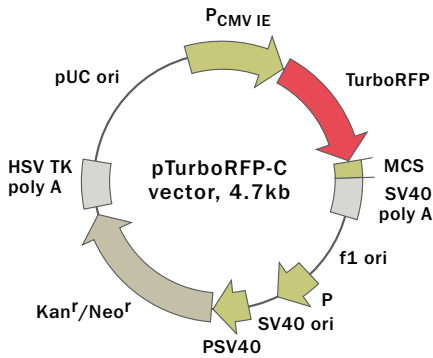


Mammalian expression vector pTurboRFP-C



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

Multiple cloning site (MCS)



- sites are blocked by methylation.

Use

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

Product	Cat.#	Size
pTurboRFP-C	FP231	20 µg

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

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

Vector description

pTurboRFP-C is an eukaryotic (mammalian) expression vector encoding red fluorescent protein TurboRFP. The vector allows generation of fusions to the TurboRFP C-terminus and expression TurboRFP fusions or TurboRFP alone in eukaryotic (mammalian) cells.

TurboRFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TurboRFP translation, Kozak consensus translation initiation site is generated upstream of TurboRFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between TurboRFP 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 TurboRFP C-terminus when inserted in the same reading frame as TurboRFP and no in-frame stop codons are present. TurboRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTurboRFP-C vector will express TurboRFP, 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

pTurboRFP-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TurboRFP or TurboRFP-tagged fusions in many cell types. 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

Kozak consensus translation initiation site: 606-616

TurboRFP

Start codon (ATG): 613-615; stop codon: 1324-1326

Last amino acid in TurboRFP: 1303-1305

MCS: 1306-1392

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1532-1537 & 1561-1566

mRNA 3' ends: 1570 & 1582

f1 single-strand DNA origin: 1629-2084

Bacterial promoter for expression of Kan^r gene

-35 region: 2146-2151;

-10 region: 2169-2174

Transcription start point: 2181

SV40 origin of replication: 2425-2560

SV40 early promoter

Enhancer (72-bp tandem repeats): 2258-2329 & 2330-2401

21-bp repeats: 2405-2425, 2426-2446 & 2448-2468

Early promoter element: 2481-2487

Major transcription start points: 2477, 2515, 2521 & 2526

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2609-2611; stop codon: 3401-3403

G->A mutation to remove PstI site: 2791

C->A (Arg to Ser) mutation to remove BssHII site: 3137

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 3639-3644 & 3652-3657

pUC plasmid replication origin: 3988-4631

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.