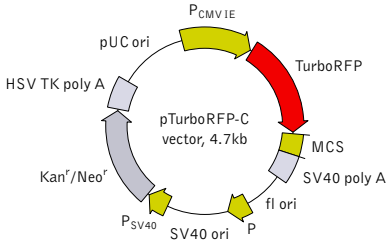
pTurboRFP-C vector

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

Product	Cat.#	Size
pTurboRFP-C vector	**FP231**	20 μg

Vector type	mammalian expression vector
Reporter	TurboRFP
Reporter codon usage	mammalian
Promoter for TurboRFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	TurboRFP expression in mammalian cells; generation of fusions to the TurboRFP C-terminus

Multiple cloning site (MCS)

TurboRFP → BspE I | Xho I | Hind III | Pst I | Kpn I | Apa I | BamH I | STOPs

. . . GAT. GAA. TCC. GGA. CTC. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGA. TCT. AGA. TAA. CTG. ATC. A . . .

Bgl II | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Xba I# | Bcl I#*

* — not unique site.
\# — 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
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
Eukaryotic 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 Pst I site: 2791
C->A (Arg to Ser) mutation to remove BssH II 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 (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

Vector description

pTurboRFP-C is a mammalian expression vector encoding red (orange) fluorescent protein TurboRFP. The vector allows generation of fusions to the TurboRFP C-terminus and expression of 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 mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the 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 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 TurboRFP-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 TurboRFP C-terminus when inserted in the same reading frame as TurboRFP and no intervening stop codons are present. TurboRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified 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.

Despite its dimeric structure, TurboRFP is still suitable for generation of fusions with proteins of interest, however we recommend to use TagFPs for these purposes.

Expression in mammalian cells

pTurboRFP-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.