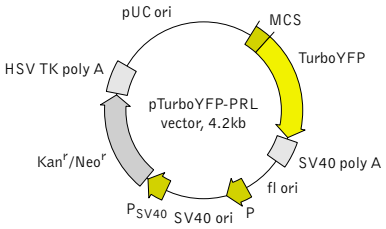
pTurboYFP-PRL vector

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

Product	Cat.#	Size
pTurboYFP-PRL vector	**FP615**	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	promoterless expression vector
Reporter	TurboYFP
Reporter codon usage	mammalian
Promoter for TurboYFP	NO
Host cells	mammalian, prokaryotic
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Monitoring of activity of different promoters and promoter/enhancer combinations

Multiple cloning site (MCS)

Afe I — Xho I — Hind III — Pst I — Kpn I — Apa I — BamH I — TurboYFP

. . . A. 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. A* . . .

Bgl II — Sac I — EcoR I — Sal I — Sac II* — Sma I/Xma I — Age I

* — not unique site.

Location of features

MCS: 12-89
TurboYFP
Kozak consensus translation initiation site: 90-100
Start codon (ATG): 97-99; Stop codon: 826-828
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 982-987 & 1011-1016
mRNA 3' ends: 1020 & 1032
f1 single-strand DNA origin: 1079-1534
Eukaryotic promoter for expression of Kan^r gene
-35 region: 1596-1601; -10 region: 1619-1624
Transcription start point: 1631
SV40 origin of replication: 1875-2010
SV40 early promoter
Enhancer (72-bp tandem repeats): 1708-1779 & 1780-1851
21-bp repeats: 1855-1875, 1876-1896 & 1898-1918
Early promoter element: 1931-1937
Major transcription start points: 1927, 1965, 1971 & 1976
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2059-2061; Stop codon: 2851-2853
G->A mutation to remove Pst I site: 2241
C->A (Arg to Ser) mutation to remove BssH II site: 2587
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3089-3094 & 3102-3107
pUC plasmid replication origin: 3438-4081

Vector description

pTurboYFP-PRL is a promoterless vector encoding yellow fluorescent protein, TurboYFP, which can be used as *in vivo* reporter of gene expression. TurboYFP 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 TurboYFP coding sequence [Kozak 1987].

Multiple cloning site (MCS) is located upstream of the Kozak consensus translation initiation site and can be used to clone a promoter or a promoter/enchancer combination of interest. Without the addition of a functional promoter, this vector will not express TurboYFP.

The vector backbone contains 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.

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

The vector will express TurboYFP under the control of functional promoter cloned into the vector's MCS. pTurboYFP-PRL 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.

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