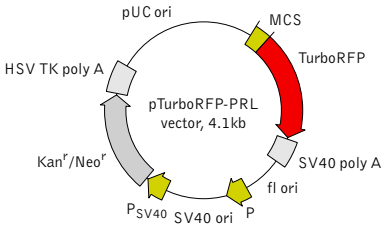
# pTurboRFP-PRL vector

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 |
|---|---|---|
| pTurboRFP-PRL vector | **FP235** | 20 $\mu$g |

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 | TurboRFP |
| Reporter codon usage | mammalian |
| Promoter for TurboRFP | NO |
| Host cells | mammalian, prokaryotic |
| Selection | prokaryotic - kanamycin<br>eukaryotic - neomycin (G418) |
| Replication | prokaryotic - pUC ori<br>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 — TurboRFP

. . . 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

## Location of features

MCS: 12-96
TurboRFP
Kozak consensus translation initiation site: 90-100
Start codon (ATG): 97-99; Stop codon: 790-792
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 946-951 & 975-980
mRNA 3' ends: 984 & 996
f1 single-strand DNA origin: 1043-1498
Eukaryotic promoter for expression of Kan$^r$ gene
-35 region: 1560-1565; -10 region: 1583-1588
Transcription start point: 1595
SV40 origin of replication: 1839-1974
SV40 early promoter
Enhancer (72-bp tandem repeats): 1672-1743 & 1744-1815
21-bp repeats: 1819-1839, 1840-1860 & 1862-1882
Early promoter element: 1895-1901
Major transcription start points: 1891, 1929, 1935 & 1940
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2023-2025; Stop codon: 2815-2817
G->A mutation to remove Pst I site: 2205
C->A (Arg to Ser) mutation to remove BssH II site: 2551
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3053-3058 & 3066-3071
pUC plasmid replication origin: 3402-4045

## Vector description

pTurboRFP-PRL is a promoterless vector encoding red (orange) fluorescent protein, TurboRFP, which can be used as *in vivo* reporter of gene expression. 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 TurboRFP 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 TurboRFP.

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 TurboRFP under the control of functional promoter cloned into the vector's MCS. pTurboRFP-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 $\mu$g/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