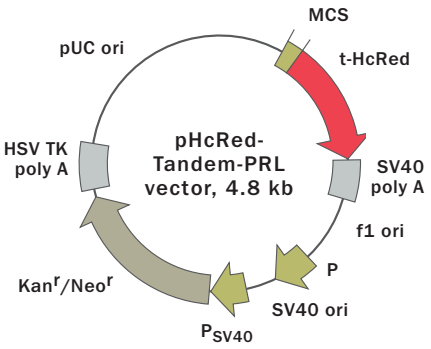
Promoterless vector pHcRed-Tandem-PRL



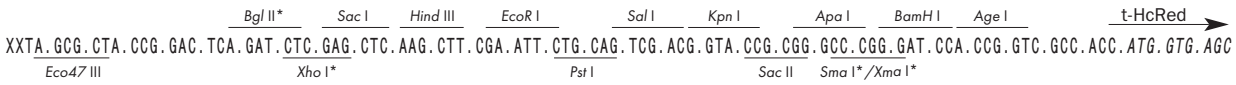
For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pHcRed-Tandem-PRL	**FP210**	20 µg

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

Vector type	promoterless vector
Reporter	t-HcRed
Reporter codon usage	mammalian
Promoter for t-HcRed	NO
Host cells	mammalian, bacterial
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)



* — not unique sites.

Use

- Monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS

Vector description

pHcRed-Tandem-PRL vector is a promoterless vector encoding far-red fluorescent protein t-HcRed, which can be used as *in vivo* reporter of gene expression. t-HcRed codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase t-HcRed mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of t-HcRed 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 t-HcRed.

The vector backbone comprises 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) allows 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.

Note: This plasmid DNA was isolated from dam$^+$-methylated *E.coli* starin. Therefore some restriction sites are bloked 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 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

MCS: 12-89
t-HcRed
Kozak consensus translation initiation site: 90-100
Start codon (ATG): 97-99;
Stop codon: 1504-1506
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1636-1641 & 1665-1670
mRNA 3' ends: 1674 & 1686
f1 single-strand DNA origin: 1733-2188
(packages the noncoding strand of t-HcRed)
Bacterial promoter expression of Kan^r gene:
-35 region: 2250-2255;
-10 region: 2273-2278
Transcription start point: 2285
SV40 origin of replication: 2529-2664
SV40 early promoter
Enhancer (72-bp tandem repeats): 2362-2433 & 2434-2505
21-bp repeats: 2509-2529, 2530-2550, & 2552-2572
Early promoter element: 2585-2591
Major transcription start points: 2581, 2619, 2625 & 2630
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2713-2715; stop codon: 3505-3507
G->A mutation to remove PstI site: 2895
C->A (Arg to Ser) mutation to remove BssHII site: 3241
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3743-3748 & 3756-3761
pUC plasmid replication origin: 4092-4735

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.