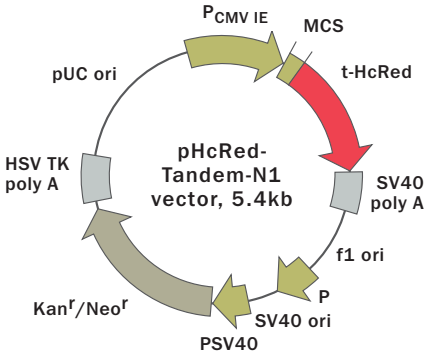
Mammalian expression vector pHcRed-Tandem-N1



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

Product	Cat.#	Size
pHcRed-Tandem-N1	**FP204**	20 µg

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

Vector type	mammalian expression vector
Reporter	t-HcRed
Reporter codon usage	mammalian
Promoter for t-HcRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

t-HcRed →

Nhe I — Bgl II* — Sac I — Hind III — EcoR I — Sal I — Kpn I — Apa I — BamH I — Age I

GCTA.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.GTG.AGC*

Eco47 III — Xho I* — Pst I — Sac II — Sma I*/Xma I*

* — not unique sites.

Use

- Generation of fusions to the t-HcRed N-terminus

- Expression of t-HcRed or its fusions in mammalian cells

Vector description

pHcRed-Tandem-N1 is an eukaryotic (mammalian) expression vector encoding far-red fluorescent protein t-HcRed. The vector is designed to generate t-HcRed-tagged fusions or to express t-HcRed in mammalian cells.

t-HcRed codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase t-HcRed translation, Kozak consensus translation initiation site is generated upstream of t-HcRed sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and t-HcRed coding sequence.

The vector backbone comprises 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 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 the t-HcRed N-terminus when inserted in the same reading frame as t-HcRed and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. t-HcRed-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Note: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. 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

pHcRed-Tandem-N1 can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Unmodified pHcRed-Tandem-N1 will express t-HcRed, when transfected into eukaryotic (mammalian) cells.

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
MCS: 591-671
t-HcRed gene
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681;
Stop codon: 2086-2088
Polyadenylation signals: 2218-2223 & 2247-2252
mRNA 3' ends: 2256 & 2268
f1 single-strand DNA origin: 2315-2770
(packages the noncoding strand of t-HcRed)
Bacterial promoter expression of Kan^r gene:
-35 region: 2832-2837; -10 region: 2855-2860
Transcription start point: 2867
SV40 origin of replication: 3111-3246
SV40 early promoter
Enhancer (72-bp tandem repeats): 2944-3015 & 3016-3087
21-bp repeats: 3091-3111, 3112-3132 & 3134-3154
Early promoter element: 3167-3173
Major transcription start points: 3163, 3201, 3207 & 3212
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3295-3297; stop codon: 4087-4089
G->A mutation to remove PstI site: 3477
C->A (Arg to Ser) mutation to remove BssHII site: 3823
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4325-4330 & 4338-4343
pUC plasmid replication origin: 4674-5317

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.