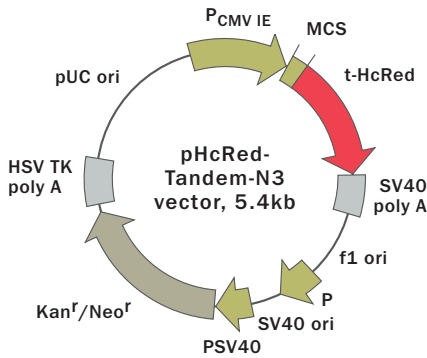


Mammalian expression vector pHcRed-Tandem-N3



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

| Product | Cat.# | Size |
|------------------|--------------|-------|
| pHcRed-Tandem-N3 | FP206 | 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)



* — 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-N3 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 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

pHcRed-Tandem-N3 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-N3 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: 2088-2090

Polyadenylation signals: 2220-2225 & 2249-2254

mRNA 3' ends: 2258 & 2270

f1 single-strand DNA origin: 2317-2772

(packages the noncoding strand of t-HcRed)

Bacterial promoter expression of Kan^r gene:

-35 region: 2834-2839;

-10 region: 2857-2862

Transcription start point: 2869

SV40 origin of replication: 3113-3248

SV40 early promoter

Enhancer (72-bp tandem repeats): 2946-3017 & 3018-3089

21-bp repeats: 3093-3113, 3114-3134 & 3136-3156

Early promoter element: 3169-3175

Major transcription start points: 3165, 3203, 3209 & 3214

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3297-3299; stop codon: 4089-4091

G->A mutation to remove PstI site: 3479

C->A (Arg to Ser) mutation to remove BssHII site: 3825

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 4327-4332 & 4340-4345

pUC plasmid replication origin: 4676-5319

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.

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