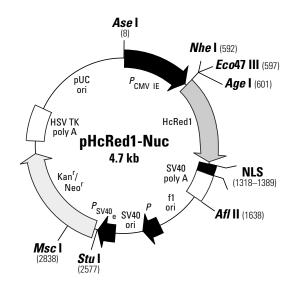
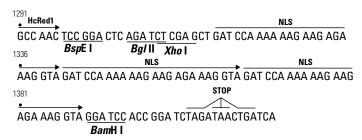
Cat. No. 632433





Plasmid map and NLS of pHcRed1-Nuc. NLS = three tandem repeats of the nuclear localization signal from simian virus large T-antigen. Unique restriction sites are shown in bold.

Description

pHcRed1-Nuc encodes HcRed1 fused with three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen (1, 2). The NLS sequences are fused to the 3'-end of the HcRed1 coding sequence. The reiteration of the NLS sequence significantly increases the efficiency with which the HcRed1 fusion translocates to the nucleus of mammalian cells (3). HcRed1, a far-red fluorescent protein whose excitation and emission maxima occur at 588 nm and 618 nm ±4 nm, respectively, derives from a non-fluorescent chromoprotein found in the reef coral *Heteractis crispa* (4). To enhance its translation efficiency in mammalian cells, the HcRed1 coding sequence has been human codon-optimized, and sequences upstream of HcRed1 have been converted to a Kozak consensus translation initiation site (5, 6)

Expression of the HcRed1 fusion is driven by the immediate early promoter of cytomegalovirus ($P_{\text{CMV IE}}$). SV40 polyadenylation signals downstream of the HcRed1 gene direct proper processing of the 3'-end of the HcRed1-NLS mRNA transcript. The vector also contains an SV40 origin for replication in any mammalian cell line that expresses the SV40 T-antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin resistance cassette—consisting of the SV40 early promoter (P_{SV40e}), the neomycin/kanamycin resistance gene of Tn5 (Neoʻ/Kanʻ), and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK poly A) gene—allows stably transfected eukaryotic cells to be selected using G418 (7). A bacterial promoter (P) upstream of this cassette drives expression of the gene encoding kanamycin resistance in E. coli.

Clontech

United States/Canada 800.662.2566 Asia Pacific +1.650.919.7300

Europe +33.(0)1.3904.6880

Japan

+81.(0)77.543.6116

Clontech Laboratories, Inc. ATakara Bio Company 1290 Terra Bella Ave. Mountain View, CA 94043 Technical Support (US) E-mail: tech@clontech.com www.clontech.com (PR34610; published 11 March 2003)

pHcRed1-Nuc **Vector Information**

Use

pHcRed1-Nuc is used for the localized expression of HcRed1 in the nucleus of mammalian cells. It allows the visualization of the nucleus in living and fixed cells using fluorescence microscopy. HcRed1 can be used for dual labeling experiments together with Enhanced Cyan, Green, or Yellow Fluorescent Proteins (ECFP, EGFP or EYFP) using standard fluorescence microscopy when the appropriate filter sets are applied, pHcRed1-Nuc is not meant to be used as a cloning vector. However, unique restriction sites at the 3' end of HcRed1, between HcRed1 and the three copies of the NLS, allow excision or insertion of DNA. The vector can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7).

Location of features

Human cytomegalovirus (CMV) immediate early promoter: 1–589

Enhancer region: 59-465; TATA box: 554-560; transcription start point: 583

C→G mutation to remove Sac I site: 569

Far-Red fluorescent protein (HcRed1) gene

Kozak consensus translation initiation site: 606-616 Start codon (ATG): 613–615 Stop codon: 1408–1410

Insertion of Val at position 2: 616-618

Last amino acid in HcRed1 coding region: 1294–1296

- Tandem repeat of the nuclear localization signal (NLS): 1318–1389
- SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1550-1555 & 1579-1584; mRNA 3' ends: 1588 & 1600

- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of pHcRed1-Nuc.)
- Bacterial promoter for expression of Kan^r gene.

-35 region: 2164-2169; -10 region: 2187-2192

Transcription start point: 2199

- SV40 origin of replication: 2443–2578
- SV40 early promoter

Enhancer (72-bp tandem repeats): 2276-2347 & 2348-2419

21-bp repeats: 2423-2443, 2444-2464 & 2466-2486

Early promoter element: 2499–2505

Major transcription start points: 2495, 2533, 2539 & 2544

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences: Start codon (ATG): 2627-2629; stop codon: 3419-3421

G→A mutation to remove Pst I site: 2809

C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155

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

Polyadenylation signals: 3657-3662 & 3670-3675

pUC plasmid replication origin: 4006–4649

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 μg/ml) to E. coli hosts.
- · E. coli replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Protocol No. PT3728-5 Clontech Laboratories, Inc. www.clontech.com Version No. PR34610 pHcRed1-Nuc Vector Information

References

- 1. Kalderon, D., et al. (1984) Cell 39:499-509.
- 2. Lanford, R. E., et al. (1986) Cell 46: 575-582.
- Fischer-Fantuzzi, L. & Vesco, C. (1988) Mol. Cell. Biol. 8:5495–5503.
- 4. Gurskaya, N. G., et al. (2001) FEBS Letters 507:16-20.
- 5. Haas, J., et al. (1996) Curr. Biol. 6:315-324.
- 6. Kozak, M. (1987) Nucleic Acids Res. 15:8125-8148.
- 7. Gorman, C. (1985) In DNA Cloning: A Practical Approach, Vol. II, Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143-190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes, nor is it intended for human use. Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, royalty-free, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com

Clontech, Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc. Clontech is a Takara Bio Company. ©2005

Protocol No. PT3728-5 www.clontech.com Clontech Laboratories, Inc.
Version No. PR34610 3