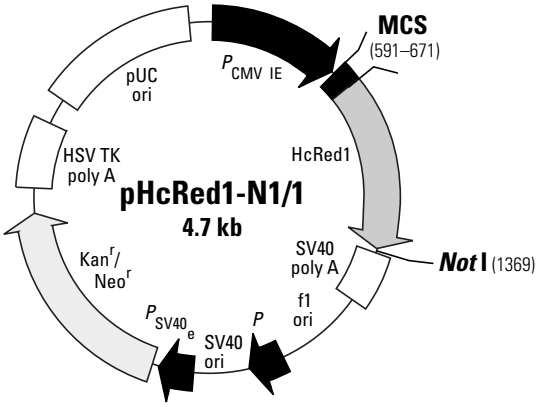
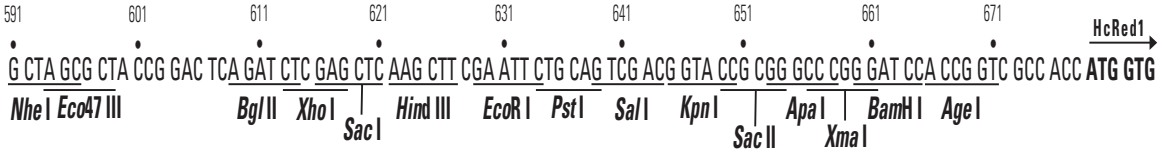
Vector Information

pHcRed1-N1/1 Vector Information PT3706-5

Cat. No. 632424





Restriction Map and Multiple Cloning Site (MCS) of pHcRed1-N1/1/1 Vector. All sites shown are unique. The *Not* I site follows the HcRed1 stop codon.

Description

pHcRed1-N1/1 encodes HcRed1, a far-red fluorescent protein that can be used to monitor gene expression and protein localization *in vivo*. HcRed1 was generated by mutagenesis of a non-fluorescent chromoprotein from the reef coral *Heteractis crispa* (1; HcRed-2A). The HcRed1 coding sequence has been human codon-optimized for higher expression in mammalian cells (2).

The multiple cloning site (MCS) in pHcRed1-N1/1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the HcRed1 coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of HcRed1. Sequences upstream of HcRed1 have been converted to a Kozak consensus translation initiation site to increase translation efficiency in eukaryotic cells (3). SV40 polyadenylation signals downstream of the HcRed1 gene direct proper processing of the 3' end of mRNA transcripts. The vector backbone contains an SV40 origin for replication in mammalian cells expressing 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 (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette confers kanamycin resistance to *E. coli*.

Use

pHcRed1-N1/1 can be used to express proteins as fusions to the N-terminus of HcRed1. If a fusion construct retains the fluorescent properties of HcRed1, its expression can be monitored by flow cytometry and its localization *in vivo* can be determined by fluorescence microscopy. The target gene should be cloned into pHcRed1-N1/1 so that it is in frame with the HcRed1 coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pHcRed1-N1/1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). pHcRed1-N1/1 can also be used as a cotransfection marker; the unmodified vector will express HcRed1.

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.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR2X112; published 18 October 2002)

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
- MCS: 591–671
- Far-red Fluorescent Protein (HcRed1) gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1363–1365
  - Insertion of Val at position 2: 682–684
  - Ala-3 to Ser mutation: 685–687
  - Thr-37 to Ala mutation: 787–789
  - Leu-123 to His mutation: 1045–1047
  - Cys-144 to Ser mutation: 1108–1110
  - Arg-169 to His mutation: 1183–1185
  - Leu-174 to His mutation: 1198–1200
  - Pro-202 to Leu mutation: 1282–1284
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1519–1524 & 1548–1553; mRNA 3' ends: 1557 & 1569
- f1 single-strand DNA origin: 1616–2071 (Packages the noncoding strand of HcRed1.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2133–2138; –10 region: 2156–2161
  - Transcription start point: 2168
- SV40 origin of replication: 2412–2547
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2245–2316 & 2317–2388
  - 21-bp repeats: 2392–2412, 2413–2433 & 2435–2455
  - Early promoter element: 2468–2474
  - Major transcription start points: 2464, 2502, 2508 & 2513
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2596–2598; stop codon: 3388–3390
  - G→A mutation to remove *Pst* I site: 2778
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3124
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3626–3631 & 3639–3644
- pUC plasmid replication origin: 3975–4618

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 JM101 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/ColE1

References:

1. Gurskaya, N. G., *et al.* (2001) *FEBS Letters* **507**:16–20.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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.