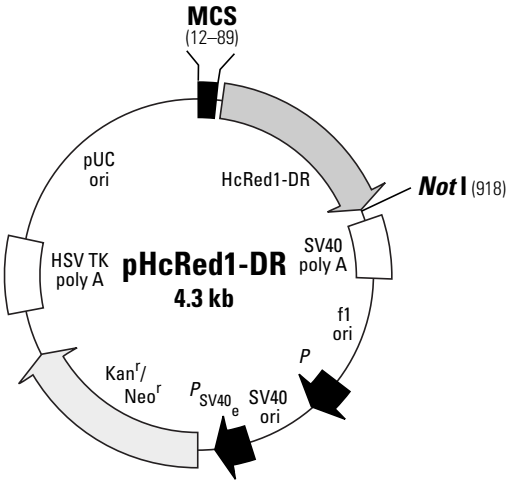
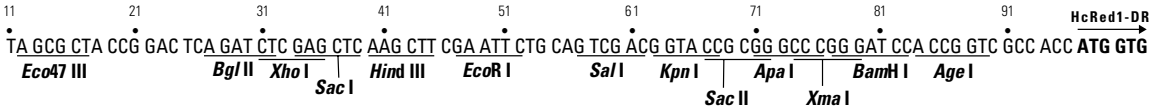
Vector Information

pHcRed1-DR Vector Information PT3701-5

Catalog No. 632422





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

Description

pHcRed1-DR is a promoterless vector that encodes HcRed1-DR, a destabilized variant of Living Colors™ far-red fluorescent protein, HcRed1 (1). In contrast to the original protein, HcRed1-DR has a short half-life, making it well suited for studies that require rapid reporter turnover. HcRed1-DR was generated from HcRed1 by fusing the C-terminus of the protein to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (2). This region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (2, 3). The original protein, HcRed1, whose excitation and emission maxima occur at 588 nm and 618 nm ±4 nm, was generated by mutagenesis of a non-fluorescent chromoprotein from the reef coral *Heteractis crispa* (4; HcRed-2A). The HcRed1-DR coding sequence has been human codon-optimized for higher expression in mammalian cells (5). Sequences upstream of the HcRed1 coding sequence have been converted to a Kozak consensus translation initiation site (6) to increase the translation efficiency in eukaryotic cells.

pHcRed1-DR can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS), located upstream of the HcRed1-DR coding sequence. SV40 polyadenylation signals downstream of the HcRed1-DR gene direct proper processing of the 3' end of the HcRed1-DR mRNA. The vector also contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (4). The Neo^r 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 this cassette confers kanamycin resistance in *E. coli*. pHcRed1-DR also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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

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Use

HcRed1-DR can be used as an *in vivo* reporter of gene expression. Because of its rapid turnover rate, its expression from a promoter of interest provides a more accurate assessment of the promoter's activity over time than does the more stable HcRed1. Promoter/enhancer elements should be inserted into the MCS upstream of the HcRed1-DR coding sequences. **Without the addition of a functional promoter, this vector will not express HcRed1-DR**. The recombinant pHcRed1-DR vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7).

Location of features

- MCS: 12–89
- Destabilized Far-red Fluorescent Protein (HcRed1-DR) gene
  - Kozak consensus translation initiation site: 90–100
  - Start codon (ATG): 97–99; Stop codon: 913–915
  - Insertion of Val at position 2: 100–102
  - Ala-3 to Ser mutation: 103–105
  - Thr-37 to Ala mutation: 205–207
  - Leu-123 to His mutation: 463–465
  - Cys-144 to Ser mutation: 526–528
  - Arg-169 to His mutation: 601–603
  - Leu-174 to His mutation: 616–618
  - Pro-202 to Leu mutation: 700–702
  - Mouse ornithine decarboxylase PEST sequence: 793–915
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1068–1073 & 1097–1102
  - mRNA 3' ends: 1105 & 1118
- f1 single-strand DNA origin: 1165–1620
  - (Packages noncoding strand of HcRed1.)
- Ampicillin resistance (β-lactamase) promoter
  - –35 region: 1682–1687; –10 region: 1705–1710
  - Transcription start point: 1717
- SV40 origin of replication: 1961–2096
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 1792–1865 & 1866–1937
  - 21-bp repeats: 1941–1961, 1962–1982 & 1984–2004
  - Early promoter element: 2017–2023
  - Major transcription start points: 2013, 2051, 2057 & 2062
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2145–2147; stop codon: 2937–2939
  - G→A mutation to remove *Pst* I site: 2327
  - C→A (Arg→Ser) mutation to remove *Bss*H II site: 2673
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3175–3180 & 3188–3193
- pUC plasmid replication origin: 3524–4170

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 XL-1 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. Living Colors HcRed (April 2002) *Clontechniques* **XVII**(2):12–13.
2. Li, X., *et al.* (1998) *J. Biol. Chem.* **273**:34970–34975.
3. Rechsteiner, M., *et al.* (1990) *Semin. Cell Biol.* **1**:433–440.
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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. This vector has not been completely sequenced.