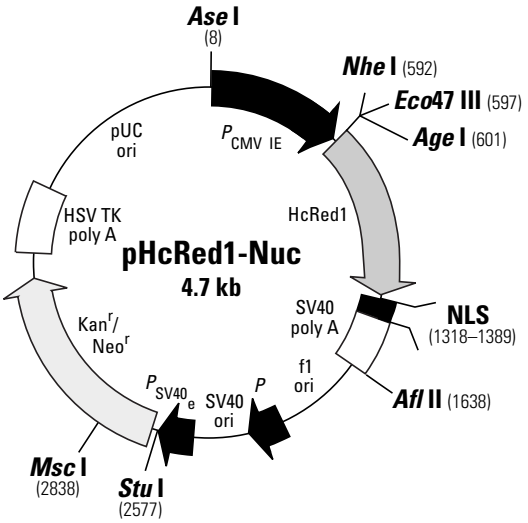
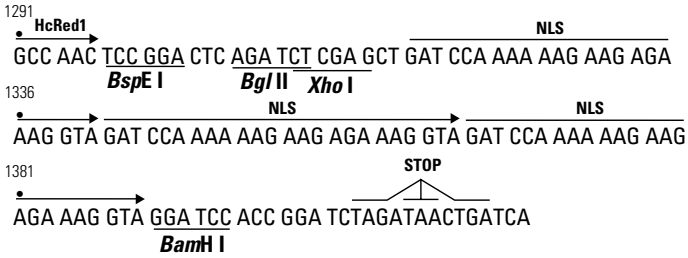
Vector Information

pHcRed1-Nuc Vector Information

PT3728-5

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 ($P_{CMV\ IE}$). 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 (Neo^r/Kan^r), 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*.

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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

References

1. Kalderon, D., *et al.* (1984) *Cell* **39**:499–509.
2. Lanford, R. E., *et al.* (1986) *Cell* **46**: 575–582.
3. 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.