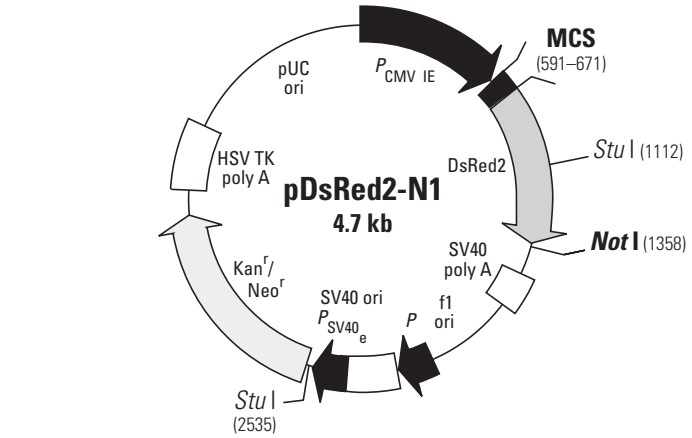
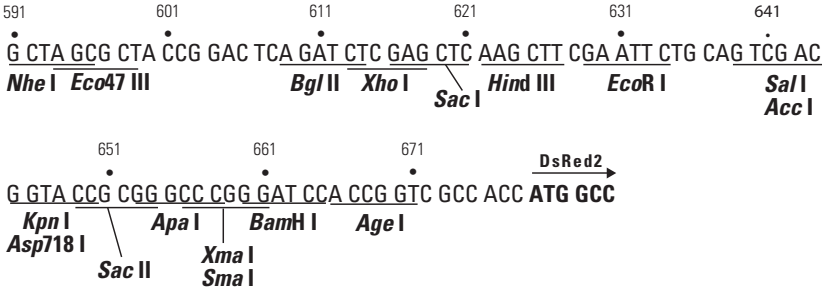
Vector Information

pDsRed2-N1 Vector Information PT3604-5

Cat. No. 632406





Restriction Map and Multiple Cloning Site (MCS) of pDsRed2-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed2 stop codon.

Description

pDsRed2-N1 encodes DsRed2, a DsRed variant that has been engineered for faster maturation and lower non-specific aggregation. Derived from the *Discosoma sp.* red fluorescent protein (drFP583; 1), DsRed2, like its progenitor DsRed1, contains a series of silent base-pair changes that correspond to human codon-usage preferences for high expression in mammalian cells (2). In addition to these changes, DsRed2 contains six amino acid substitutions: V105A, I161T, and S197A, which result in the more rapid appearance of red fluorescence in transfected cell lines; and R2A, K5E, and K9T, which prevent the protein from aggregating. (DsRed2 may, however, form the same tetrameric structure as DsRed1 [3].) In mammalian cell cultures when DsRed2 is expressed constitutively, red-emitting cells can be detected by fluorescence microscopy within 24 hours of transfection. Large insoluble aggregates of protein, often observed in bacterial and mammalian cell systems expressing DsRed1, are dramatically reduced in cells expressing DsRed2. The faster-maturing, more soluble red fluorescent protein is also well tolerated by host cells; mammalian cell cultures transfected with DsRed2 show no obvious signs of reduced viability—in those cell lines tested, cells expressing DsRed2 display the same morphology (e.g., adherence, light-refraction) and growth characteristics as non-transfected controls.

The multiple cloning site (MCS) in pDsRed2-N1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed2 coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed2. Sequences upstream of DsRed2 have been converted to a Kozak consensus translation initiation site to increase translation efficiency in eukaryotic cells (4). SV40 polyadenylation signals downstream of the DsRed2 gene direct proper processing of the 3' end of the DsRed2 mRNA. 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*.

(121712; published 17 December 2012)

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

Use

pDsRed2-N1 can be used to construct fusions to the N-terminus of DsRed2. If a fusion construct retains the fluorescent properties of the native DsRed2 protein, 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 pDsRed2-N1 so that it is in frame with the DsRed2 coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pDsRed2-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5). Unmodified pDsRed2-N1 can also be used to express DsRed2 in a cell line of interest (*e.g.*, for use as a transfection marker).

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
- *Discosoma sp.* Red Fluorescent Protein (DsRed2) gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1354–1356
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1508–1513 & 1537–1542; mRNA 3' ends: 1546 & 1558
- f1 single-strand DNA origin: 1605–2060 (Packages the noncoding strand of DsRed2.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2122–2127; –10 region: 2145–2150
  - Transcription start point: 2157
- SV40 origin of replication: 2401–2536
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2234–2305 & 2306–2377
  - 21-bp repeats: 2381–2401, 2402–2422 & 2424–2444
  - Early promoter element: 2457–2463
  - Major transcription start points: 2453, 2491, 2497 & 2502
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2585–2587; stop codon: 3377–3379
  - G→A mutation to remove *Pst* I site: 2767
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3113
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3615–3620 & 3628–3633
- pUC plasmid replication origin: 3964–4607

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/ColE1

Red Fluorescent Protein (DsRed2)

- Excitation/Emission Maxima: 558 nm / 583 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Yarbrough, D., *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98**:462–467.
4. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.