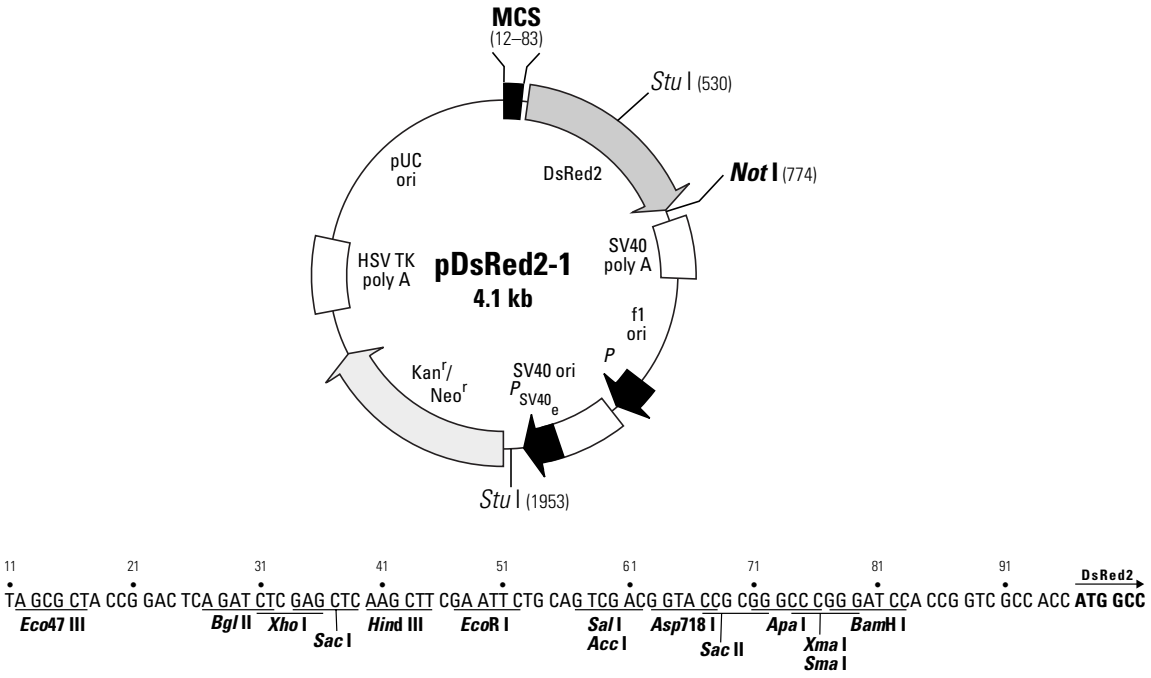
Vector Information

pDsRed2-1 Vector Information PT3602-5

Cat. No. 632405



Restriction Map and Multiple Cloning Site (MCS) of pDsRed2-1. Unique restriction sites are in bold. The *Not* I site is part of the DsRed2 stop codon.

Description

pDsRed2-1 encodes DsRed2, a DsRed variant 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.

pDsRed2-1 is a promoterless DsRed2 vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS). Sequences upstream of DsRed2 have been converted to a Kozak consensus translation initiation site (4) to increase translation efficiency in eukaryotic cells. 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 expresses kanamycin resistance in *E. coli*.

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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

DsRed2 can be used as an *in vivo* reporter of gene expression. Promoters should be cloned into the pDsRed2-1 MCS upstream from the DsRed2 coding sequence. Without addition of a functional promoter, this vector will not express DsRed2. The recombinant DsRed2 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5).

Location of features

- MCS: 12–83
- *Discosoma sp.* human codon-optimized Red Fluorescent Protein (DsRed2) gene
  Kozak consensus translation initiation site: 90–100
  Start codon (ATG): 97–99; Stop codon: 772–774
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 926–931 & 955–960
  mRNA 3' ends: 964 & 976
- f1 single-strand DNA origin: 1023–1478
  (Packages noncoding strand of DsRed2.)
- Ampicillin resistance (b-lactamase) promoter
  –35 region: 1540–1545; –10 region: 1563–1568
  Transcription start point: 1575
- SV40 origin of replication: 1819–1954
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 1650–1723 & 1724–1795
  21-bp repeats: 1799–1819, 1820–1840 & 1842–1862
  Early promoter element: 1875–1881
  Major transcription start points: 1871, 1909, 1915 & 1920
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2003–2005; stop codon: 2795–2797
  G→A mutation to remove *Pst* I site: 2185
  C→A (Arg→Ser) mutation to remove *Bss*H II site: 2531
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3033–3038 & 3046–3051
- pUC plasmid replication origin: 3382–4025

Sequencing Primer Locations

- DsRed1-N Sequencing Primer (No.632387; 5'-GTACTGGAACTGGGGGGACAG-3'): 297–277
- DsRed1-C Sequencing Primer (No.632388; 5'-AGCTGGACATCACCTCCCACAACG-3'): 689–712

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

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.

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.

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