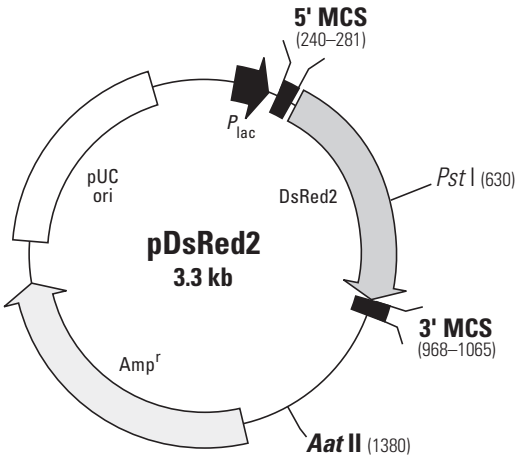
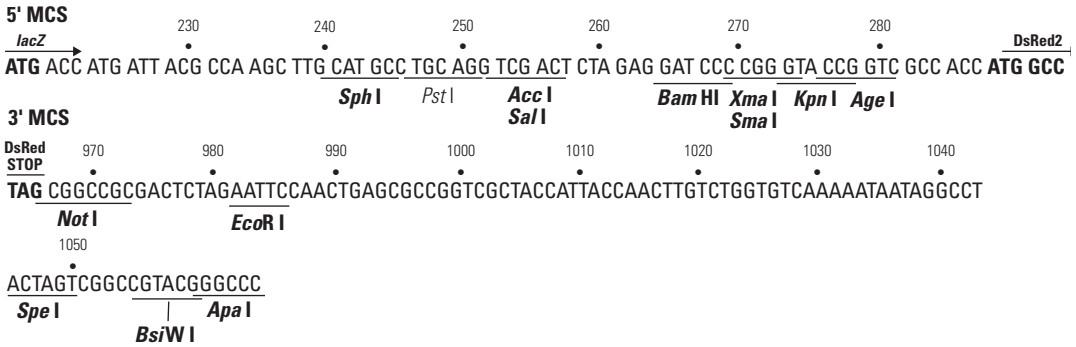
Vector Information

pDsRed2 Vector Information PT3601-5

Cat. No. 632404





Restriction Map and Multiple Cloning Site (MCS) of pDsRed2. Unique restriction sites are in bold.

Description

pDsRed2 is a prokaryotic expression vector that 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 corresponding to human codon-usage preferences for high expression in mammalian cells (2). In addition to these changes, DsRed2 contains six amino acid substitutions: A105V, 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.

In pDsRed2, the DsRed2 coding sequence is flanked by separate and distinct multiple cloning sites at the 5' and 3' ends so that it is easy to excise the gene for use in other applications. Alternatively, the DsRed2 coding sequence can be amplified by PCR. The DsRed 2 gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that DsRed2 is expressed from the *lac* promoter (P_{lac}) in *E. coli* host cells. A Kozak consensus sequence is located immediately upstream of the DsRed2 gene to enhance translational efficiency in eukaryotic systems (4). The entire DsRed2 expression cassette in pDsRed2 is supported by a pUC backbone, which contains a high-copy number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

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Use

pDsRed2 is primarily intended to serve as a source of DsRed2 cDNA. The flanking MCS regions make it possible to excise the DsRed2 coding sequence and insert it into other expression vectors of choice. The vector can also be used in bacteria to produce DsRed2 protein.

Location of features

- *lac* Promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*-DsRed2 fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 964–966
- 5' Multiple Cloning Site: 240–281
- *Discosoma sp.* Red Fluorescent Protein (DsRed2) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 964–966
- 3' Multiple cloning site: 968–1065
- Ampicillin resistance gene
  - Promoter
    - –35 region: 1441–1446; –10 region: 1464–1469
  - Transcription start point: 1476
  - Ribosome binding site: 1499–1503
  - β-lactamase coding sequences
    - Start codon (ATG): 1511–1513; Stop codon: 2369–2371
    - β-lactamase signal peptide: 1511–1579
    - β-lactamase mature protein: 1580–2368
- pUC plasmid replication origin: 2519–3162

Sequencing Primer Location

- DsRed1-C Sequencing Primer (Cat. No. 632388; 5'-AGCTGGACATCACCTCCCACAACG-3'): 881–904

Propagation in *E. Coli*

- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to ampicillin (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.

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The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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