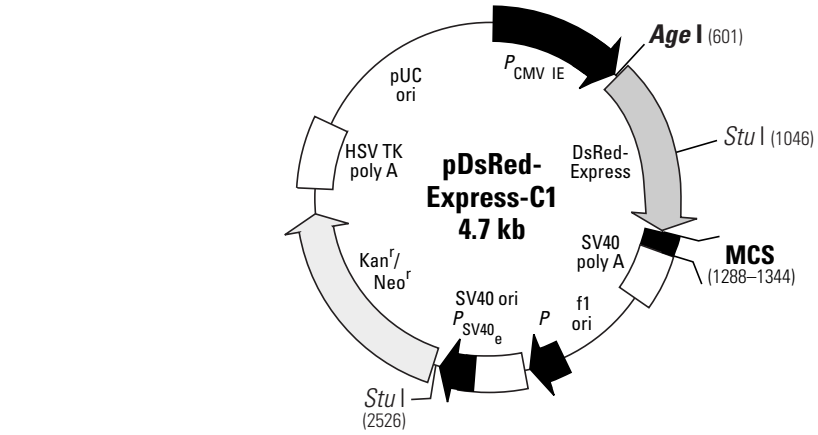
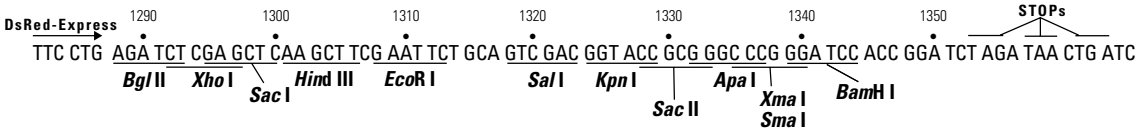
Vector Information

pDsRed-Express-C1 Vector Information PT3726-5

Cat. No. 632430





Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Express-C1. Unique restriction sites are in bold.

Description

pDsRed-Express-C1 is a mammalian expression vector that encodes DsRed-Express, a variant of *Discosoma sp.* red fluorescent protein (DsRed; 1). DsRed-Express contains nine amino acid substitutions (listed on page 2), which improve the solubility of the protein and reduce the time from transfection to detection of red fluorescence (2). In addition, these substitutions reduce the level of residual green emission (2). When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection (DsRed-Express excitation and emission maxima = 557 nm and 579 nm, respectively). Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (2). The DsRed-Express coding sequence is human codon-optimized for high expression in mammalian cells (3).

The multiple cloning site (MCS) in pDsRed-Express-C1 is positioned between the DsRed-Express coding sequence and the SV40 polyadenylation signal (SV40 poly A). Genes cloned into the MCS will be expressed as fusions to the C-terminus of DsRed-Express if they are in the same reading frame as DsRed-Express and there are no intervening stop codons. A Kozak consensus translation initiation site upstream of DsRed-Express increases the translation efficiency in eukaryotic cells (4). SV40 poly A signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts. The vector backbone also 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 (Neor)—consisting 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—allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*.

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

pDsRed-Express-C1 can be used to construct fusions to the C-terminus of DsRed-Express. If a fusion construct retains the fluorescent properties of the native DsRed-Express 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 pDsRed-Express-C1 so that it is in frame with the DsRed-Express coding sequences, with no intervening in-frame stop codons. The recombinant DsRed-Express vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (5). pDsRed-Express-C1 can also be used as a cotransfection marker; the unmodified vector will express DsRed-Express.

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
- *Discosoma sp.* human codon-optimized Red Fluorescent Protein (DsRed-Express) gene
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615; Stop codon: 1357–1359
  - CGC→GCC (Arg-2 to Ala) mutation: 616–618
  - AAG→GAG (Lys-5 to Glu) mutation: 625–627
  - AAC→GAC (Asn-6 to Asp) mutation: 628–630
  - ACC→TCC (Thr-21 to Ser) mutation: 673–675
  - CAC→ACC (His-41 to Thr) mutation: 733–735
  - AAC→CAG (Asn-42 to Gln) mutation: 736–738
  - GTG→GCC (Val-44 to Ala) mutation: 742–744
  - TGC→TCC (Cys-117 to Ser) mutation: 961–963
  - ACC→GCC (Thr-217 to Ala) mutation: 1261–1263
  - Last amino acid in DsRed-Express: 1285–1287
- MCS: 1288–1344
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1498–1503 & 1527–1532; mRNA 3' ends: 1536 & 1648
- f1 single-strand DNA origin: 1595–2050 (Packages the noncoding strand of DsRed-Express.)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2112–2117; –10 region: 2135–2140
  - Transcription start point: 2147
- SV40 origin of replication: 2391–2526
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2224–2295 & 2296–2367
  - 21-bp repeats: 2371–2991, 2392–2412 & 2414–2431
  - Early promoter element: 2447–2453
  - Major transcription start points: 2443, 2481, 2487 & 2492
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2576–2578; stop codon: 3368–3370
  - G→A mutation to remove *Pst* I site: 2758
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3104
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3606–3611 & 3619–3624
- pUC plasmid replication origin: 3955–4598

Sequencing primer locations

- DsRed1-C Sequencing Primer (Cat. No. 632388; 5'-AGCTGGACATCACCTCCCACAACG-3'): 1205–1228

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

Excitation and emission maxima of DsRed-Express

- Excitation maximum = 557 nm
- Emission maximum = 579 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Bevis, B. J. & Glick B. S. (2002) *Nature Biotech.* **20**:83–87.
3. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
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 Laboratories, Inc. This vector has not been completely sequenced.