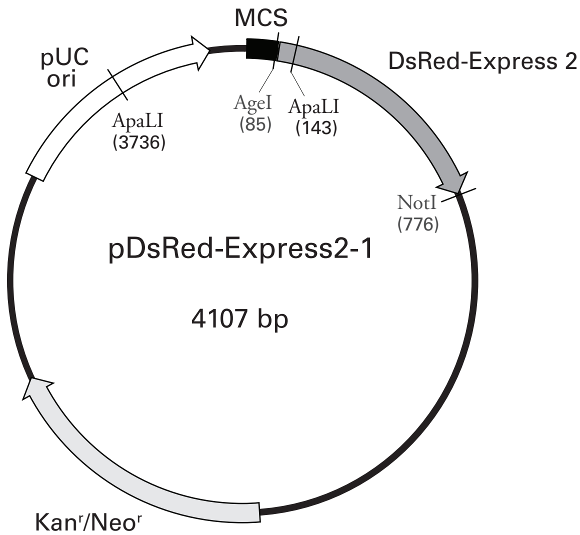
pDsRed-Express2-1 Vector Information

PT4075-5

Catalog No. 632536

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                                BglII      SacI
   Eco47III                           XhoI      HindIII   EcoRI           SalI    KpnI
                                                                          AccI        SacII
11 TAGCGCTACC GGACTCAGAT CTCGAGCTCA AGCTTCGAAT TCTGCAGTCG ACGGTACCGC
   ATCGCGATGG CCTGAGTCTA GAGCTCGAGT TCGAAGCTTA AGACGTCAGC TGCCATGGCG

   Bsp120I  BamHI
     SmaI
 SacI XmaI
   ApaI        AgeI
71 GGGCCCGGGA TCCACCGGTC
   CCCGGGCCCT AGGTGGCCAG
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pDsRed-Express2-1 Vector Map and Multiple Cloning Sites (MCS).

Description

pDsRed-Express2-1 is a promoterless reporter vector that allows the functional analysis of promoters and promoter/enhancer combinations cloned into its multiple cloning site (MCS). The vector encodes DsRed-Express2, a variant of the *Discosoma sp.* red fluorescent protein, DsRed (1). DsRed-Express2 retains the fast maturation and high photostability characteristic of its predecessor, DsRed-Express (2), and has been engineered (through additional amino acid substitutions) for increased solubility (3). Although it most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express2 displays a greatly reduced tendency to aggregate, resulting in reduced cyto- and phototoxicity, and making DsRed-Express2 much better suited for *in vivo* applications involving sensitive cells, such as primary or stem cells. DsRed-Express2 also exhibits extremely low residual green fluorescence, which allows cells expressing the protein to be effectively separated from other fluorescently labeled cell populations by flow cytometry.

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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

The sequence immediately upstream of DsRed-Express2 has been converted to a Kozak consensus sequence (4) to enhance translational efficiency in eukaryotic cells. SV40 polyadenylation signals down-stream of the DsRed-Express2 gene direct proper processing of the 3' end of the DsRed-Express2 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 largeT 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 (5). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

pDsRed-Express2-1 is designed to be used as an *in vivo* reporter of gene expression. Promoters should be cloned into the pDsRed-Express2-1 MCS, located upstream of the DsRed-Express2 coding sequence. Without the addition of a functional promoter, this vector will not express DsRed-Express2.

The pDsRed-Express2-1 vector can be transfected into mammalian cells using any standard transfection method. Mammalian cells expressing DsRed-Express2 (excitation and emission maxima: 554 and 591 nm, respectively) can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection. If required, stable transfectants can be selected using G418.

Location of features

- MCS (multiple cloning site): 12–89
- DsRed-Express2 (*Discosoma sp.* red fluorescent protein variant)
  Kozak consensus translation initiation site: 90–100
  Start codon (ATG): 97–99; Stop codon: 772–774
- SV40 early polyA$^+$ signals
  Polyadenlyation signals: 926–931 & 955–960; mRNA 3' ends: 964 & 976
- f1 origin of replication (for packaging the noncoding strand of DsRed-Express2): 1023–1478
- SV40 origin of replication: 1819–1957
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences: 2003–2797
- pUC origin of replication: 3382–4015

Propagation in *E. coli*

- Recommended host strain: 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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of DsRed-Express2

- Excitation maximum = 554 nm
- Emission maximum = 591 nm

References

1. Matz, M. V. et al. (1999) *Nat. Biotechnol.* **17**(10):969-973.
2. Bevis, B. J. & Glick, B. S. (2002) *Nat. Biotechnol.* **20**(1):83–87. Erratum in *Nat. Biotechnol.* (2002) **20**(11):1159
3. Strack, R. L. *et al.* (2008) *Nat. Methods* **5**(11):955–957.
4. Kozak, M. (1987) Nucleic Acids Res. **15**(20): 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 vector sequence was 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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