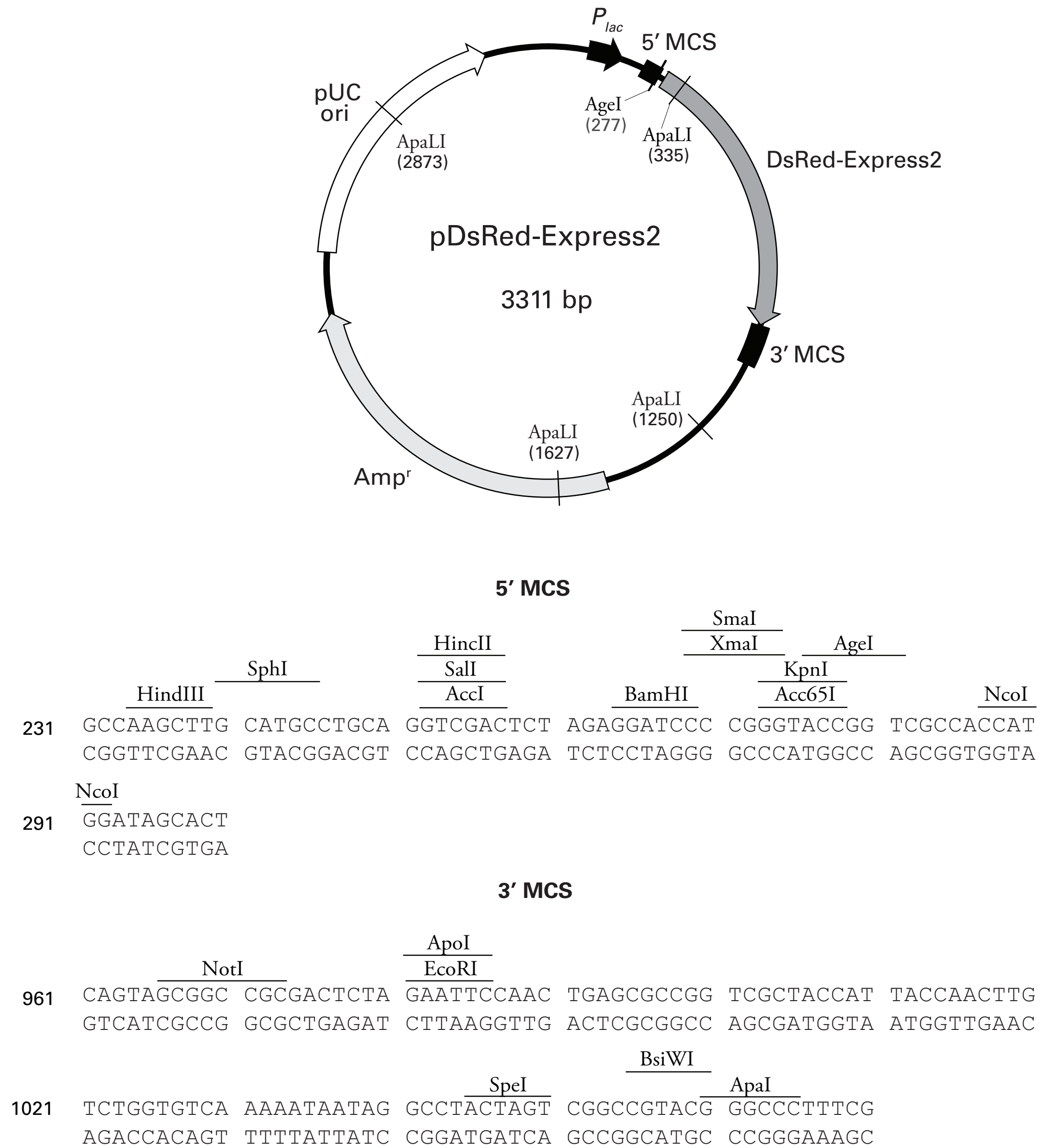
Vector Information

pDsRed-Express2 Vector Information PT4074-5

Catalog No. 632535


P lac
5′ MCS
AgeI (277)
ApaLI (335)
DsRed-Express2
pUC ori
ApaLI (2873)
pDsRed-Express2
3311 bp
3′ MCS
ApaLI (1250)
ApaLI (1627)
Amp r
5′ MCS
HindIII SphI HincII SalI AccI BamHI SmaI XmaI KpnI Acc65I AgeI NcoI
231 GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGG TCGCCACCAT
CGGTTCGAAC GTACGGACGT CCAGCTGAGA TCTCCTAGGG GCCCATGGCC AGCGGTGGTA
NcoI
291 GGATAGCACT
CCTATCGTGA
3′ MCS
NotI ApoI EcoRI
961 CAGTAGCGGC CGCGACTCTA GAATTCCAAC TGAGCGCCGG TCGCTACCAT TACCAACTTG
GTCATCGCCG GCGCTGAGAT CTTAAGGTTG ACTCGCGGCC AGCGATGGTA ATGGTTGAAC
SpeI BsiWI ApaI
1021 TCTGGTGTCA AAAATAATAG GCCTACTAGT CGGCCGTACG GGCCCTTTCG
AGACCACAGT TTTTATTATC CGGATGATCA GCCGGCATGC CCGGGAAAGC

pDsRed-Express2 Vector Map and Multiple Cloning Sites (MCS).

Description

pDsRed-Express2 is a prokaryotic expression vector that 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 and reduced cytotoxicity (3). Although it most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express2 displays a greatly reduced tendency to aggregate; this results in minimal cytotoxicity, which makes 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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In pDsRed-Express2, the DsRed-Express2 coding sequence is flanked by separate and distinct multiple cloning sites (i.e., the 5' MCS and 3' MCS) that make it easy to excise the gene for use in other cloning applications. In *E. coli*, DsRed-Express2 is expressed from the *lac* promoter (P_{lac}) as a fusion with several amino acids, including the first five amino acids of the LacZ protein. Note, however, that if the DsRed-Express2 coding sequence is excised using a restriction site in the 5' MCS, the protein will no longer be expressed as a fusion (as it is when it is expressed from the *lac* promoter). A Kozak consensus sequence is located immediately upstream of the DsRed-Express2 coding sequence to enhance translational efficiency in eukaryotic cells (4).The entire DsRed-Express2 expression cassette in pDsRed-Express2 is supported by a pUC19 backbone, which contains a high-copy number origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Use

pDsRed-Express2 is primarily intended to serve as a source of DsRed-Express2 cDNA. The flanking MCS regions make it possible to excise the DsRed-Express2 coding sequence and insert it into other vector systems. The vector can also be used to express DsRed-Express2 in bacteria.

Cells expressing DsRed-Express2 (excitation and emission maxima: 554 nm and 591 nm, respectively) can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection.

For Western analysis, DsRed-Express2 can be detected with either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or the Living Colors DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393).

Location of features

- P_{lac} (*lac* Promoter): 95–178
  CAP binding site: 111–124
  –35 region: 143–148; –10 region: 167–172
  lac operator: 179–199
- *lacZ*-DsRed-Express2 fusion expressed in *E. coli*
  Ribosome binding site: 206–209
  Start codon (ATG): 217–219; Stop codon 961–963
- 5' MCS (5' multiple cloning site): 234–292
- DsRed-Express2 (*Discosoma sp.* red fluorescent protein variant)
  Kozak consensus translation initiation site: 282–292
  Start codon (ATG): 289–291; Stop codon: 964–966
- 3' MCS (3' multiple cloning site): 966–1065
- Amp^r (Ampicillin resistance gene): 1511–2371
- pUC origin of replication: 2519–3161

Propagation in *E. coli*

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

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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