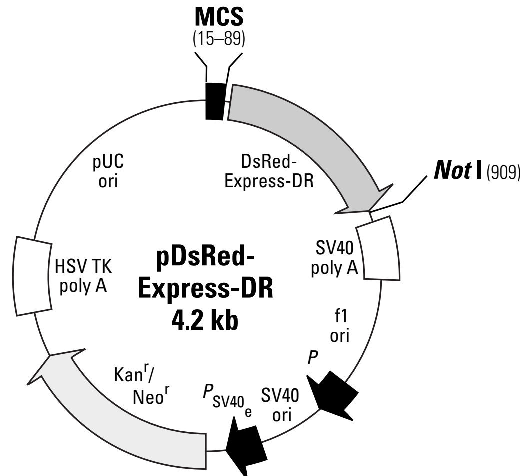
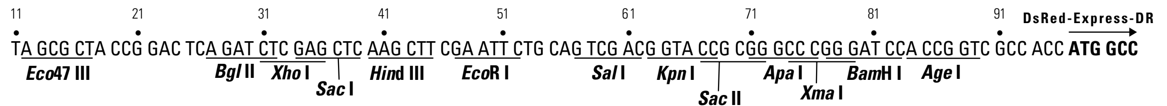
# pDsRed-Express-DR Vector Information

PT3700-5

Cat. No. 632423




**Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Express-DR.** All sites shown are unique. The *Not* I site follows the DsRed-Express-DR stop codon.

## Description

pDsRed-Express-DR is a promoterless vector that encodes DsRed-Express-DR, a destabilized variant of *Discosoma sp.* red fluorescent protein (DsRed). In contrast to the original protein, which is extremely stable, DsRed-Express-DR has a short half-life, making it well suited for studies that require rapid reporter turnover. Though its geneology can be traced to wild-type DsRed (1), DsRed-Express-DR is directly derived from DsRed-Express, a DsRed variant engineered for rapid fluorescence development (2, 3). DsRed-Express contains nine amino acid substitutions (listed on page 2), which improve the protein's solubility and decrease the time from transfection to detection of red fluorescence (2). The substitutions also reduce the level of residual green emission (2). The destabilized variant, DsRed-Express-DR, was constructed by fusing the C-terminus of the protein to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (4). This region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (4, 5). The DsRed-Express-DR gene is human codon-optimized for high expression in mammalian cells (6), and sequences upstream of the gene have been converted to a Kozak consensus translation initiation site (7) to enhance translation efficiency in eukaryotic cells.

pDsRed-Express-DR can be used to monitor transcription from different promoters and promoter/ enhancer combinations inserted into the multiple cloning site (MCS), located upstream of the DsRed-Express-DR coding sequence. SV40 polyadenylation signals downstream of the DsRed-Express-DR gene direct proper processing of the 3' end of the DsRed-Express-DR mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large 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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## Use

DsRed-Express-DR can be used as an *in vivo* reporter of gene expression. Because of its rapid turnover rate, its expression from a promoter of interest provides a more accurate assessment of the promoter's activity over time than does the more stable DsRed-Express. Promoter/enhancer elements should be inserted into the MCS upstream of the DsRed-Express-DR coding sequence. **Without the addition of a functional promoter, this vector will not express DsRed-Express-DR**. The recombinant pDsRed-Express-DR vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (8).

## Location of features

- MCS: 12–89
- Destabilized *Discosoma sp.* Red Fluorescent Protein (DsRed-Express-DR) gene
  - Kozak consensus translation initiation site: 90–100
  - Start codon (ATG): 97–99; Stop codon: 904–906
  - CGC→GCC (Arg-2 to Ala) mutation: 100–102
  - AAG→GAG (Lys-5 to Glu) mutation: 109–111
  - AAC→GAC (Asn-6 to Asp) mutation: 112–114
  - ACC→TCC (Thr-21 to Ser) mutation: 157–159
  - CAC→ACC (His-41 to Thr) mutation: 217–219
  - AAC→CAG (Asn-42 to Gln) mutation: 220–222
  - GTG→GCC (Val-44 to Ala) mutation: 226–228
  - TGC→TCC (Cys-117 to Ser) mutation: 445–447
  - ACC→GCC (Thr-217 to Ala) mutation: 745–747
  - Mouse ornithine decarboxylase PEST sequence: 784–906
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1059–1064 & 1088–1093
  - mRNA 3' ends: 1097 & 1109
- f1 single-strand DNA origin: 1156–1611
  - (Packages noncoding strand of DsRed-Express-DR.)
- Ampicillin resistance (β-lactamase) promoter
  - –35 region: 1673–1678; –10 region: 1696–1701
  - Transcription start point: 1708
- SV40 origin of replication: 1952–2087
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 1783–1856 & 1857–1928
  - 21-bp repeats: 1932–1952, 1953–1973 & 1975–1995
  - Early promoter element: 2008–2014
  - Major transcription start points: 2004, 2042, 2048 & 2053
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2136–2138; stop codon: 2928–2930
  - G→A mutation to remove *Pst* I site: 2318
  - C→A (Arg→Ser) mutation to remove *Bss*H II site: 2664
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3166–3171 & 3179–3184
- pUC plasmid replication origin: 3515–4158

## 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

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4. Li, X., *et al.* (1998)*J. Biol. Chem.* **273**:34970–34975.
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6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. 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.

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