



**pDsRed-Express2-N1 Vector Map and Multiple Cloning Sites (MCS).**

**Description**

pDsRed-Express2-N1 is a mammalian expression vector designed to express a protein of interest fused to the N-terminus of 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. (**Please note:** Because DsRed-Express2 likely forms tetramers, its suitability as a fusion partner will largely depend on how its tetramerization affects the function of the protein to which it is fused.) 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.

The multiple cloning site (MCS) in pDsRed-Express2-N1 is positioned upstream of the DsRed-Express2 coding sequence. A Kozak consensus sequence (4), located between the MCS and the DsRed-Express2 coding sequence, enhances the translational efficiency of the unfused DsRed-Express2 protein in eukaryotic cells. SV40 polyadenylation signals downstream of the DsRed-Express2 coding sequence direct proper processing of the 3' ends of the DsRed-Express2 and fusion gene mRNA transcripts.

(PR8Z2651; published 2 December 2008)



**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 vector backbone also 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. This vector also has a neomycin-resistance cassette (Neo<sup>r</sup>) that allows G418 selection of stably transfected eukaryotic cells (5). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSVTK) polyadenylation signals. A bacterial promoter upstream of this cassette allows kanamycin resistance in *E. coli*.

### Use

To construct a fusion protein, the gene of interest must be cloned into pDsRed-Express2-N1 so that it is in-frame with the DsRed-Express2 coding sequence; the gene must include an initiation codon (ATG), and lack in-frame stop codons. pDsRed-Express2-N1 can also be used as a cotransfection marker, as the unmodified vector will express DsRed-Express2 in mammalian cells.

pDsRed-Express2-N1 can be transfected into mammalian cells using any standard transfection method. Fusions that retain the fluorescence properties of the native DsRed-Express2 protein (excitation and emission maxima: 541 and 591, respectively) can be monitored by flow cytometry and localized by fluorescence microscopy. If required, stable transfectants can be selected using G418.

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

### Location of features

- $P_{CMVIE}$  (human cytomegalovirus immediate early promoter): 1–589
- MCS (multiple cloning site): 591–671
- DsRed-Express2 (*Discosoma sp.* red fluorescent protein variant)
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1354–1356
- SV40 early polyA<sup>+</sup> signals: 1508–1513 & 1537–1542; mRNA 3' ends: 1546 & 1558
- f1 origin of replication: 1605–2060 (complementary)
- SV40 origin of replication: 2401–2539
- Kan<sup>r</sup>/Neo<sup>r</sup> (kanamycin/neomycin resistance gene)
  - Neomycin phosphotransferase coding sequences:
    - Start codon (ATG): 2585–2587; stop codon: 3377–3379
- pUC origin of replication: 3964–4607

### Propagation in *E. coli*

- Recommended host strain: DH5 $\alpha$ , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to kanamycin (50  $\mu$ g/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.

**Notice to Purchaser**

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

CMV Sequence:

DsRed-Express & DsRed-Express2:

Living Colors® Products AcGFP1, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow and their variants:

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com) or click here for more information.

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2008 Clontech Laboratories, Inc..