



```

          PacR7I
          XhoI
    NheI   Eco47III   BglII   SacI   EcoRI   SalI   SacII
    591  GCTAGCGCTA  CCGGACTCAG  ATCTCGAGCT  CAAGCTTCGA  ATTCTGCAGT  CGACGGTACC
        CGATCGCGAT  GGCCTGAGTC  TAGAGCTCGA  GTTCGAAGCT  TAAGACGTCA  GCTGCCATGG

          SmaI
    SacII   BamHI
    651  GCGGGCCCGG  GATCCGCCCC
        CGCCGGGCC  CTAGGCGGGG
  
```

pIRES2 DsRed-Express2 Vector Map and Multiple Cloning Site (MCS).

Description

pIRES2 DsRed-Express2 is a bicistronic expression vector that allows the simultaneous expression of your protein of interest and DsRed-Express2 from the same mRNA transcript. The vector is designed to allow efficient flow cytometric detection of transiently transfected mammalian cells expressing either DsRed-Express2 and a protein of interest, or DsRed-Express2 alone (at lower signal intensity).

DsRed-Express2 is a variant of the *Discosoma sp.* red fluorescent protein, DsRed (1). It 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.

(PR9Y3392; published 14 November 2009)



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

Bicistronic expression of the protein of interest and DsRed-Express2 is facilitated by the encephalomyocarditis virus (EMCV) internal ribosome entry site 2 (IRES2), located between the multiple cloning site (MCS) and the DsRed-Express2 coding region. This IRES allows cap-independent translation of DsRed-Express2 from an internal start site at the IRES/DsRed-Express2 junction (4, 5). Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter (P_{CMVIE}) located just upstream of the MCS.

SV40 polyadenylation signals downstream of the DsRed-Express2 coding sequence direct proper processing of the 3' ends of the bicistronic mRNA. 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^r) that allows G418 selection of stably transfected eukaryotic cells (6). 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 this cassette allows kanamycin resistance in *E. coli*.

Use

When cloning into the pIRES2 DsRed-Express2 MCS, your gene of interest must contain an initiation codon (ATG) and a stop codon. Cells expressing the gene of interest can be quickly identified by screening for DsRed-Express2 fluorescence.

pIRES2 DsRed-Express2 and its derivatives can be introduced into mammalian cells using any standard transfection method. Cells expressing DsRed-Express2 (excitation and emission maxima: 554 and 591, respectively) can be detected by flow cytometry or microscopy 24 hr after transfection. However, in some cases, up to 48 hr may be required for detection. If required, stable transfectants can be selected using G418. Please refer to the Living Colors[®] User Manual provided with this vector for additional information on detection of DsRed-Express2.

Location of features

- P_{CMVIE} (human cytomegalovirus immediate early promoter): 1–589
- MCS (multiple cloning site): 591–665
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 666–1250
- DsRed-Express2 (*Discosoma sp.* red fluorescent protein variant)
Start codon (ATG): 1254–1256; Stop codon: 1929–1931
- SV40 early polyA⁺ signals: 2083–2088 & 2112–2117; mRNA 3' ends: 2121 & 2133
- f1 origin of replication: 2181–2636 (complementary)
- SV40 origin of replication: 2977–3112
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
Neomycin phosphotransferase coding sequences: 3161–3955
- pUC origin of replication: 4540–5183

Propagation in *E. Coli*

- Recommended host strain: DH5 α , 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 μ 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. Jackson, R.J. *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
5. Jang, S.K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
6. 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.

Reef Coral Fluorescent Proteins:

The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 and 7,442,522.

Living Colors® Fluorescent Protein Products:

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.

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.

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2009 Clontech Laboratories, Inc.