

							Saci									
	XhoI											Ac	ccI	KpnI		
	End DsRed-Express2		BglII				HindIII		EcoRI			SalI		Acc65I		
1279	CTG	TTC	CAG	AGA	TCT	CGA	GCT	CAA	GCT	TCG	AAT	TCT	GCA	GTC	GAC	GGT

				SmaI			
		SacII		XmaI			
	KpnI		Bsp120	)I			
	Acc65I		ApaI		BamHI		
1327	ACC	GCG	GGC	CCG	GGA	TCC	

pDsRed-Express2-C1 Vector Map and Multiple Cloning Sites (MCS).

# Description

pDsRed-Express2-C1 is a mammalian expression vector designed to express a protein of interest fused to the C-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-C1 is positioned downstream of the DsRed-Express2 coding sequence. A Kozak consensus sequence (4), located immediately upstream of DsRed-Express2, enhances translational efficiency in eukaryotic cells. SV40 polyA signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts.

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Clontech Laboratories, Inc. ATakara Bio Company 1290Terra Bella Ave. Mountain View, CA 94043 Technical Support (US) E-mail: tech@clontech.com www.clontech.com pDsRed-Express2-C1 **Vector Information** 

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 (Neor) 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-C1 so that it is inframe with the DsRed-Express2 coding sequence; it should also contain a proper stop codon at the 3' end of its coding region. pDsRed-Express2-C1 can also be used as a cotransfection marker, as the unmodified vector will express DsRed-Express2 in mammalian cells.

pDsRed-Express2-C1 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® DsRed Polyclonal Antibody (Cat. No. 632496) or the Living Colors DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393).

### **Location of features**

- P<sub>CMV IF</sub> (human cytomegalovirus immediate early promoter): 1–589
- DsRed-Express2 (Discosoma sp. red fluorescent protein variant)

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615; Last amino acid: 1285-1287

- MCS (multiple cloning site): 1288–1344
- SV40 early polyA+ signals: 1499-1504 & 1528-1533; mRNA 3' ends: 1537 & 1549
- f1 origin of replication: 1596–2051 (complementary)
- SV40 origin of replication: 2392–2530
- Kan<sup>r</sup>/Neo<sup>r</sup> (kanamycin/neomycin resistance gene)

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2576-2578; stop codon: 3368-3370

• pUC origin of replication: 3955-4598

#### Propagation in *E. coli*

- Recommended host strain: DH5α, HB101, and other general purpose strains.
- 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

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- 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.

Protocol No. PT4077-5 www.clontech.com Clontech Laboratories, Inc. Version No. PR8Z2652 pDsRed-Express2-C1 Vector Information

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CMV Sequence:

DsRed-Express & DsRed-Express2:

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

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