

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 (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 in-frame 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_{CMVIE} (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^r/Neo^r (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: DH5 α , HB101, and other general purpose strains.
- 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. 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.

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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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