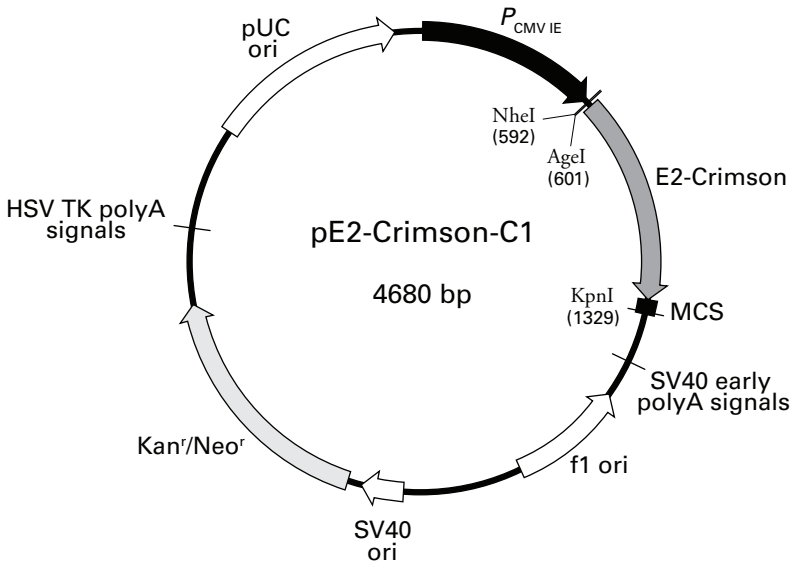
Vector Information

pE2-Crimson-C1 Vector Information PT5071-5

Catalog No. 632555

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                                                                  XhoI
          End of E2-Crimson      BglII          SacI       HindIII        EcoRI
1279      CTG TTC CAG AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA
          GAC AAG GTC TCT AGA GCT CGA GTT CGA AGC TTA AGA CGT

                                                        XmaI
                        KpnI          Bsp120I
          SalI                  SacII         SmaI
          AccI          Acc65I          ApaI          BamHI
1318      GTC GAC GGT ACC GCG GGC CCG GGA TCC
          CAG CTG CCA TGG CGC CCG GGC CCT AGG
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pE2-Crimson-C1 Vector Map and Multiple Cloning Sites (MCS).

Description

pE2-Crimson-C1 is a mammalian expression vector designed to express a protein of interest fused to the C-terminus of E2-Crimson, a far-red fluorescent protein derived from the tetrameric red fluorescent protein DsRed-Express2 (1, 2). E2-Crimson retains the reduced cyto- and phototoxicity, increased solubility, fast maturation, and high photostability characteristic of DsRed-Express2. Unlike other far-red fluorescent proteins, E2-Crimson is not cytotoxic in bacterial and mammalian cells, making it well-suited for *in vivo* applications involving sensitive cells, such as primary or stem cells. E2-Crimson has an emission maximum at 646 nm, and absorbance and excitation maxima at 611 nm, giving it the furthest red-shifted excitation spectrum of any available fluorescent protein (1). The protein can be efficiently excited with a standard 633 nm laser, which is useful in multi-color labeling experiments with orange and green fluorescent proteins.

The multiple cloning site (MCS) in pE2-Crimson-C1 is positioned downstream of the E2-Crimson coding sequence. A Kozak consensus sequence (3), located immediately upstream of E2-Crimson, enhances translational efficiency in eukaryotic cells. SV40 polyA signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts.

(PR993340; published 21 September 2009)

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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 (4). 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

To construct a fusion protein, the gene of interest must be cloned into pE2-Crimson-C1 so that it is in-frame with the E2-Crimson coding sequence; it should also contain a proper stop codon at the 3' end of its coding region. pE2-Crimson-C1 can also be used as a cotransfection marker, as the unmodified vector will express E2-Crimson in mammalian cells.

pE2-Crimson-C1 can be transfected into mammalian cells using any standard transfection method. Fusions that retain the fluorescence properties of the native E2-Crimson protein (excitation and emission maxima: 611 and 646, respectively) can be monitored by flow cytometry and localized by fluorescence microscopy. E2-Crimson matures faster than any previously described far-red fluorescent protein (the half-time for fluorophore maturation is 26 minutes at 37°C; 1). Cells expressing E2-Crimson fusions that retain the native protein's fluorescence properties can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection. If required, stable transfectants can be selected using G418.

For western analysis, E2-Crimson 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

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- E2-Crimson (*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
- HSV TK polyA signals: 3606–3611 & 3619–3624
- 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 E2-Crimson

- Excitation maximum = 611 nm
- Emission maximum = 646 nm

References

1. Strack, R. L. *et al.* (2009) *Biochemistry* **48**(35):8279–8281.
2. Bevis, B. J. & Glick, B. S. (2002) *Nat. Biotechnol.* **20**(1):83–87. Erratum in *Nat. Biotechnol.* (2002) **20**(11):1159
3. Kozak, M. (1987) Nucleic Acids Res. **15**(20): 8125-8148
4. 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:

E2-Crimson:

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