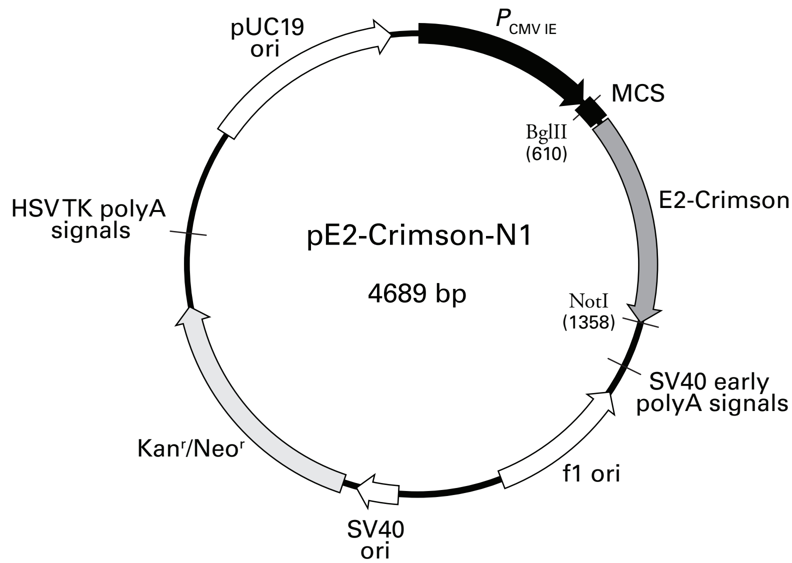
pE2-Crimson-N1 Vector Information PT5070-5

Catalog No. 632554

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                                                        SacI
             NheI                             XhoI
                  AfeI                BglII                HindIII     EcoRI
589   CCG CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT
      GGC GAT CGC GAT GGC CTG AGT CTA GAG CTC GAG TTC GAA GCT TAA

                        Acc65I/KpnI
                 SalI               SacII      SmaI/XmaI                Kozak Sequence
      EcoRI      AccI                    ApaI/Bsp120I  BamHI     SpeI
634   CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CTA GTC GCC ACC
      GAC GTC AGC TGC CAT GGC GCC CGG GCC CTA GGT GAT CAG CGG TGG

      Kozak Sequence
      Start of E2-Crimson
679   ATG GAT AGC
      TAC CTA TCG
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pE2-Crimson-N1 Vector Map and Multiple Cloning Sites (MCS).

Description

pE2-Crimson-N1 is a mammalian expression vector designed to express a protein of interest fused to the N-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.

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The multiple cloning site (MCS) in pE2-Crimson-N1 is positioned upstream of the E2-Crimson coding sequence. A Kozak consensus sequence (3), located between the MCS and the E2-Crimson coding sequence, enhances the translational efficiency of the unfused E2-Crimson protein in eukaryotic cells. SV40 polyadenylation signals downstream of the E2-Crimson coding sequence direct proper processing of the 3' ends of the E2-Crimson and fusion gene mRNA transcripts.

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 (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 pE2-Crimson-N1 so that it is in-frame with the E2-Crimson coding sequence; the gene must include an initiation codon (ATG), and lack in-frame stop codons. pE2-Crimson-N1 can also be used as a cotransfection marker, as the unmodified vector will express E2-Crimson in mammalian cells.

pE2-Crimson-N1 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
- MCS (multiple cloning site): 591–671
- E2-Crimson (*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 signals: 1508–1513 & 1537–1542; mRNA 3' ends: 1546 & 1558
- f1 origin of replication: 1605–2060 (complementary)
- SV40 origin of replication: 2401–2539
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2585–2587; stop codon: 3377–3379
- HSVTK polyA signals: 3615–3620 and 3628–3633
- pUC origin of replication: 3964–4607

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.

CMV Sequence:

E2-Crimson: