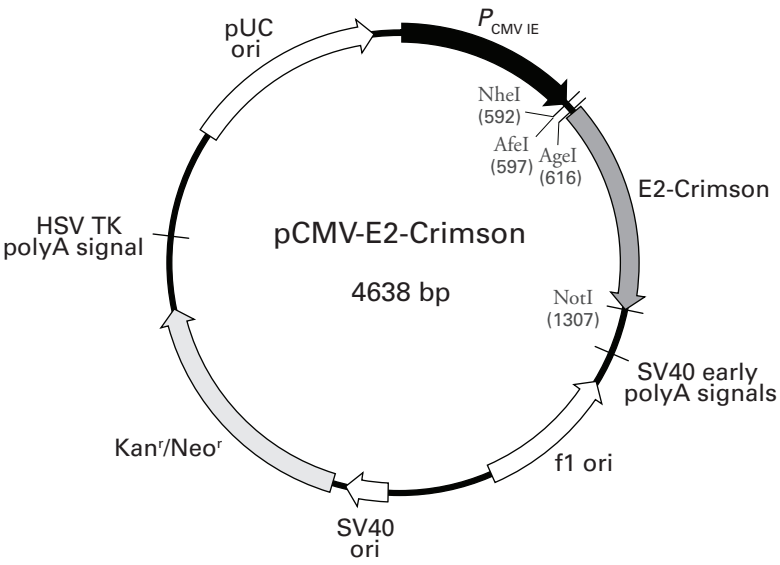
Vector Information

pCMV-E2-Crimson Vector Information PT5072-5

Catalog No. 632556



pCMV-E2-Crimson Vector Map.

Description

pCMV-E2-Crimson is a mammalian expression vector designed to be used for whole cell labeling or as a marker of cotransfection. In mammalian cells, the vector constitutively expresses 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 E2-Crimson gene is positioned just downstream of the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). As a result, mammalian cells transfected with this vector will constitutively express the red fluorescent protein. A Kozak consensus sequence (3) has been placed immediately upstream of the E2-Crimson coding sequence to enhance translational efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the E2-Crimson gene direct proper processing of the 3' end of the E2-Crimson mRNA. The vector backbone 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. A neomycin resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (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 the cassette expresses kanamycin resistance in *E. coli*.

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Use

pCMV-E2-Crimson can be used for whole cell labeling or as a cotransfection marker. The vector can be transfected into mammalian cells using any standard transfection method. 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 (excitation and emission maxima: 611 nm and 646 nm, respectively) can be detected by fluorescence microscopy or flow cytometry 8–12 hours after transfection. If required, stable transfectants can be selected using G418. After cotransfection with pCMV-E2-Crimson and an expression construct of interest, cells can also be sorted by flow cytometry to enrich for transfected cells.

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: 621–631
  Start codon (ATG): 628–630; Stop codon: 1303–1305
- SV40 early polyA signals: 1457–1462 & 1486–1491
- f1 origin of replication: 1554–2009 (complementary)
- SV40 origin of replication: 2350–2488
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences: 2534–3328
- HSV TK polyA signal: 3564–3582
- pUC origin of replication: 3913–4556

Propagation in *E. coli*

- Recommended host strain: DH5α, 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 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.