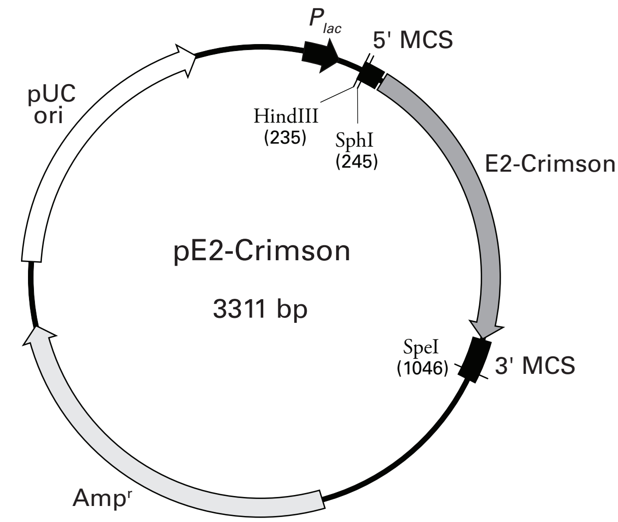
Vector Information



5' MCS

```
                                        SalI                           Acc65I/KpnI
                  SphI                  AccI                     SmaI/XmaI
      HindIII                           HincII          BamHI                 AgeI
232   CCA AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG GTC GCC
      GGT TCG AAC GTA CGG ACG TCC AGC TGA GAT CTC CTA GGG GCC CAT GGC CAG CGG

          Start of E2-Crimson
286   ACC ATG GAT AGC
      TGG TAC CTA TCG
```

3' MCS

```
                    NotI
      End of E2-Crimson              ApoI/EcoRI
958   TTC CAG TAG CGG CCG CGA CTC TAG AAT TCC AAC TGA GCG CCG GTC GCT ACC ATT
      AAG GTC ATC GCC GGC GCT GAG ATC TTA AGG TTG ACT CGC GGC CAG CGA TGG TAA

                                                               BsiWI
                                                 SpeI                    ApaI
1012  ACC AAC TTG TCT GGT GTC AAA AAT AAT AGG CCT ACT AGT CGG CCG TAC GGG CCC
      TGG TTG AAC AGA CCA CAG TTT TTA TTA TCC GGA TGA TCA GCC GGC ATG CCC GGG
```

pE2-Crimson Vector Map and Multiple Cloning Sites (MCS).

Description

pE2-Crimson is a prokaryotic expression vector that encodes E2-Crimson, a far-red fluorescent protein derived from the tetrameric red fluorescent protein DsRed-Express2 (1). E2-Crimson retains the fast maturation, high photostability, increased solubility, and reduced cytotoxicity characteristic of DsRed-Express2 (2). Like DsRed-Express2, E2-Crimson displays a greatly reduced tendency to aggregate. This results in minimal cytotoxicity in bacterial and mammalian cells, making E2-Crimson 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). E2-Crimson can be efficiently excited with a standard 633 nm laser, which is useful in multi-color labeling experiments with orange and green fluorescent proteins.

(PR993338; published 18 September 2009)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

In pE2-Crimson, the E2-Crimson coding sequence is flanked by separate and distinct multiple cloning sites (i.e., the 5' MCS and 3' MCS) that make it easy to excise the gene for use in other cloning applications. In *E. coli*, E2-Crimson is expressed from the *lac* promoter (P_{lac}) as a fusion with several amino acids, including the first five amino acids of the LacZ protein. Note, however, that if the E2-Crimson coding sequence is excised using a restriction site in the 5' MCS, the protein will no longer be expressed as a fusion (as it is when it is expressed from the *lac* promoter). The entire E2-Crimson expression cassette in pE2-Crimson is supported by a pUC19 backbone, which contains a high-copy number origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Use

pE2-Crimson is primarily intended to serve as a source of E2-Crimson cDNA. The flanking MCS regions make it possible to excise the E2-Crimson coding sequence and insert it into other vector systems. The vector can also be used to express E2-Crimson in bacteria.

With a half-time for chromophore maturation of 26 minutes at 37°C, E2-Crimson matures faster than any previously described far-red fluorescent protein (1). Cells expressing E2-Crimson (excitation and emission maxima: 611 nm and 646 nm, respectively) can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection.

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_{lac} (*lac* Promoter): 95–178
- *lacZ*-E2-Crimson fusion expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 964–966
- 5' MCS (5' multiple cloning site): 234–281
- E2-Crimson (*Discosoma sp.* red fluorescent protein variant)
  - Start codon (ATG): 289–291; Stop codon: 964–966
- 3' MCS (3' multiple cloning site): 966–1065
- Amp^r (Ampicillin resistance gene; β-lactamase): 1511–2371
- pUC origin of replication: 2519–3161

Propagation in *E. coli*

- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to ampicillin (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

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.

Notice to Purchaser
Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

E2-Crimson:

Living Colors® Fluorescent Protein Products:
Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com or click here for more information.