

	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	

SacI

	Accost Rom														
			SalI				cII SmaI/Xn			aI			Kozak Sequence		
	EcoRI AccI						Apa	I/Bsp12	/Bsp120I		BamHI				
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

679 Kozak Sequence
Start of E2-Crimson
ATG GAT AGC
TAC CTA TCG

pE2-Crimson-N1 Vector Map and Multiple Cloning Sites (MCS).

Acc65I/KnnI



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# 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.

pE2-Crimson-N1 **Vector Information** 

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 (Neor) 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<sub>CMV IF</sub> (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<sup>r</sup>/Neo<sup>r</sup> (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

Protocol No. PT5070-5 Clontech Laboratories, Inc. www.clontech.com Version No. PR993339 pE2-Crimson-N1 Vector Information

### 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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