

pTimer Vector

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Catalog No.

632402

Amount

20 µg

Lot Number

Specified on product label.

Product Information

pTimer is a prokaryotic expression vector that encodes DsRed1-E5, a fluorescent protein that changes color as it ages. Shortly after translation, DsRed1-E5 emits green light; its red fluorophore emerges later, hours after translation. Because of its predictable color shift, DsRed1-E5 acts as a timer, useful for monitoring changes in gene activity in living cells.

DsRed1-E5 is a mutant of the red fluorescent protein, DsRed1 (1). The cDNA for the wild-type protein, DsRed, was originally isolated by Matz *et al.*, who refer to the protein as drFP583 (2). DsRed1-E5 contains two amino acid substitutions (V105A and S197T), which increase its fluorescence intensity and endow it with a distinct spectral property: As the protein ages, it changes color (3). When first synthesized, DsRed1-E5 is bright green (excitation & emission maxima = 483 nm & 500 nm). As time passes, the green fluorophore undergoes additional changes that cause its fluorescence to shift to longer wavelengths—when fully matured, the protein is bright red (excitation & emission maxima = 558 nm & 583 nm). In mammalian cells transfected with a Tet-inducible DsRed1-E5 expression vector, the green-to-red transition starts about 3 hours after the protein first becomes fluorescent (3). In addition to the amino acid replacements, DsRed1-E5's coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for high expression in mammalian cells (4).

pTimer is primarily intended to serve as a convenient source of the DsRed1-E5 cDNA. The DsRed1-E5 coding sequence is flanked by separate and distinct multiple cloning sites at the 5' and 3' ends so that the gene can easily be excised for use in other expression systems. Alternatively, the DsRed1-E5 coding sequence can be amplified by PCR.

The DsRed1-E5 gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that DsRed1-E5 is expressed from the *lac* promoter (P_{lac}) in *E. coli* host cells. A Kozak consensus sequence is located immediately upstream of DsRed1-E5 to enhance translational efficiency should you wish to express the gene in eukaryotic systems (5). The entire DsRed1-E5 expression cassette in pTimer is supported by a pUC backbone, which contains a high-copy number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

When expressed in multicellular organisms, DsRed1-E5's invariable green-to-red shift can be used as a timer to track the on-off phases of gene expression during embryogenesis and cell differentiation (3).

Package Contents

- 20 µg pTimer Vector

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pTimer Vector

Storage Conditions

- Store at -20°C .
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/ μl

Shipping Conditions

- Dry ice (-70°C)

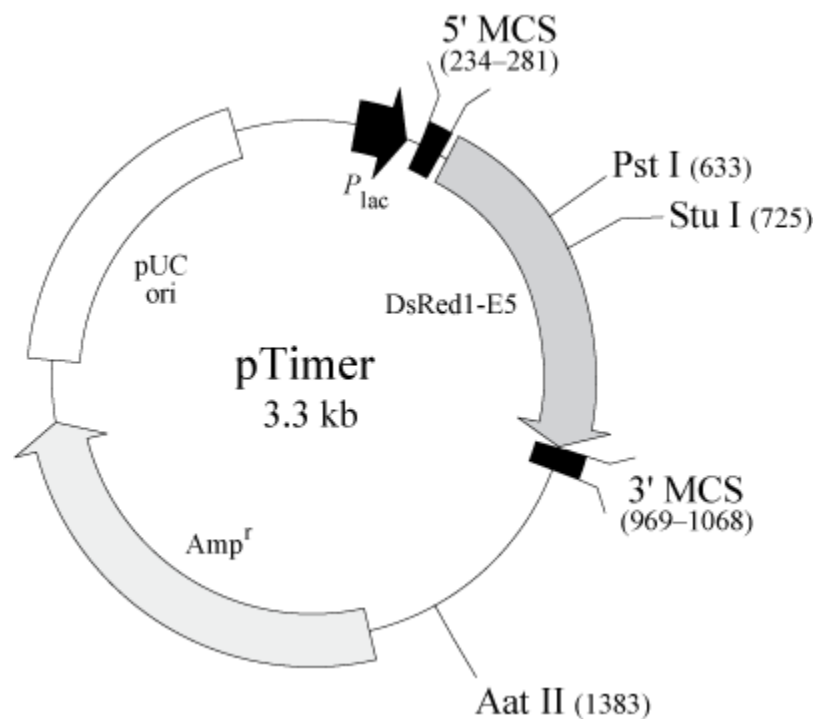


Figure 1. pTimer vector map.

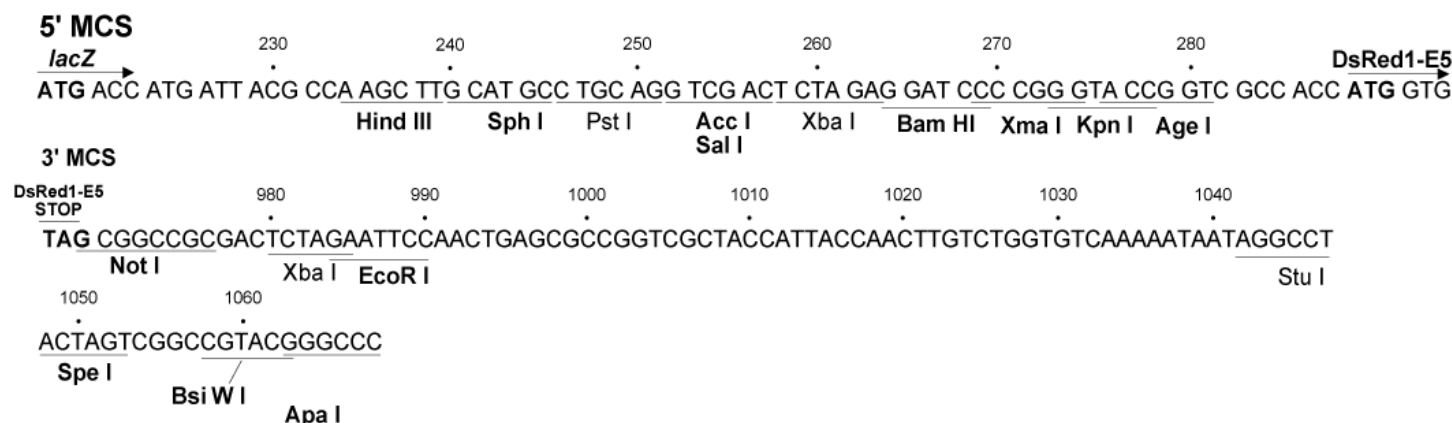


Figure 2. pTimer multiple cloning sites. Unique restriction sites are in bold. Notice that XbaI sites flank the DsRed1-E5 coding sequence and can therefore be used to excise the entire DsRed1-E5 coding sequence. No other XbaI sites exist in the vector sequence.

Location of Features

- P_{lac} (*lac* promoter): 95–178
- *lacZ*-DsRed1-E5 fusion protein expressed in *E. coli*: 217–969
- 5' MCS (5' multiple cloning site): 234–281
- Kozak consensus sequence: 282–292
- DsRed1-E5 gene: 289–969
- 3' MCS (3' multiple cloning site): 969–1068
- Amp^r (Ampicillin resistance gene; β -lactamase): 1514–2374
- pUC plasmid replication origin: 2522–3165

Additional Information

Recommended Primer Locations:

- 472–492 and 884–907 (DsRed1-C Sequencing Primer, Cat No. 632388, can be used at 884–907)

Propagation in *E. coli*

- Recommended host strain: DH5 α
- Selectable marker: plasmid confers resistance to ampicillin (50 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Living Colors® Red Fluorescent Protein (October 1999) *Clontechniques* **XIV**(4):2–6.
2. Matz, M. V., *et al.* (1999) *Nat. Biotechnol.* **17**:969–973.
3. Terskikh, A., *et al.* (2000) *Science* **290**:1585–1588.
4. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.

5. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzyme	Fragment(s) (kb)
StuI	3.0 & 0.3
AatII	3.3

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280} : 1.8–2.0

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LICENSING STATEMENTS:

The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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