

## pTimer-1 Vector

### Table of Contents

Product Information .....	1
Location of Features .....	3
Additional Information .....	3
Quality Control Data.....	3

### Catalog No.

632403

### Amount

20 µg

### Lot Number

Specified on product label.

### Product Information

pTimer-1 is a promoterless vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the MCS located upstream of the DsRed1-E5 coding sequence. 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. pTimer-1 can be used to study the on-off activity of any *cis*-acting regulatory element cloned into the MCS upstream of DsRed1-E5.

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).

To further increase translation efficiency in eukaryotic cells, a sequence upstream of DsRed1-E5 has been converted to a Kozak consensus sequence (5). SV40 polyadenylation signals downstream of the DsRed1-E5 gene direct proper processing of the 3' end of the DsRed1-E5 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 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<sup>r</sup>) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Without addition of a functional promoter, this vector will not express DsRed1-E5.

### Package Contents

- 20 µg pTimer-1 Vector

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pTimer-1 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)

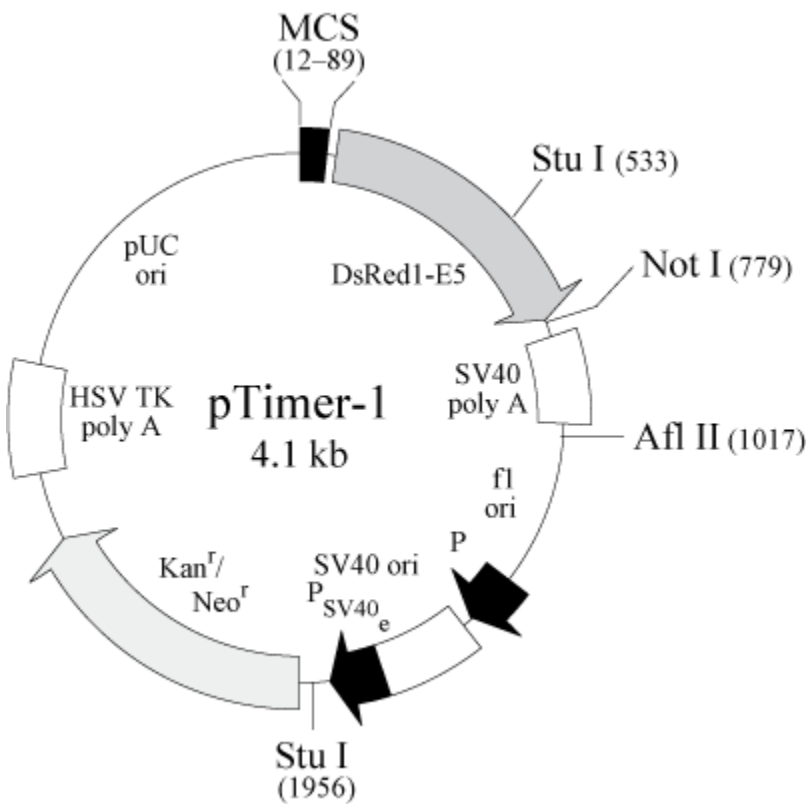


Figure 1. pTimer-1 vector map.

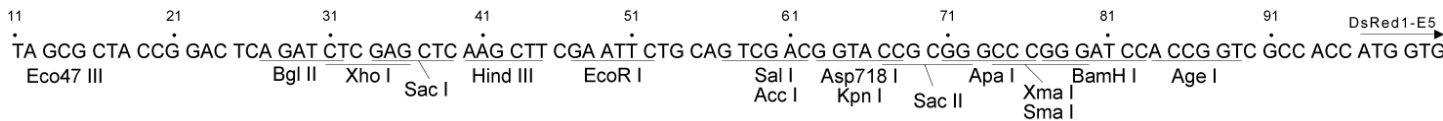


Figure 2. pTimer-1 multiple cloning site.

## Location of Features

- MCS (multiple cloning site): 12–89
- Kozak consensus sequence: 90–100
- DsRed1-E5 gene: 97–777
- SV40 early polyA signals: 929–934 & 958–963
- f1 origin of replication: 1026–1481
- *P* (promoter for Kan<sup>r</sup>): 1543–1571
- SV40 origin of replication: 1822–1957
- *P*<sub>SV40e</sub> (SV40 early promoter and enhancer): 1653–1884
- Kan<sup>r</sup>/Neo<sup>r</sup> (kanamycin/neomycin resistance gene): 2006–2800
- HSV TK (Herpes simplex virus thymidine kinase polyadenylation signals): 3036–3041 & 3049–3054
- pUC origin of replication: 3385–4028

## Additional Information

### Recommended Primer Locations:

- 300–280 and 692–715 (DsRed1-C Sequencing Primer, Cat No. 632388, can be used at 692–715)

### Propagation in *E. coli*

- Suitable host strains: 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) 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. Tersikh, 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)
NotI	4.1
StuI	2.7 & 1.4

# Certificate of Analysis

Cat. No. 632403

pTimer-1 Vector

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

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## pTimer-1 Vector

### CATALOG NO.

632403

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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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This document has been reviewed and approved by the Clontech Quality Assurance Department.

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