ptdTomato Vector

Catalog No.
632531

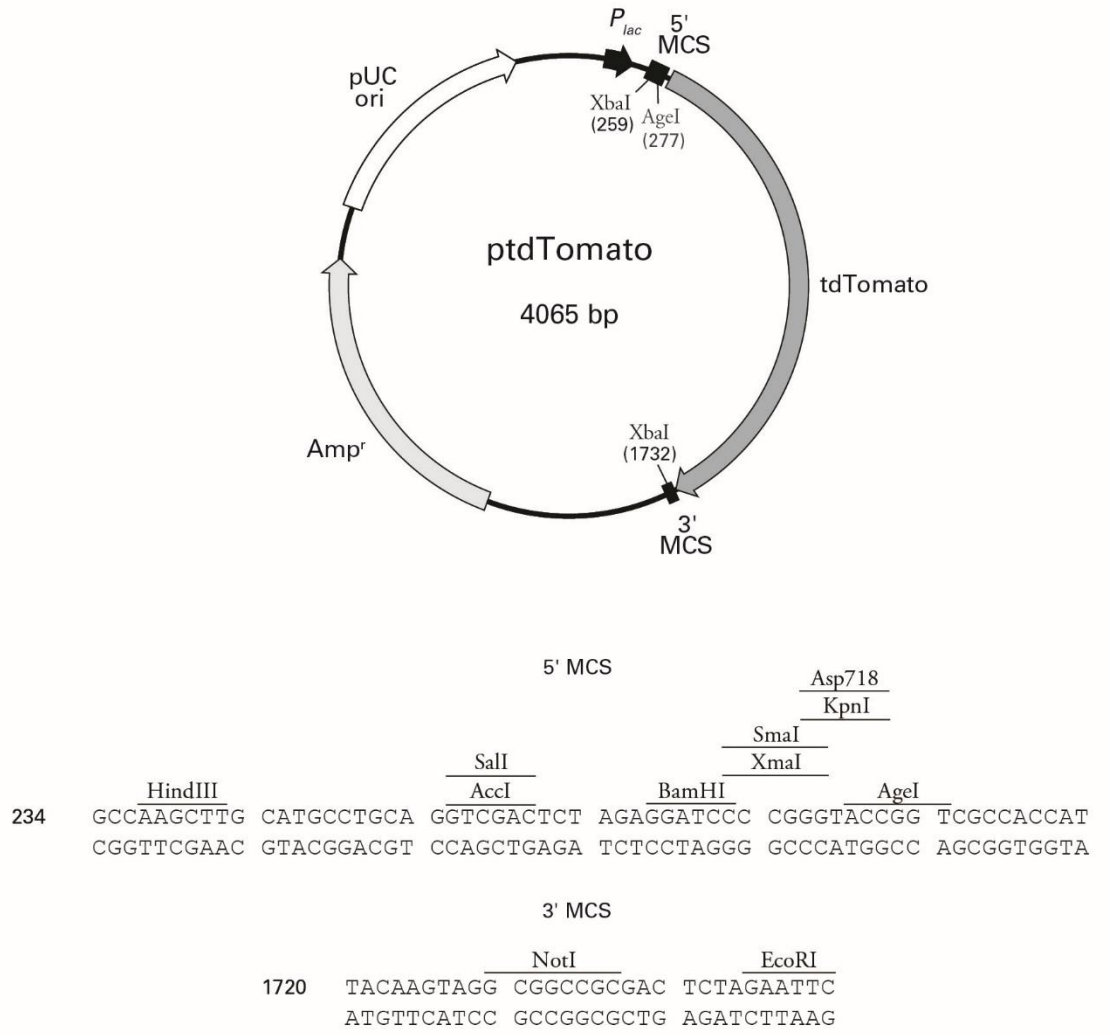

Figure 1. ptdTomato Vector map and multiple cloning sites (MCS).

Description

ptdTomato encodes tdTomato, a member of the family of fruit fluorescent proteins (Shaner et al. 2004) derived from the *Discosoma sp.* Red fluorescent protein, DsRed (Haas et al. 1996). Because the Tomato protein has a tendency to dimerize, the vector was designed with two copies of the Tomato coding region linked together to allow intramolecular dimerization. As a result, each tdTomato RNA transcript encodes a tandem dimer of the Tomato protein (excitation and emission maxima equal 554 nm and 581 nm, respectively).

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.565.6999

Vector Map

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ptdTomato

In ptdTomato, the tdTomato coding sequence (Haas et al. 1996) is flanked by restriction sites (5' MCS and 3' MCS) that make it easy to excise the gene for use in other cloning applications. In *E. coli*, tdTomato 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 tdTomato 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 expressed from the *lac* promoter).

A Kozak consensus sequence is located immediately upstream of the tdTomato coding sequence to enhance translational efficiency in eukaryotic cells (Kozak 1987). In the tdTomato vector, the entire tdTomato expression cassette is supported by a pUC19 backbone, which contains a high copy-number origin of replication and an ampicillin resistance gene for propogation and selection in *E. coli*.

Use

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

For Western analysis, either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393) can be used to detect the tdTomato protein.

Location of Features

- P_{lac} (*lac* promoter): 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - *lac* operator: 179–199
- *lacZ*-tdTomato fusion expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219
- 5' MCS (5' multiple cloning site): 234–281
- tdTomato
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 1717–1719
- 3' MCS (3' multiple cloning site): 1720–1740
- Amp^r (ampicillin resistance gene): 2265–3125
- pUC origin of replication: 3273–3915

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 tdTomato

- Excitation maximum = 554 nm
- Emission maximum = 581 nm

References

Haas, J., *et al.*, Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6**(3):315–324 (1996).

Kozak, M., An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15**(20):8125–8148 (1987).

Shaner, N.C., *et al.*, Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nature Biotech.* **22**(12):1567–1572 (2004).

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Takara Bio USA, Inc. This vector has not been completely sequenced.

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