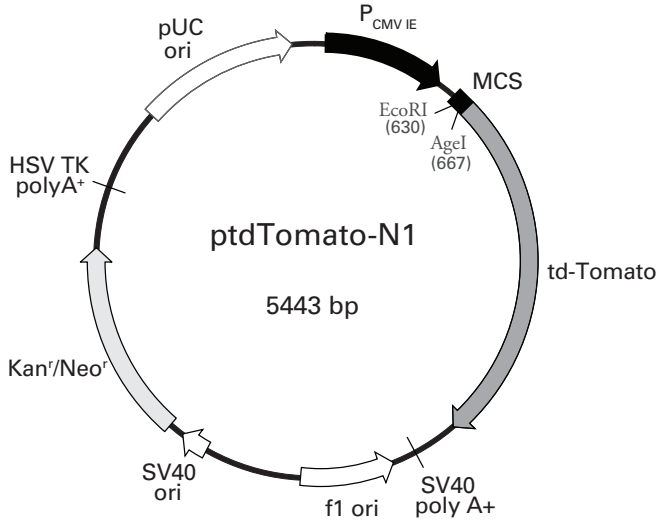
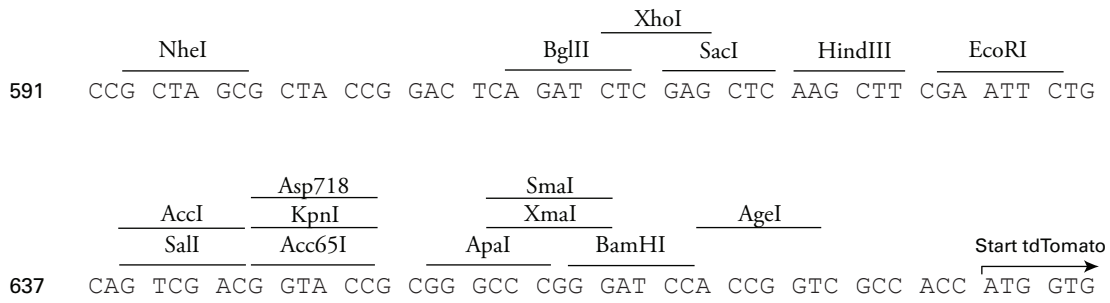
Vector Information

ptdTomato-N1 Vector Information PT4067-5

Catalog No. 632532





ptdTomato-N1 Vector Map and Multiple Cloning Site (MCS).

Description

ptdTomato-N1 is a mammalian expression vector designed to express a protein of interest fused to the N-terminus of tdTomato. tdTomato is a member of the family of fruit fluorescent proteins (1) derived from the *Discosoma sp.* red fluorescent protein, DsRed (2). 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 554nm and 581nm, respectively). Expression of td Tomato as a tandem dimer prevents the fused protein of interest from being forced into a dimeric complex. Fusions that retain the fluorescence properties of tdTomato can be monitored by flow cytometry and localized by fluorescence microscopy.

The multiple cloning site (MCS) in ptdTomato-N1 is positioned upstream of the tdTomato coding sequence. A Kozak consensus sequence, located between the MCS and the tdTomato coding sequence, enhances translational efficiency of the unfused tdTomato protein in eukaryotic cells (3). SV40 polyadenylation signals downstream of the tdTomato coding sequence direct proper processing of the 3' end of the tdTomato (or fusion gene) mRNA.

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 largeT antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. The vector also has a neomycin

(PR882589; published 10 October 2008)

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resistance cassette (Neo^r) that allows G418 selection of stably transfected eukaryotic cells. This cassette consists of the SV40 early promoter, the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette confers kanamycin resistance in *E. Coli*.

Use

The gene of interest must be cloned into ptdTomato-N1 so that it is in-frame with the tdTomato coding sequence. The gene must include an initiation codon (ATG), and lack in-frame stop codons.

ptdTomato-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). ptdTomato-N1 can also be used as a cotransfection marker, as the unmodified vector will express tdTomato in mammalian cells.

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

Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- MCS (multiple cloning site): 591–671
- tdTomato
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 2107–2109
  - Last amino acid: 2104-2106
- SV40 early polyA$^+$ signals: 2262–2267 & 2291–2296
- f1 origin of replication: 2359–2814 (complementary)
- SV40 origin of replication: 3155–3293
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 3339–3341; stop codon: 4131–4133
- HSV TK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 4369–4374 & 4382–4387
- pUC origin of replication: 4718–5361

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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/Col E1

Excitation and emission maxima of tdTomato

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

References

1. Shaner, N. C., *et al.* (2004) *Nature Biotechnol.* **22**(12):1567-72.
2. Matz, M. V., *et al.* (1999) *Nature Biotechnol.* **17**(10):969–973.
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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