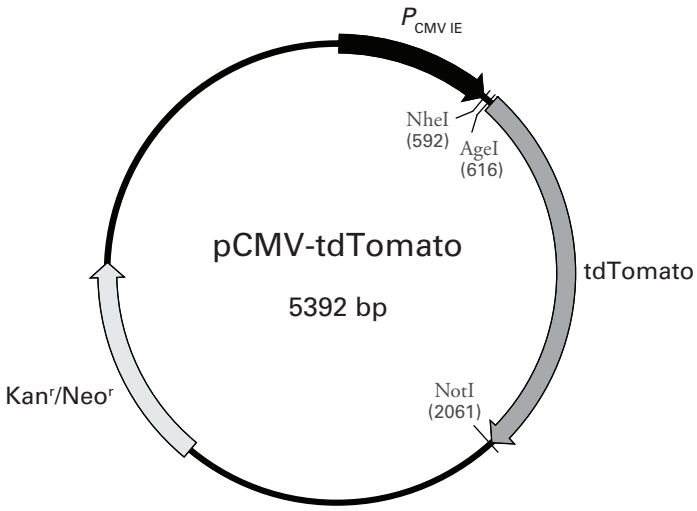
Vector Information

pCMV-tdTomato Vector Information

PT4069-5

Catalog No. 632534



pCMV-tdTomato Vector Map. The *Not* I site is downstream of the tdTomato stop codon.

Description

pCMV-tdTomato encodes tdTomato, 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).

The tdTomato coding sequence is positioned just downstream of the constitutively active cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). As a result, mammalian cells transfected with this vector will constitutively express tdTomato. A Kozak consensus sequence is located immediately upstream of the tdTomato coding sequence to enhance translational efficiency in eukaryotic cells (3). SV40 polyadenylation signals downstream of the tdTomato coding sequence direct proper processing of the 3' end of the tdTomato 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. A neomycin resistance cassette (Neo^r) 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 (HSVTK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

pCMV-tdTomato is designed for use as a marker for cotransfection or cell tracking. The red fluorescence of tdTomato can be detected by fluorescence microscopy, allowing direct visual imaging. In addition, flow cytometry can be used to enrich transfected cell populations. pCMV-tdTomato can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4).

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Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- tdTomato:
  Kozak consensus translation initiation site: 621–631
  Start codon (ATG): 628–630; Stop codon: 2056–2058
- Kan^r/Neo^r (kanamycin/neomycin resistance gene):
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3288–3290; stop codon: 4080–4082

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: ~500
- 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 Biotech. **22**(12):1567-1572.
2. Bevis, B. J. & Glick, B. S. (2002) *Nature Biotech.* **20**:83–87.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**: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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