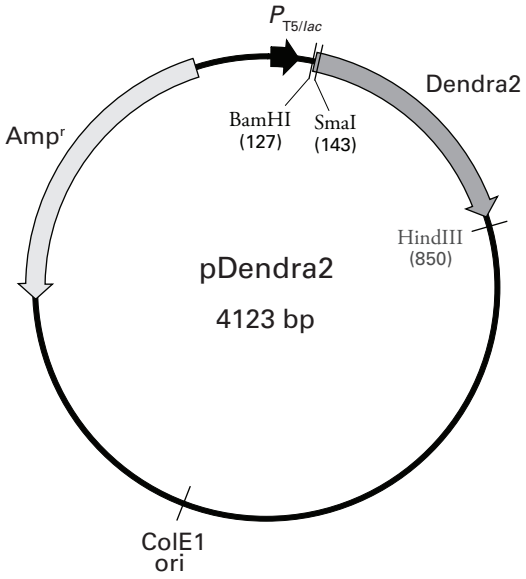
Vector Information

pDendra2 Vector Information

PT5021-5

Catalog No. 632544



pDendra2 Vector Map.

Description

pDendra2 is a prokaryotic expression vector encoding the monomeric, green-to-red photoswitchable fluorescent protein Dendra2. Dendra2 is a human codon-optimized variant of the octocoral *Dendronephthya sp.* fluorescent protein, Dendra, that has been engineered for faster maturation and brighter fluorescence both before and after photoswitching (1, 2). The Dendra2 coding sequence is flanked by restriction sites for convenient gene excision and subcloning. The Dendra2 coding sequence can be also be amplified by PCR.

In *E. coli*, Dendra2 expression is controlled by the T5 promoter/*lac* operator ($P_{T5/lac}$). The vector backbone contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Use

pDendra2 is primarily intended as a source of Dendra2 cDNA. The flanking restriction sites make it possible to excise the Dendra2 coding sequence and insert it into other vector systems. The vector can also be used to express Dendra2 in bacteria.

Note: pDendra2 was propagated in a *dam*$^+$ *E.coli* strain. Therefore, some restriction sites are blocked by methylation. In order to cut the vector at these blocked sites, you will first need to transform it into a *dam*$^-$ host strain and repurify the DNA.

Dendra2 matures efficiently at both 20°C and 37°C, which makes it useful in a wide range of experimental systems, from cultured mammalian cells to cold-blooded animals. Mammalian cells transiently transfected with Dendra2 expression vectors display an evenly distributed green signal without aggregation 10–12 hrs after transfection. No cell toxicity is observed.

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Dendra2 undergoes irreversible photoconversion from green to red fluorescence in response to intense irradiation at 405 nm or 488 nm. Because photoconversion can occur in response to intense irradiation at 460–500 nm (i.e., wavelengths used for protein visualization), the protein should be visualized with low intensity light that allows green signal detection without undesirable photoconversion.

Dendra2 can be photoconverted by irradiation with either UV-violet light (360–420 nm) or blue light (460–500 nm). We recommend using either a 405 nm diode laser or a 488 nm Ar laser; although a 405 nm laser provides more efficient photoconversion than a 488 nm laser, intense UV-violet light can be harmful to cells.

While nonphotoconverted (green) Dendra2 possesses excitation/emission maxima at 409/507 nm, photoconverted (red) Dendra2 possesses excitation/emission maxima at 553/573 nm. Thus, a TRITC filter set can be used to visualize photoconverted Dendra2. Under a confocal microscope, red fluorescence can be obtained using a 543 nm excitation laser line and detected at 560-650 nm.

Location of features
- $P_{T5/lac}$ (T5 promoter/*lac* operator): 7–87
- Dendra2: 132–821
- ColE1 origin of replication: 2301
- Amp^r (ampicillin resistance gene; β-lactamase): 3059–3919 (complementary)

Propagation in *E. coli*
- Recommended host strain: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of nonphotoconverted (green) Dendra2
- Excitation maximum = 490 nm
- Emission maximum = 507 nm

Excitation and emission maxima of photoconverted (red) Dendra2
- Excitation maximum = 553 nm
- Emission maximum = 573 nm

References
1. Gurskaya, N.G. *et al.* (2006) *Nat. Biotechnol.* **24**(4):461-465.
2. Haas, J. *et al.* (1996) *Curr. Biol.* **6**(3):315-324.

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.