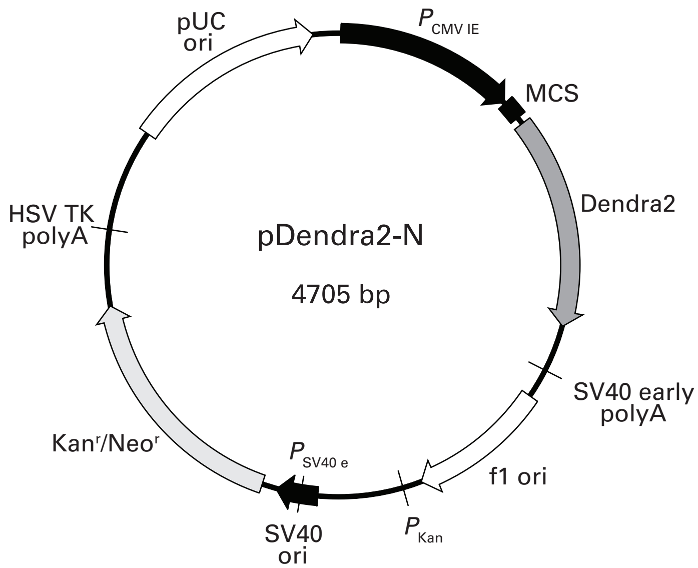
pDendra2-N Vector Information

PT5022-5

Catalog No. 632545

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                             Eco47III                          BglII     SacI
      PCMV IE      NheI                                              XhoI           HindIII   EcoRI
586   GAT  CCG  CTA  GCG  CTA  CCG  GAC  TCA  GAT  CTC  GAG  CTC  AAG  CTT  CGA

                PstI                        SacII          BamHI
      EcoRI           SalI     KpnI              ApaI                   AgeI  Kozak sequence
631   ATT  CTG  CAG  TCG  ACG  GTA  CCG  CGG  GCC  CGG  GAT  CCA  CCG  GTC  GCC

      Kozak sequence
           Start Dendra2
676   ACC  ATG  AAC
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pDendra2-N Vector Map and Multiple Cloning Site (MCS).

Description

pDendra2-N is a mammalian expression vector designed to express a protein of interest fused to the N-terminus of Dendra2, a monomeric, green-to-red photoswitchable fluorescent protein. 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 multiple cloning site (MCS) in pDendra2-N is positioned between the cytomegalovirus immediate early promoter ($P_{CMV IE}$) and the Dendra2 coding sequence. A Kozak consensus sequence is located immediately upstream of the Dendra2 gene to enhance translational efficiency in eukaryotic systems when Dendra2 is expressed by itself (i.e., not as a fusion; 3). SV40 polyadenylation signals downstream of the Dendra2 coding sequence direct proper processing of the 3' end of the Dendra2 mRNA.

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The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large 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^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter ($P_{SV40\ e}$), the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSVTK) gene. A bacterial promoter (P_{Kan^r}) upstream of the cassette confers kanamycin resistance in *E. coli*.

Use

A gene of interest or an intracellular localization signal must be cloned into pDendra2-N so that it is in-frame with the Dendra2 coding sequence. The inserted sequence must include an initiation codon (ATG), and lack stop codons.

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

The pDendra2-N vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). pDendra2-N can also be used as a cotransfection marker, as the unmodified vector will express Dendra2 in mammalian cells.

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.

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

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- MCS (multiple cloning site): 591–671
- Kozak consensus translation initiation site: 672–682
- Dendra2 (human codon-optimized): 679–1371
- SV40 early polyA signals: 1524–1529 & 1553–1558
- f1 origin of replication: 1621–2076
- P_{Kan^r} (bacterial promoter for Kan^r gene expression): 2138–2166
- SV40 origin of replication: 2417–2552
- $P_{SV40\ e}$ (SV40 early promoter) 2473–2479
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 2601–3395
- HSVTK polyA (herpes simplex virus thymidine kinase polyadenylation signals): 3631–3636 & 3644–3649
- pUC origin of replication: 3980–4623

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 the JM109 or XL1-Blue strains.
- 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/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.
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.