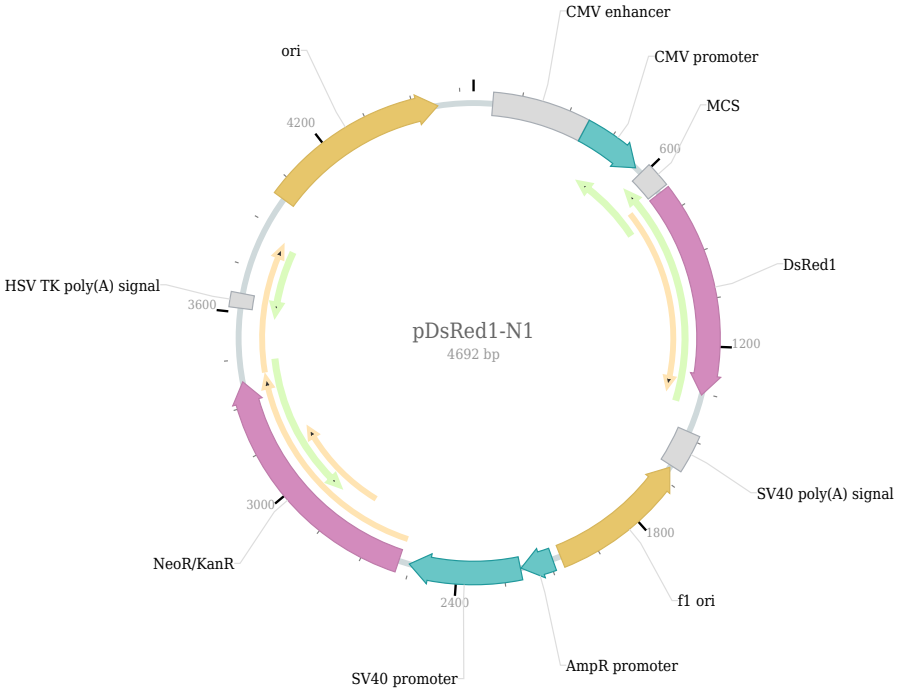
pDsRed1-N1

Vector ID	V012426
Antibiotic Resistance	Kanamycin
Length	4692 bp
Vector Type	Fluorescent Protein Reporter Vectors

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Promoter	CMV
Comment	pDsRed1-N1 encodes a novel red fluorescent protein (RFP; 1) that has been optimized for high expression in mammalian cells (excitation maximum = 558 nm; emission maximum = 583 nm). RFP was isolated from an IndoPacific sea anemone-relative, Discosoma sp; DsRed1's coding sequence contains 144 silent base pair changes, which correspond to human codon-usage preferences for high expression in mammalian cells (2). Sequences upstream of DsRed1 have been converted to a Kozak consensus translation initiation site (3) to increase translation efficiency in eukaryotic cells. The MCS is between the immediate early promoter of CMV (PCMV IE) and the DsRed1 coding sequence. Genes cloned into the MCS as described below are expressed as fusions to the N-terminus of DsRed1. SV40 polyadenylation signals downstream of the DsRed1 gene direct proper processing of the 3' end of the DsRed1 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neor) 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 (HSV TK) gene. A bacterial promoter upstream of the cassette confers kanamycin resistance to E. coli. The pDsRed1-N1 backbone also has a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.Fusions to the N terminus of DsRed1 typically do not alter the fluorescence properties of native DsRed1, allowing in vivo localization of the fusion protein.The target gene should be cloned into pDsRed1-N1 in frame with the DsRed1 coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pDsRed1-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). Unmodified pDsRed1-N1 can also be used to express DsRed1 in a cell line of interest (e.g., for use as a transfection marker).

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 μl for 5 μg plasmid, and 100 μl for 100 μg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.