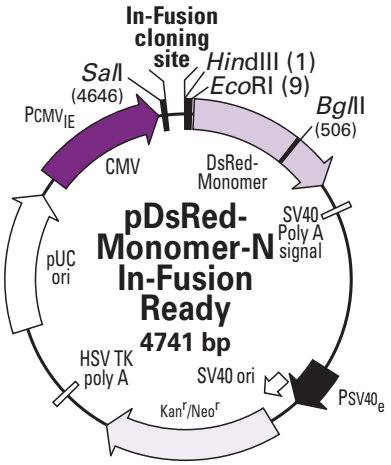
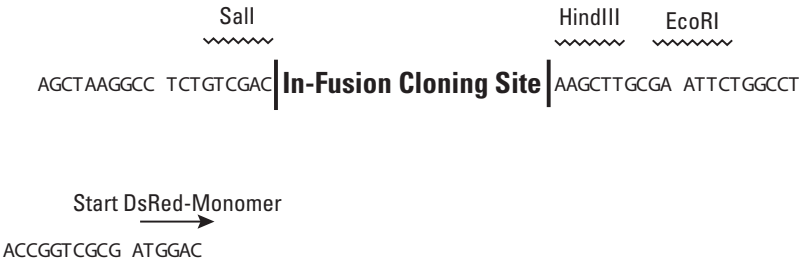
Vector Information

pDsRed-Monomer-N In-Fusion® Ready Vector Information PT3863-5

Catalog No. 632498





Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer-N In-Fusion Ready (Linear) Vector. All sites shown are unique.

Description

pDsRed-Monomer-N In-Fusion Ready vector is a *linearized* mammalian expression vector that encodes DsRed-Monomer (DsRed. M1), a monomeric mutant derived from the tetrameric *Discosoma sp*. red fluorescent protein, DsRed (1). DsRed-Monomer contains a total of forty-five amino acid substitutions. When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection. DsRed-Monomer has an excitation maximum at 557 nm and an emission maximum at 592 nm. The DsRed-Monomer coding sequence is human codon-optimized for high expression levels in mammalian cells (2).The linearized vector allows direct cloning of PCR products without any need for restriction digestion when using the In-Fusion HD Cloning Plus (638909). This is accomplished by the use of a specific 15 nucleotide long sequence within the 5′ ends of the sense and antisense primers that overlap with the cut ends of the linearized vector.

The primers that will be used to amplify In-Fusion Ready PCR products require the following 15 nucleotides on their 5′ends:

Sense primer: 5′ AAGGCCTCTGTCGAC followed by sequence of amplification target 3′

Antisense primer: 5′ AGAATTCGCAAGCTT followed by sequence of amplification target 3′

If the sequence of the gene of interest is added in-frame immediately after the 15 nucleotides mentioned above, this sequence will automatically be in-frame with the DsRed-Monomer sequence downstream and therefore be expressed as a fusion protein to the N-terminus of DsRed-Monomer.

A Kozak consensus translation initiation site upstream of DsRed-Monomer increases the translation efficiency in eukaryotic cells (3). SV40 polyadenylation signals downstream of the DsRed-Monomer gene direct proper processing of the 3' end of the mRNA. transcript. The vector backbone contains an SV40 origin of replication in mammalian cells expressing

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

the SV40 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 selection of stably transfected eukaryotic cells using G418. This cassette contains 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 allows the plasmid to confer kanamycin resistance in *E. coli*.

Use

Fusions to the N-terminus of DsRed-Monomer retain the fluorescent properties of the native protein and allow monitoring of the fusion protein localization *in vivo*. The PCR-amplified gene of interest is directly cloned into the pDsRed-Monomer-N In-Fusion Ready Vector so that it is in-frame with the DsRed-Monomer coding sequences with no intervening in-frame stop codons. This can be accomplished by using the suggested primers. The inserted gene must include the initiating ATG codon. This recombinant vector can be transfected into mammalian cells using any standard transfection method. Stable transformants can be selected using G418 (2).

The DsRed1-N Sequencing Primer (Cat. No. 632387) can be used to sequence genes cloned adjacent to the 5′ end of the DsRed-Monomer coding region.

For Western blotting, the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) can be used to recognize the DsRed-Monomer protein. For optimal results for immunoprecipitation, we suggest you titrate the amount of antibody needed for quantitative recovery of the antigen. As a starting point, however, we recommend using the antibody at a 1:1,000 dilution.

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 4039–4627
  Enhancer region: 4097–4503; TATA box: 4592–4598
  Transcription start point: 4621
  CØG mutation to remove *Sac* I site: 4607
- Human codon-optimized DsRed-Monomer gene
  First codon: 30–32; Stop codon: 702–704
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 857–862 & 886–891; mRNA 3′ ends: 895 & 907
- SV40 origin of replication: 1750–1885
- SV40 early promoter: 1583–1793
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: Start codon (ATG): 1934–1936; Stop codon: 2726–2728
  GØA mutation to remove *Pst* I site: 2116
  CØA (Arg to Ser) mutation to remove *Bss*H II site: 2462
- pUC plasmid replication origin: 3313–3956

Sequencing primer location

- DsRed1-N Sequencing Primer (5′-GTACTGGAACTGGGGGGACAG-3′): 227–207

Note: This primer is available from Clontech (Cat. No. 632387).

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~ 500
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of DsRed-Monomer

- Excitation maximum = 557 nm
- Emission maximum = 592 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
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 attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.