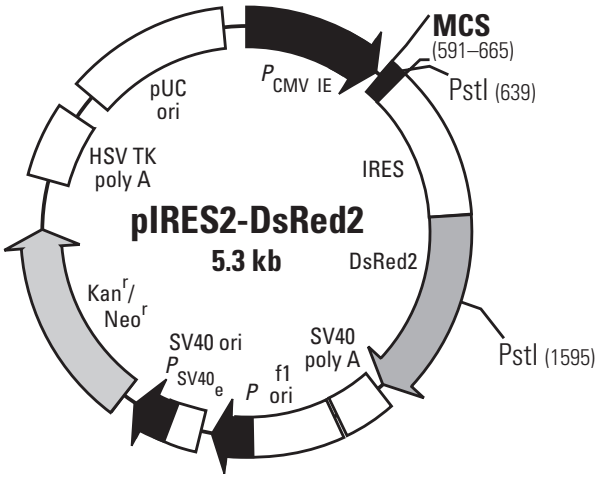
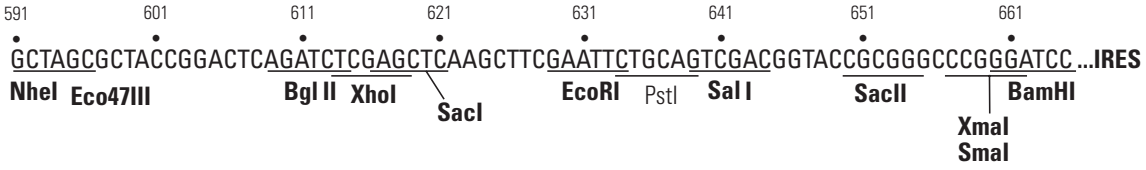
Vector Information

pIRES2-DsRed2 Vector Information PT3663-5

Catalog No. 632420





Restriction Map and Multiple Cloning Site (MCS) of pIRES2-DsRed2 Vector. Unique restriction sites are in bold.

Description

pIRES2-DsRed2 contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and the *Discosoma sp.* red fluorescent protein (DsRed2; 3, 4) coding region. This permits both the gene of interest (cloned into the MCS) and the DsRed2 gene to be translated from a single bicistronic mRNA. pIRES2-DsRed2 is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing DsRed2 and the protein of interest. This vector can express DsRed2 alone at lower signal intensity. The vector can also be used to obtain stably transfected cell lines by drug and clonal selection.

DsRed2 is a human codon-optimized (5) DsRed variant engineered for faster maturation and lower non-specific aggregation. The MCS in pIRES2-DsRed2 is located between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the DsRed2 gene direct proper processing of the 3' end of the bicistronic mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSVTK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pIRES2-DsRed2 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pIRES2-DsRed2 can be used to quickly identify cells expressing a gene of interest by screening for DsRed2 fluorescence. Genes inserted into the MCS should include the initiating ATG codon. Selection of DsRed2-positive cells is possible 24 hr after transfection by flow cytometry or fluorescence microscopy. However, in some cases, up to 48 hr may be required for detection of red-emitting cells. pIRES2-DsRed2 and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

(PR732215; published 30 March 2007)

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

Please refer to the Living Colors® User Manual Volume II (PT3404-1) provided with this vector for additional information on detection of DsRed2.

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; Transcription start point: 583
  C→G mutation to remove SacI site: 569
- MCS: 591–665
- IRES sequence: 666–1250
- *Discosoma sp.* Red Fluorescent Protein (DsRed2) gene
  Start codon (ATG): 1254–1256; Stop codon: 1929–1931
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2083–2088 & 2112–2117; mRNA 3' ends: 2121 & 2133
- f1 single-strand DNA origin: 2180–2635 (Packages the noncoding strand of DsRed2.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2697–2702; –10 region: 2720–2725
  Transcription start point: 2732
- SV40 origin of replication: 2976–3111
- SV40 early promoter/enhancer
  72 bp tandem repeats: 2809–2952; 21 bp repeats (3): 2956–3019
  Early promoter element: 3032–3038
- Kanamycin/neomycin resistance gene: 3158–3952
  G→A mutation to remove *Pst* I site: 3342; C→A (Arg to Ser) mutation to remove *Bss*H II site: 3688
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4190–4208
- pUC plasmid replication origin: 4539–5182

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 JM101 or XL1-Blue.
- 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

References

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6. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

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