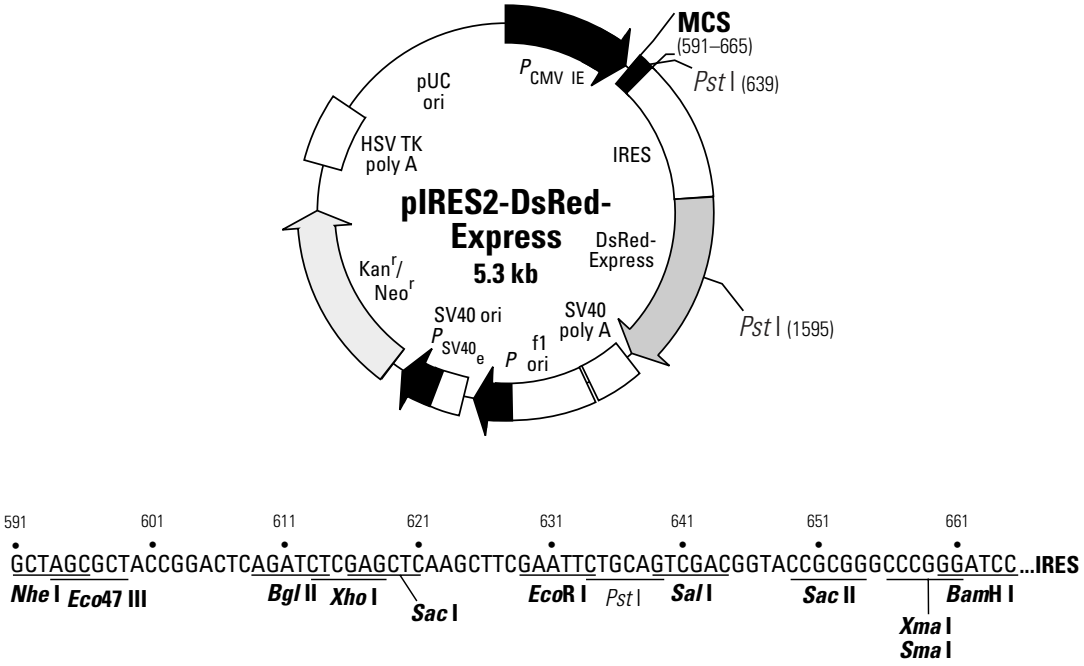

Restriction Map and Multiple Cloning Site (MCS) of pIRES2-DsRed-Express Vector. Unique restriction sites are in bold.

Description

pIRES2-DsRed-Express contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and a mutant of the *Discosoma* sp. red fluorescent protein DsRed-Express coding region (3–5). This permits both the gene of interest (cloned into the MCS) and the DsRed-Express gene to be translated from a single bicistronic mRNA. pIRES2-DsRed-Express is designed for the efficient selection by flow cytometry of transiently transfected mammalian cells expressing DsRed-Express and the protein of interest. This vector can also be used to express DsRed-Express alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

DsRed-Express is a human codon-optimized (4) DsRed variant of *Discosoma* sp. red fluorescent protein (6). Its coding sequence contains nine amino acid substitutions which improve solubility of the protein and reduce the time from transfection to detection of red fluorescence (exitation and emission maxima = 557 nm and 579 nm, respectively). In addition, these substitutions reduce the level of residual green emission (5). Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (3, 5).

The MCS in pIRES2-DsRed-Express is between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the DsRed-Express 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 (HSV TK) 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-DsRed-Express backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR42578; published 10 February 2004)

Use

pIRES2-DsRed-Express can be used to quickly identify cells expressing a gene of interest by screening for DsRed-Express fluorescence. Genes inserted into the MCS should include the initiating ATG codon. When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by flow cytometry or microscopy 24 hours after transfection. However, in some cases, up to 48 hours may be required for detection of red-emitting cells. pIRES2-DsRed-Express and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7).

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

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 *Sac* I site: 569
- MCS: 591–665
- IRES sequence: 666–1250
- *Discosoma sp.* Red Fluorescent Protein (DsRed-Express) 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 DsRed-Express.)
- 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: 3160–3954
  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) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

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The CMV promoter is covered under U.S. Patent Nos. 5,168,062, and 5,385,839 assigned to the University of Iowa Research Foundation.

The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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