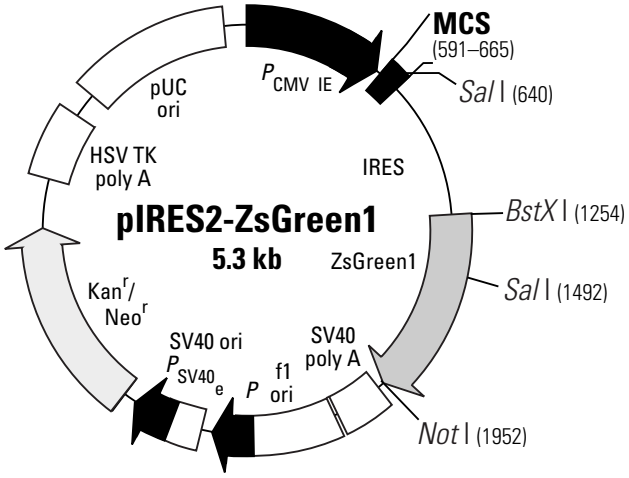
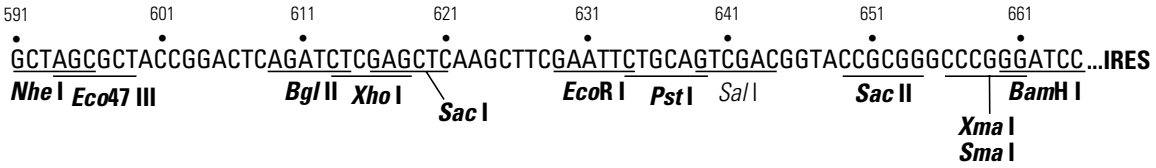
Vector Information

pIRES2-ZsGreen1 Vector Information PT3824-5

Cat. No. 632478





Restriction Map and Multiple Cloning Site (MCS) of pIRES2-ZsGreen1 Vector. Unique restriction sites are in bold.

Description

pIRES2-ZsGreen1 contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between an MCS and the *Zoanthus* sp. green fluorescent protein (ZsGreen; 3, 4) coding region. This design permits both the gene of interest (cloned into the MCS) and the ZsGreen1 gene to be translated from a single bicistronic mRNA. pIRES2-ZsGreen1 is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing ZsGreen1 and the protein of interest. This vector can be used to obtain stably transfected cell lines without time-consuming drug and clonal selection.

ZsGreen1 is a human codon-optimized (5) ZsGreen variant that encodes the brightest commercially available green fluorescent protein. The MCS in pIRES2-ZsGreen1 is located between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the ZsGreen1 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-ZsGreen1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pIRES2-ZsGreen1 can be used to quickly identify cells expressing a gene of interest by screening for ZsGreen1 fluorescence. Genes inserted into the MCS should include an initiating ATG codon. Selection of ZsGreen1-positive cells is possible 24–36 hours after transfection by flow cytometry or fluorescence microscopy. pIRES2-ZsGreen1 and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

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Please refer to the Living Colors® User Manual Volume II (PT3404-1) provided with this vector for additional information on the detection of ZsGreen1.

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
- *Zoanthus* sp. Green Fluorescent Protein (ZsGreen1) gene
  - Start codon (ATG): 1254–1256
  - Stop codon: 1947–1949
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 2102–2107 & 2131–2136
  - mRNA 3' ends: 2140 & 2152
- f1 single-strand DNA origin: 2199–2654 (Packages the noncoding strand of ZsGreen1.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2716–2721
  - –10 region: 2739–2744
  - Transcription start point: 2751
- SV40 origin of replication: 2995–3130
- SV40 early promoter/enhancer
  - 72 bp tandem repeats: 2828–2971
  - 21 bp repeats (3): 2975–3038
  - Early promoter element: 3051–3057
- Kanamycin/neomycin resistance gene: 3179–3973
  - G→A mutation to remove *Pst* I site: 3361
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3707
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4209–4227
- pUC plasmid replication origin: 4558–5201

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418 (500 µg/ml)

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: high

References

1. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
2. Jang, S. K., *et al.* (1990) *J. Virol.* **62**(8):2636–2643.
3. Living Colors® pIRES2-ZsGreen1 Vector (2005) *Clontechniques* **XX**(1):8.
4. Matz M. V., *et al.* (1999) *Nature Biotechnol.* **17**(10):969–973.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**(3):315–324.
6. Gorman, C. (1985). In *DNA Cloning: A Practical Approach, Vol. II.,* Ed. Glover, D.M., (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.