Vector Information

pIRES2-AcGFP1-Nuc Vector Information

PT3907-5

Catalog No. 632515

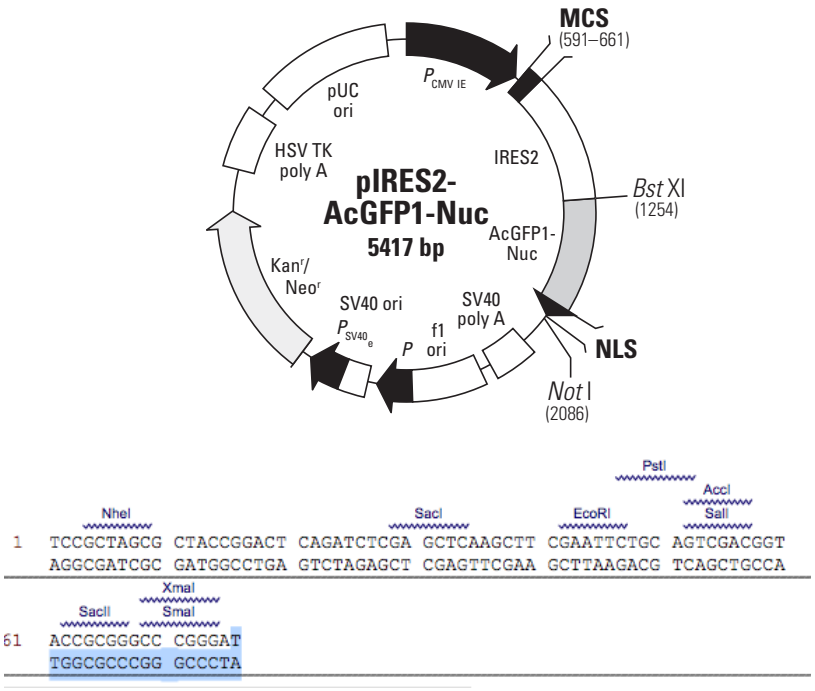

Figure 1. Restriction Map and Multiple Cloning Site (MCS) of pIRES2-AcGFP1-Nuc Vector.

Description

pIRES2-AcGFP1-Nuc contains the internal ribosome entry site (IRES; 1,2) of the encephalomyocarditis virus (ECMV) between the MCS and the *Aequorea coerulescens* green fluorescent protein (AcGFP1) coding region, fused to three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen (3,4). The reiteration of the NLS sequence significantly increases the efficiency of translocation of AcGFP1 into the nucleus of mammalian cells (5). The IRES2 sequence permits both the gene of interest (cloned into the MCS) and the AcGFP1-Nuc gene to be translated from a single bicistronic mRNA. pIRES2-AcGFP2-Nuc is designed for the efficient nuclear labeling of transiently transfected mammalian cells expressing AcGFP1-Nuc and the protein of interest.

AcGFP1 is a green fluorescent protein (GFP) from *Aequorea coerulescens* (excitation maximum = 475 nm; emission maximum = 505 nm). AcGFP1 contains silent mutations that create an open reading frame comprised almost entirely of preferred human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and consequently the expression of AcGFP1 in mammalian and plant cells.

The MCS in pIRES2-AcGFP1-Nuc lies between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. Additional features include SV40 polyadenylation signals downstream of the AcGFP1-Nuc gene to 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 (6). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pIRES2-AcGFP1-Nuc backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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

(PR671845; published 5 July 2006)

Use

pIRES2-AcGFP1-Nuc can be used to quickly identify cells expressing a gene of interest because, in addition to the gene of interest, transfected cells will also express AcGFP1-Nuc localized in the nucleus. This is especially advantageous for microscopic imaging and screening applications using nuclear labeling as a means of counting cells, as well as for monitoring nuclear translocation events. When transfecting with pIRES2-AcGFP1-Nuc, only cells expressing a protein of interest will be counted and imaged, because these are the only cells containing a green fluorescent nucleus.

Genes inserted into the MCS should include the initiating ATG and stop codons. Detection of AcGFP1-Nuc positive cells is possible 24 hours after transfection by flow cytometry or fluorescence microscopy. pIRES2-AcGFP1-Nuc and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

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: 591–661
- IRES sequence: 666–1250
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  Start codon (ATG): 1254–1256; last codon of AcGFP1: 1968–1970
  Tandem repeat of the nuclear localization signal (NLS): 1971–2081
  Stop codon: 2082–2084
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2236–2286
- f1 single-strand DNA origin: 2223–2678 (packages the noncoding strand of AcGFP1-Nuc)
- SV40 origin of replication: 3129–3264
- SV40 early promoter/enhancer: 2962–3230
- Kanamycin/neomycin resistance gene: 3313–4107
- *Herpes simplex* virus (HSV) thymidine kinase (TK) polyadenylation signals: 4343–4361
- pUC plasmid replication origin: 4692–5335

Propagation in *E. coli*

- Suitable host strains: DH5α 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. (1988) *J. Virol.* **62**(8):2636-2643
3. Kalderon, D., et al. (1984) *Cell* **39**(3):499–509
4. Lanford, R.E., et al. (1986) *Cell* **46**(4):575–582
5. Fischer-Fantuzzi, L. & Vesco, C. (1988) *Molec. Cell. Biol.* **8(**12):5495–5503
6. Gorman, C. (1985) In DNA Cloning: A Practical Approach, Vol. II, Ed. D.M. Glover. (IRL Press, Oxford, UK) 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. This vector has not been completely sequenced.

General Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

This product is the subject of pending U.S. and foreign patents.

This product contains a Living Colors® fluorescent protein.

Not-For-Profit entities may place orders in the normal manner by contacting your local representative or Clontech Customer Service at 800.662.2566, extension 1. Clontech Laboratories, Inc. grants not-for-profit research entities a worldwide, non-exclusive, royalty-free, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties. Any other use of this product will require a license from Clontech Laboratories, Inc. Please contact the licensing hot line by phone at 800.662.2566, or by e-mail at licensing@clontech.com.

For-Profit entities that wish to use this product in non-commercial or commercial applications are required to obtain a license from Clontech Laboratories, Inc. For license information, please contact the licensing hot line by phone at 800.662.2566, or by e-mail at licensing@clontech.com.