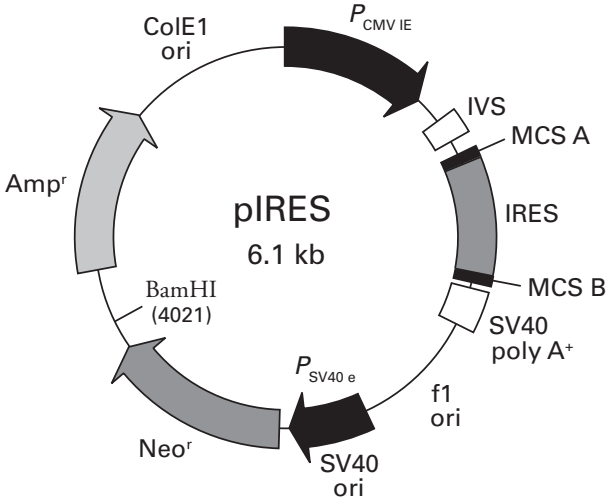
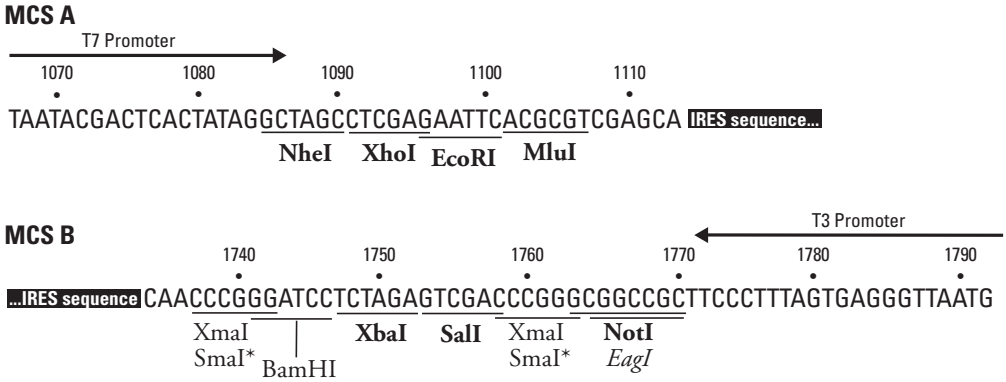
Vector Information

pIRES Vector Information PT3266-5

Cat. No. 631605





pIRES Vector Map and Multiple Cloning Sites (MCS). Unique restriction sites are in bold. The XmaI/SmaI sites can be treated as unique sites for cloning purposes.

Description

pIRES is a mammalian expression vector that allows high level expression of two genes of interest from the same bicistronic mRNA transcript. The vector contains the encephalomyocarditis virus (ECMV) internal ribosome entry site (IRES) flanked by two multiple cloning sites (MCS A and B), an arrangement that allows cap-independent translation of the gene cloned into MCS B (1–3). pIRES utilizes a partially disabled IRES sequence (1) that reduces the rate at which the gene cloned into MCS B is translated relative to that of MCS A.

Expression of the bicistronic transcript is driven by the constitutively active cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located upstream of MCS A. An intervening sequence (IVS) known to enhance the stability of mRNA (4) is located between $P_{CMV\ IE}$ and MCS A, and is efficiently spliced out following transcription. SV40 polyadenylation signals downstream of MCS B direct proper processing of the 3' end of the mRNA. Bacteriophage T7 and T3 promoters are located upstream of MCS A and downstream of MCS B, respectively. pIRES includes a neomycin resistance gene (Neo^r) to aid in the selection of transfected cells. Neo^r is expressed from the SV40 enhancer/promoter, and a synthetic polyadenylation signal directs proper processing of the 3' end of the Neo^r mRNA. The SV40 origin allows for replication in mammalian cells expressing the SV40 T antigen. The vector also contains an ampicillin resistance gene (Amp^r), and a ColE1 origin of replication for selection and propagation in *E. coli*, and an f1 origin for single-stranded DNA production.

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Use

Genes cloned into either MCS must contain ATG start codons. pIRES and derivatives can be introduced into mammalian cells by any standard transfection method. Transformed cells can be selected by growth in medium containing the antibiotic G418. Sense or antisense RNA can be transcribed from the T7 and T3 promoters, respectively.

Location of features

- $P_{CMV\ IE}$:
  CMV IE enhancer: 1–659
  CMV IE promoter: 669–750
- Intervening sequence (IVS): 890–1022
- T7 RNA polymerase promoter: 1067–1085
- Multiple cloning site A: 1085–1107
- IRES sequence: 1130–1725
- Multiple cloning site B: 1737–1763
- T3 RNA polymerase promoter: 1771–1792 (complementary)
- SV40 polyadenylation signal: 1802–2023
- f1 origin of replication: 2118–2573
- Neo^r expression cassette: 2637–4004
  SV40 enhancer/early promoter: 2637–3054
  SV40 origin of replication: 2953–3018
  Neo^r structural gene: 3098–3892
    Start codon (ATG): 3098–3100
    Stop codon: 3890–3892
  Synthetic polyadenylation signal: 3956–4004
- Ampicillin resistance (β-lactamase) gene: 4415–5275
  Start codon (ATG): 4415–4417
  Stop codon (TAA): 5273–5275

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

References

1. Rees, S., *et al.* (1996) *BioTechniques* **20**(1):102–110.
2. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
3. Jang, S. K., *et al.* (1990) *J. Virol.* **62**(8):2636–2643.
4. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947

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.