



**Restriction Map and Multiple Cloning Site (MCS) of pIRES.** Unique restriction sites are in bold. The Xma I/Sma I sites can be treated as unique sites for cloning purposes.

**Description**

pIRES is a mammalian expression vector that allows you to express two genes of interest at high levels by cloning them into multiple cloning sites (MCS) A and B. These MCSs are located on either side of the internal ribosome entry site (IRES) from the encephalomyocarditis virus (ECMV), which allows translation of two consecutive open reading frames from the same messenger RNA (1–3). pIRES utilizes a partially disabled IRES sequence (1) which will have the affect of a reduced rate of translation initiation at the second, downstream cloned gene relative to that of the first. The MCSs and IRES sequence are downstream of the immediate early promoter of cytomegalovirus ( $P_{CMV IE}$ ). The intervening sequence (IVS) between  $P_{CMV IE}$  and the MCS is an intron that is efficiently spliced out following transcription. SV40 polyadenylation signals downstream of the MCS direct proper processing of the 3' end of the mRNA from your gene of interest. Bacteriophage T7 and T3 promoters are located upstream and downstream of MCS A and B, respectively. pIRES uses the neomycin resistance gene ( $Neo^r$ ) to permit selection of transformed 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 also allows for replication in mammalian cells expressing the SV40T antigen. The vector backbone also contains the  $\beta$ -lactamase gene for ampicillin resistance and a ColE1 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

## 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 media containing the antibiotic G418. Sense or antisense RNA can be transcribed from the T7 and T3 promoters, respectively.

## Location of features

- $P_{CMVIE}$ :
  - 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: 1130–1710
- Multiple cloning site B: 1722–1748
- T3 RNA polymerase promoter: 1777–1756
- SV40 fragment containing polyadenylation signal: 1787–2008
- f1 origin of replication: 2103–2558
- Neo<sup>r</sup> expression cassette: 2622–3989
  - SV40 enhancer/early promoter: 2622–3039
  - SV40 origin of replication: 2938–3003
  - Neo<sup>r</sup> structural gene: 3083–3877
    - Start codon (ATG): 3083–3085
    - Stop codon: 3875–3877
  - Synthetic polyadenylation signal: 3941–3989
- Ampicillin resistance ( $\beta$ -lactamase) gene: 4400–5260
  - Start codon (ATG): 4400–4402
  - Stop codon (TAA): 5258–5260

## Propagation in *E. coli*

- Suitable host strains: DH5 $\alpha$ , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

## References

1. Rees, S., *et al.* (1996) *BioTechniques* **20**:102–110.
2. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**:477–483.
3. Jang, S. K., *et al.* (1990) *J. Virol.* **62**:2636–2643.

## Notice to Purchaser

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.

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