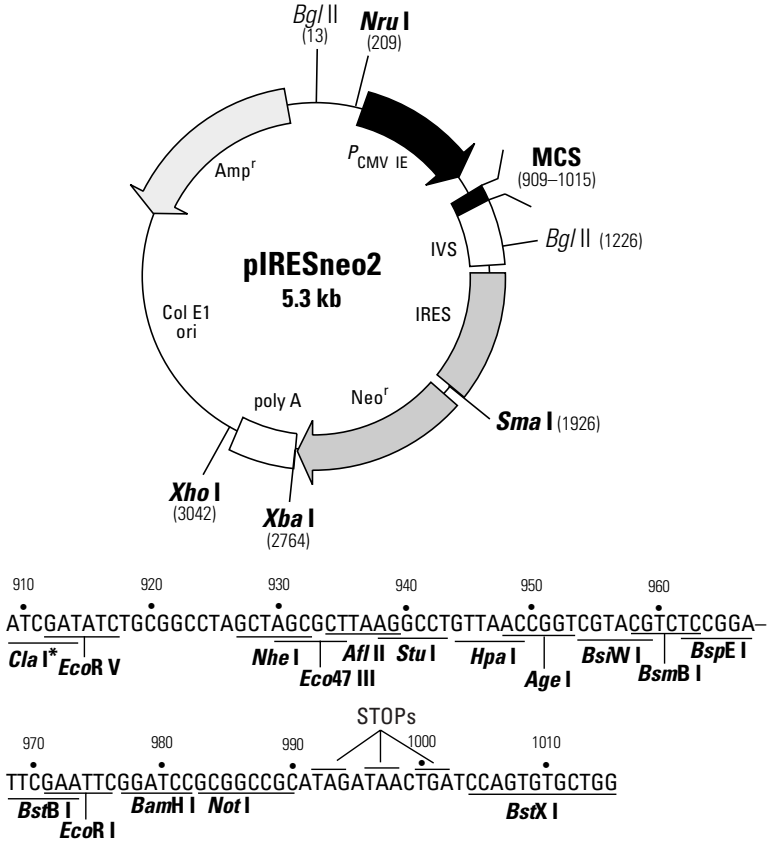

Restriction Map and Multiple Cloning Site (MCS) of pIRESneo2 Vector. Unique restriction sites are in bold. The *Cla* I site (*) in the MCS is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pIRESneo2 contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA (1–3). After selection with G418, nearly all surviving colonies will stably express the gene of interest, thus decreasing the need to screen large numbers of colonies to find functional clones. To select for cells that express high levels of the gene of interest, the selective pressure for antibiotic resistance was increased by moving the initiation codon of the neomycin phosphotransferase gene to a less optimal position for translation as directed by the IRES sequence (attenuated IRES; 1). The expression cassette of pIRESneo2 contains the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed by a multiple cloning site (MCS) that precedes stop codons in all three reading frames, a synthetic intron known to enhance the stability of the mRNA (4), the ECMV IRES followed by the neomycin phosphotransferase (NPT II) gene, and the polyadenylation signal of the bovine growth hormone. Ribosomes can enter the bicistronic mRNA at the 5' end to translate the gene of interest and at the ECMV IRES to translate the antibiotic resistance marker.

Use:

When using the pIRESneo2 Vector, the antibiotic exerts selective pressure on the entire expression cassette; thus, a high dose of antibiotic will select for cells expressing a high level of the gene of interest. This selective pressure also ensures that the expression of the gene of interest will be stable over time in culture. Unless your expression experiments require a pure population of cells, you can use the pool of cells surviving selection instead of isolating and characterizing clonal cell lines. We recommend selecting mammalian cultures in 500–1,300 μg/ml G418 (#8056-1) depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of G418 to determine optimal selection concentration.

(PR9X258; published 17 September 1999)

Location of features:

- Human cytomegalovirus (CMV) major immediate early promoter
  Enhancer region: 309–715; TATA box: 804–810
- Multiple cloning site (MCS): 909–1015
- Synthetic intron (IVS): 1015–1310
- Attenuated internal ribosome entry site (IRES) from encephalomyocarditis virus (ECMV): 1336–1949
- Neomycin phosphotransferase coding sequence (NPT II): 1951–2747
- Fragment containing the bovine growth hormone poly-A signal: 2764–3040
- Col E1 origin of replication: 3538–4137
- Ampicillin resistance (β-lactamase) gene:
  Promoter: –35 region: 5229–5225; –10 region: 5206–5195
  Transcription start point: 5194
  Ribosome binding site: 5171–5168
  β-lactamase coding sequence:
  Start codon: 5159–5157; stop codon: 4301–4299
  β-lactamase signal peptide: 5159–5151
  β-lactamase mature polypeptide: 5090–4302

Propagation in *E. coli*:

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: high

References:

1. Rees, S., *et al*. (1996) *BioTechniques* **20**:102–104.
2. Jackson, R. J., *et al*. (1990) *Trends Biochem. Sci.* **15**:477–483.
3. Jang, S. K., *et al*. (1988) *J. Virol.* **62**:2636–2643.
4. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**: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.