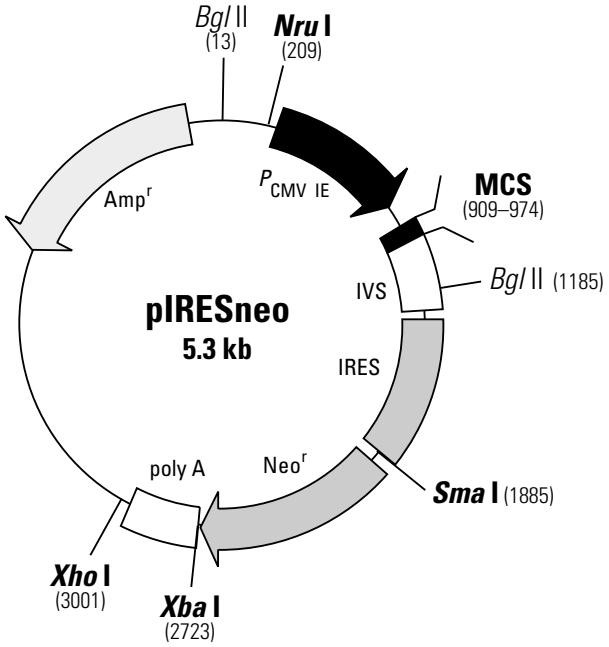

900 910 920 930 940 950 960 970

CGAGCTCGGATCGATATCTGCGGCCGCGTCGACGGAATTCAGTGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAA

Cla I* *Eco*R V *Not* I *Eco*R I *Bam*H I *Bst*X I

Restriction Map and Multiple Cloning Site (MCS) of pIRESneo 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

pIRESneo (originally described as pCIN4 by Rees *et al.;* 1) 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 (2, 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. The expression cassette of pIRESneo contains the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed by a multiple cloning site (MCS), 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 either at the 5' end to translate the gene of interest or at the ECMV IRES to translate the antibiotic resistance marker.

Use

When using the pIRESneo Vector, the antibiotic exerts selective pressure on the whole expression cassette; thus, a high dose of antibiotic will select only 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 of G418 (#8056-1) depending on the cell line (be sure to establish a kill curve for each lot of G418 to determine optimal selection concentration).

(PR93695; published 18 March 1999)

Location of features

- $P_{CMV\ IE}$ promoter: 232–820
- Multiple cloning site (MCS): 909–974
- Synthetic intron (IVS): 974–1269
- Internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV): 1295–1880
- Neomycin phosphotransferase gene (NPT II): 1906–2709
- Fragment containing the bovine growth hormone poly A signal: 2710–2998
- Ampicillin resistance gene: 5117–4257

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.

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**(4):937–947.

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.