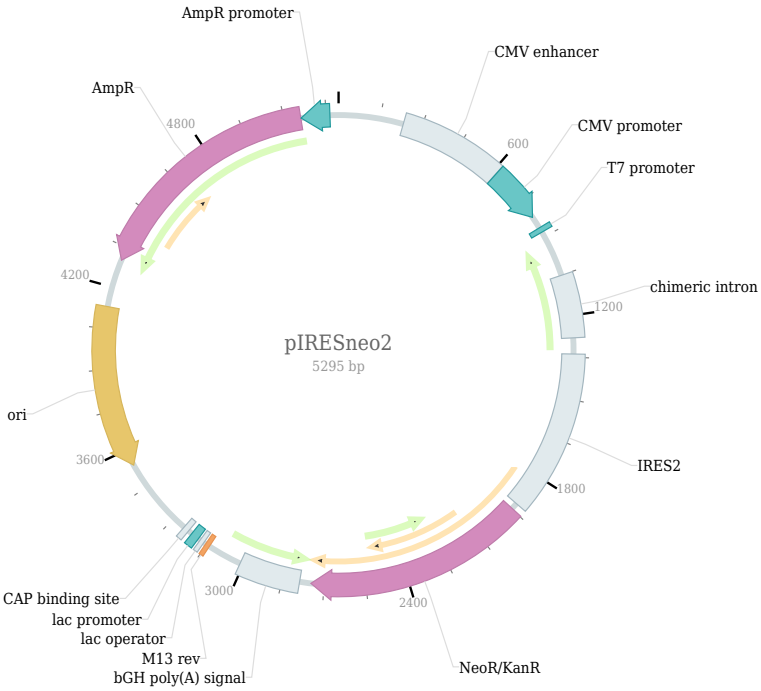
pIRESneo2

Vector ID	V001630
Antibiotic Resistance	Ampicillin
Length	5295 bp
Vector Type	Mammalian Expression Vectors

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Promoter	CMV
5' Primer	CMV-F: 5'-CGCAAATGGGCGGTAGGCGTG-3'
3' Primer	pIRESrv: 5'-GCCCTAGATGCATGCTCG-3'
Comment	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.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.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.