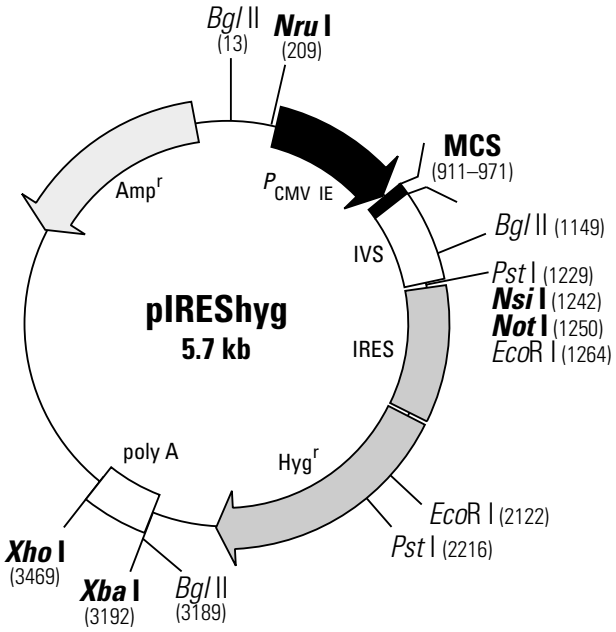
Vector Information

pIREShyg Vector Information PT3059-5

GenBank Accession #: U89672 Catalog #6061-1



Restriction map and multiple cloning site (MCS) of pIREShyg Vector. Unique restriction sites are in bold.

Description

pIREShyg 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 hygromycin B, 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 pIREShyg 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 hygromycin B phosphotransferase 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 pIREShyg 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 250–600 µg/ml of Hygromycin B (#8057-1) depending on the cell line (be sure to establish a kill curve for each lot of Hygromycin B to determine optimal selection concentration).

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Location of features

- $P_{CMV\ IE}$ promoter: 232–820
- Synthetic intron (IVS): 938–1233
- Internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV): 1270–1856
- Hygromycin B phosphotransferase gene: 1869–2903
- Fragment containing the bovine growth hormone poly A signal: 3192–3468
- Ampicillin resistance gene: 5584–4727

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

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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.