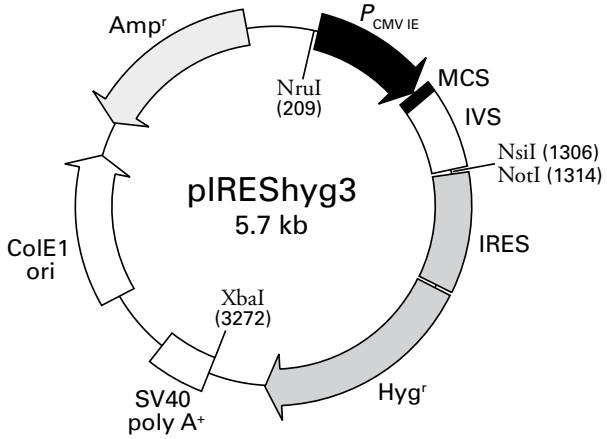
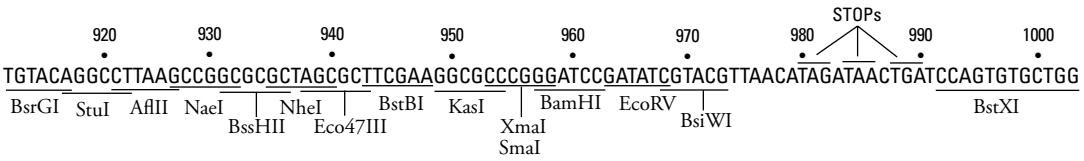
Vector Information

pIREShyg3 Vector Information

PT3644-5

Cat. No. 631620





pIREShyg3 Vector Map and Multiple Cloning Site (MCS). All sites shown are unique.

Description

pIREShyg3 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. To select for cells that express high levels of the gene of interest, the selective pressure for antibiotic resistance was increased by shifting the hygromycin B phosphotransferase gene downstream to a less optimal position for translation as directed by the IRES sequence (3). By decreasing the level of expression of the antibiotic resistance marker, the selective pressure on the entire expression cassette is increased, resulting in selection for cells that express the entire transcript, including the gene of interest, at high levels.

The expression cassette of pIREShyg3 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 hygromycin B phosphotransferase gene, and the polyadenylation signal from SV40. 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.

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Use

When using the pIREShyg3 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 250–600 µg/ml Hygromycin B (Cat. No. 631309) depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of Hygromycin B to determine optimal selection concentration.

Location of features

- Human cytomegalovirus (CMV) major immediate early promoter
  Enhancer region: 309–715; TATA Box: 804–810
- T7 RNA polymerase promoter: 863–879
- Multiple cloning site (MCS): 912–1003
- Synthetic intron (IVS): 1002–1297
- Internal ribosome entry site (IRES) from encephalomyocarditis virus (ECMV): 1333–1924
- Hygromycin B phosphotransferase gene: 1948–2979
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 3421–3426 & 3450–3455; mRNA 3' ends: 3459 & 3471
- ColE1 origin of replication: 4012–4611
- Ampicillin resistance (β-lactamase) gene:
  Promoter: –35 region: 5703–5698; –10 region: 5680–5675
  β-lactamase coding sequence:
  Start codon: 5633–5631; stop codon: 4775–4773

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: ColE1
- Copy number: high

References

1. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
2. Jang, S. K., *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
3. Rees, S., *et al.* (1996) *BioTechniques* **20**(1):102–104.
4. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):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 Laboratories, Inc. This vector has not been completely sequenced.