PT3646-5 Catalog No. 631619



Eco47III				
EcoRV	Nhe	AflII	AgeI	
CGATATCTGC	GGCCTAGCTA	GCGCTTAAGG	CCTGTTAACC GGT	CGTACGT
GCTATAGACG	CCGGATCGAT	CGCGAATTCC	GGACAATTGG CCAC	GCATGCA
BstBI BamHI				
BspEI	EcoRI	NotI	Stop Stop Stop	BstXI
CTCCGGATTC	GAATTCGGAT	CCGCGGCCGC	ATAGATAACT GATO	CCAGTGT GCTGGA
GAGGCCTAAG	CTTAAGCCTA	GGCGCCGGCG	TATCTATTGA CTAC	GGTCACA CGACCT
	CGATATCTGC GCTATAGACG Bs BspEI CTCCGGATTC	EcoRVNheCGATATCTGCGGCCTAGCTAGCTATAGACGCCGGATCGATBstBIBamilBspEIEcoRICTCCGGATTCGAATTCGGAT	EcoRVNhelAfIIICGATATCTGCGGCCTAGCTAGCGCTTAAGGGCTATAGACGCCGGATCGATCGCGAATTCCBstBIBamHIBspEIEcoRINotICTCCGGATTCGAATTCGGATCCGCGGCCGC	EcoRV Nhel AflII Agel CGATATCTGC GGCCTAGCTA GCGCTTAAGG CCTGTTAACC GGT GCTATAGACG CCGGATCGAT CGCGAATTCC GGACAATTGG CCAC BstBI BamHI BspEI EcoRI NotI Stop Stop

pIRESpuro3 Vector Map and Multiple Cloning Site (MCS). All sites shown are unique.



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Description

pIRESpuro3 is a bicistronic mammalian expression vector that allows you to simultaneously express a protein of interest and puromycin resistance (Puro¹) from a single, bicistronic mRNA transcript. Selective pressure exerted by puromycin on the entire expression cassette ensures that: a high dose of antibiotic will select for cells expressing a high level of the gene of interest; and expression of the gene of interest will be stable over time (1). After selection with puromycin, nearly all surviving colonies will stably express the gene of interest, decreasing the need to screen large numbers of colonies for functional clones.

Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter (P_{CMVIE}), located upstream of the multiple cloning site (MCS). An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), positioned between the MCS and the puromycin-N-acetyl-transferase gene (Puro^r; 2), facilitates capindependent translation of Puro^r from an internal start site at the IRES/Puro^r junction (3, 4). Between the MCS and the IRES, the vector contains stop codons in all three reading frames, and a synthetic intron known to enhance the stability of the mRNA (IVS; 5).

(PR0Z3757; published 8 December 2010)

pIRESpuro3

Use

We recommend selecting mammalian clones in 5–10 μ g/ml puromycin (Cat Nos. 631305 & 631306), depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of puromycin to determine the optimal effective dose. Unless your expression experiments require a pure population of cells, you can use the pool of cells that survive selection instead of isolating and characterizing clonal cell lines.

Location of features

- P_{CMV IE} (human cytomegalovirus immediate early promoter) Enhancer region: 309–715; TATA Box: 804–810
- P_{T7} (T7 RNA Polymerase promoter): 863–879
- MCS (multiple cloning site): 912–1015
- IVS (synthetic intron): 1015–1310
- IRES (encephalomyocarditis virus internal ribosome entry site): 1336–1926
- Puro^r (puromycin resistance gene; puromycin-N-acetyl-transferase): 1959–2555
- SV40 early polyadenylation signals: 2822-2827 & 2851-2856
- pUC origin of replication: 3411-4010
- Amp^r (ampicillin resistance gene; β-lactamase): 4172–5032 (complementary)

Propagation in *E. coli*

- Suitable host strains: DH5 α^{TM} and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

- 1. Rees, S., et al. (1996) BioTechniques 20(1):102–104.
- 2. de la Luna, S., et al. (1988) Gene 62(1):121-126.
- 3. Jackson, R. J., et al. (1990) Trends Biochem. Sci. **15**(12):477–483.
- 4. Jang, S. K., *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
- 5. Huang, M.T. F. & Gorman, C. M. (1990) Nucleic Acids Res. 18(4):937–947.

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