

pORF-LacZ

An expression vector containing the *E. coli* LacZ reporter gene

Catalog # porf-lacz

For research use only

Version # 02G10-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pORF-LacZ.
- GT100 genotype is: *F*-, *mcrA*, Δ (*mrr-hsdRMS-mcrBC*), Φ 80lacZ Δ M15, *MacX74*, *recA1*, *endA1*.
- 4 pouches of *E. coli* FastMedia™ Amp.

Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* FastMedia™ Amp at room temperature. FastMedia™ pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pORF is a ready-made expression vector containing a gene of interest.

pORF may be used for:

Obtaining a gene to subclone into another vector. Two unique restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Gene expression in mammalian cells. Cells may be transiently transfected with pORF. The secreted protein may be harvested in the cell culture supernatant as all secreted proteins in pORF possess a signal sequence.

LacZ gene may be cut out by using *Bam*H I and *Nhe* I enzymes

*Bam*H I is compatible with *Bgl* II, *Bst*Y I, *Bcl* I and *Dpn* II.

Nhe I is compatible with *Xba* I, *Spe* I, and *Avr* II.

PLASMID FEATURES

• **EF-1 α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1 α (EF-1 α) promoter¹ and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1 α utilizes a type 2 promoter that encodes for a "house keeping" gene. The promoter is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat² has been coupled to the EF-1 α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **Intron II17 5'UTR:** InvivoGen utilizes an inducible promoter for the second transcriptional unit that is spliced out as an intron in mammalian cells. *LacI* expression causes overproduction of Lac repressor protein acting on the bacterial promoter to repress the expression of the gene. This safeguard is essential when the second transcription gene product is toxic to *E. coli*. Treatment with IPTG enables the expression of the second transcription unit in bacteria constitutively expressing *LacI*.

• **LacZ Gene**

LacZ gene from the ATG to the stop codon.

Size: 3054 bp

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **Amp (ampicillin resistance gene):** The ampicillin resistance gene allows the selection of bacteria carrying the pORF plasmid.

References

1- Kim et al (1990). Gene 2: 217-223.

2- Takebe et al (1988). Mol. Cell Biol. 1: 466-472.

METHODS

Growth of pORF-transformed bacteria:

Use sterile conditions to do the following:

- 1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
- 2- Streak bacteria taken from this suspension on an ampicillin LB agar plate prepared with the *E. coli* FastMedia™ Amp agar provided (see below).
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with ampicillin using the FastMedia™ Amp liquid provided (see below).
- 5- Extract the pORF plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* FastMedia™ Amp:

E. coli FastMedia™ Amp is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Amp is a TB (liquid) or LB (solid) based medium with ampicillin, and contains stabilizers.

E. coli FastMedia™ Amp can be ordered separately (catalog code # fas-am-1, fas-am-s, fas-am-x).

Method:

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave FastMedia™.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

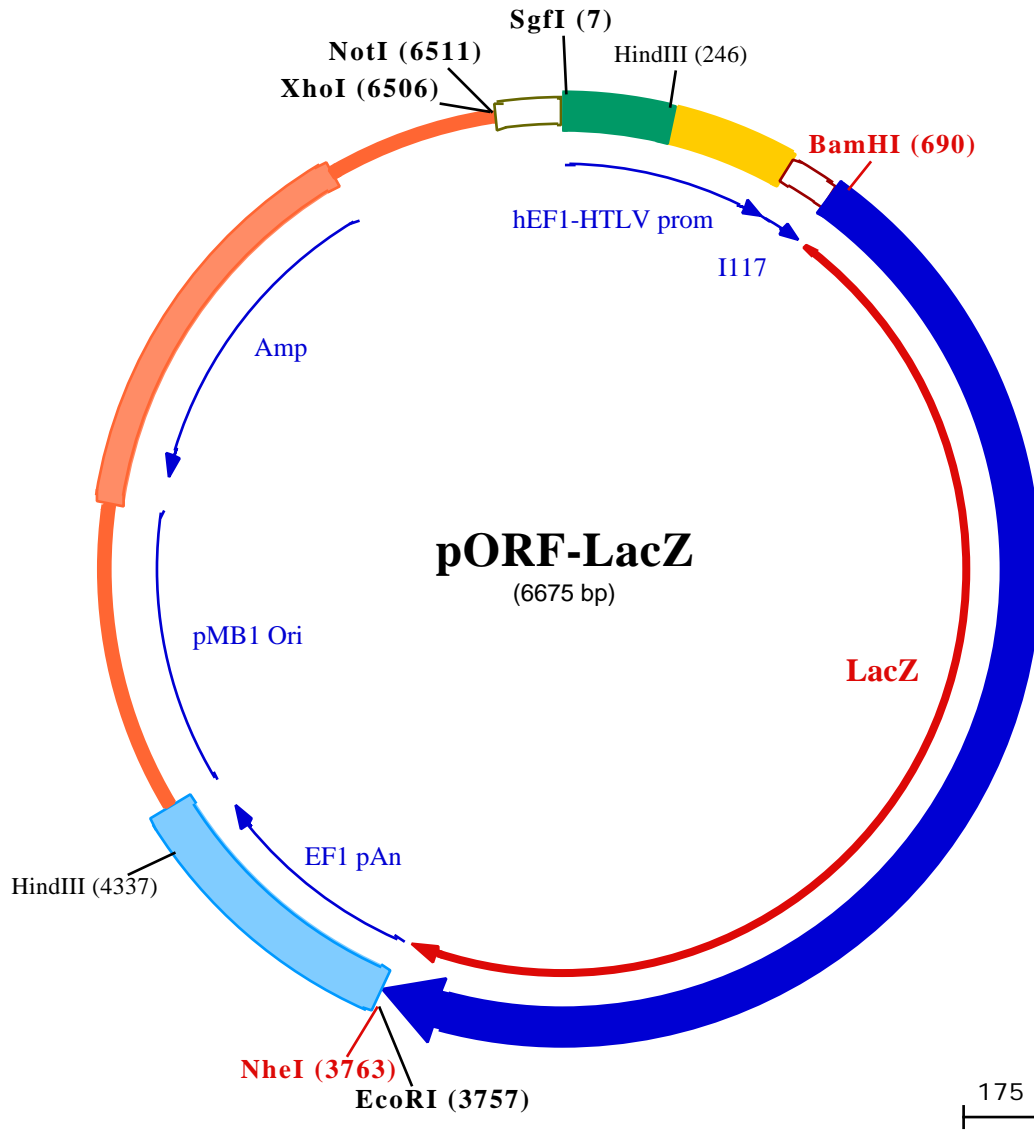
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SgfI (7)

1 GGATCTGCGATCGTCCGGTCCCGTCACTGGGCGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGGCAATTGAACCGGTGCCTA

101 GAGAAAGTGGCGCGGGTAAACTGGGAAAAGTATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)

201 GTGAACGTTCTTTTTTCGCAACGGGTTTGGCCGAGAACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGGTCTTCGCCGCTCCCGCTGTGGTGCCTCCTGAACTGCGTCCCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCCACGCTTTGCCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCGCTACgt aagt gat at ct act agatt t at caaaaagagt gtt gact tgt gagcgt caca

BamHI (690)

601 ttgatacttagattcatcgagaggacacgtcgactactaaccttcttctcttctcctacagCTGAGATCACCGGTAGGAGGGCCATCATGGATCCCGTCC

1 MetAspProValV

701 TTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCG

801 CACCGATCGCCCTTCCCAACAGTGGCGCAGCCTGAATGGCGAATGGCGCTTTGCTGGTTCCGGCACAGAAAGCGGTGCCGAAAGCTGGCTGGAGTGC

901 GATCTTCTGAGGCGGATACTGTCGTGTCCTCAAACTGGCAGATGCACGGTTACGATGGCGCCATCTACACCAACGTAACCTATCCATTACGGTCA

1001 ATCCGCGTGTGTTCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATATTTT

1101 TGATGGCGTAACTCGCGTTCATCTGTGGTGAACGGGGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCATT

1201 TTACGGCCGGAGAAAACCGCTCGCGGTGATGGTGCCTGGAGTGACCGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGATTTTCC

1301 GTGACGTCGTTGCTGCATAAAACCGACTACACAACTCAGCAGATTTCCATGTTGCCACTCGCTTTAATGATGATTTACGGCGCTACTGGAGCTGA

1401 AGTTCAGATGTGCGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTAAACCGCAGGTCGCCAGCGGCACCGCGCTTTCGGCGGT

1501 GAAATTCGATGAGCGTGGTGGTTATGCCGATCGGTCACACTACGCTGAAACGTCGAAAACCCGAAACTGTGGAGCGCGAAATCCCAATCTCTATC

1601 GTGCGGTGGTTGAACGTCACACCGCCGACGGCAGCGTGATTGAAGCAGAAGCCTGGCATGTCGGTTTCCGGAGGTGCGGATTGAAAATGGTCTGCTGCT

1701 GCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCAGGAGCATATCCTCTGCATGGTCAGGTCATGGATGAGCAGCAGTGGTGACGATATC

1801 CTGCTGATGAAGCAGAACAACCTTAAACGCGCTGCGCTTCCGATTATCCGAAACCTCCGCTGGTACACGCTGTGCGACCGCTACGGCCTGATGTGG

1901 TGGATGAAGCCAATATTGAAACCCACGGCATGGTGCATGAATCGTCTGACCGATGATCCGCGCTGGTACCGCGATGAGCGAACCGGTAACCGGAAT

2001 GGTGCAGCGCATCGTAATCACCGAGTGTGATCATCTGGTCGGGGAATGAATCAGGCCAGCGCGCTAATCACGACCGCTGTATCGCTGGATCAA

2101 TCTGTGATCCTTCCCGCCCGTGCAGTATGAAGGGCGGAGCCGACACCAGCCACCGATATTATTTGCCCGATGTACGCGCGGTGATGAAGACC

2201 AGCCCTTCCCGCTGTGCCAATGGTCCATCAAAAATGGCTTTCGCTACCTGGAGAGACCGCCCGCTGATCCTTTGCGAATACGCCACGCGATGGG

2301 TAACAGTCTGGCGTTTCGCTAAACTGCGAGCTGGCGGTTCTCGTACATCCCGTTTACAGGCGGCTTCGCTGGAGCTGGGTGGATCAGTCCGCTGATT

2401 AAATATGATGAAAACCGCAACCGTGGTGGCTTACGGCGGTGATTTTGGCGATACGCCAAGCATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCG

2501 ACCGCACGCCCATCCAGCGTGCAGGAAGCAAAACACCAGCAGCAGTTCCTCAGTTCGTTTATCCGGGCAACCATCGAAGTGACCAGCGAATACCT

2601 GTTCCGTCATAGCGATAACGAGCTCCTGCAGTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCTCTGGATGTCGCTCCACAAGT

2701 AAACAGTTGATTGAACCTGCTGAACCTACCGCAGCCGAGAGCGCCGGCAACTCTGGCTCACAGTACGCGTGTGCAACCGAACCGCAGCCGATGGTCAG

2801 AAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGCTGGCGGAAAACCTCAGTGTGACGCTCCCGCCGCTCCACGCCATCCCGATCTGACCCAGC

2901 CGAAATGGATTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCAGTCAGGCTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACG

3001 CTGACCGCGTGGCGATCAGTTCACCGTGCACCGCTGGATAACGACATGGCGTAAGTGAAGCGACCCGATTCACCTAACCGCTGGCTCGACCGT

3101 GGAAGGCGGCGGCCATTACCAGGCGGAAGCAGCGTGTGTCAGTGCAGCGCAGATACACTTGTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCA

3201 GCATCAGGGGAAAACCTTATTTATCAGCCGAAAACCTACCGGATGATGGTAGTGGTCAAAATGGCGATTACCGTGTGATGTTGAAGTGGCGAGCGATACA

3301 CCGCATCCGGCGGATTTGGCCTGAACCTGACGCTGGCGAGGTAGCAGGCGGGTAAACTCGGATTAGGGCCGAAGAAAACCTACCGACCGCC

3401 TTAAGCGCGCTGTTTGGACCGCTGGGATCTGCCATTTGTCAGACATGTATACCCCGTACGCTTCCCGAGCGAAAACGGTCTGGCTGGCGGACCGCGCA

3501 ATTGAATTTAGCCACACCGTGGCGCGGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAACCGACCGATCCGATCTGCTG

3601 CACGCGGAAGAAGGCACATGGCTGAATATCGACGCTTTCATATGGGGATTGGTGGCGAGCAGTCTGGAGCCCGTCAAGTATCGCGGGAATACAGCTGA

972 HisAlaGluGluGlyThrTrpLeuAsnI leAspGlyPheHisMetGlyI leGlyGlyAspAspSerTrpSerProSerValSerAlaGluLeuGlnLeuS

NheI (3763)

EcoRI (3757)

3701 GCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAAATAATAATCTAGTCGAGAATTCGCTAGCATTATCCCTAATACCTGCCACCCACTCTTAA
 1005 ▶erAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys•••
 3801 TCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTCATTTGGCCATTAAAGTTTAGTAGTAAAAGACTGGTAAATGATAACAATGCATCGTAAAACCTT
 3901 CAGAAGGAAAGGAGAATGTTTTGTGGACCACCTTTGGTTTTCTTTTTTGCCTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAA
 4001 ACAACTTGACCAAAAAATTTGTACAGAATTTTGAGACCCATTAATAAAGTTAAATGAGAAACCTGTGTGTTCTTTTGGTCAACACCGAGACATTTAGGTG
 4101 AAAGACATCTAATTCTGGTTTTACGAATCTGGAACTTCTTGAAAATGTAATCTTGAGTTAACACTCTGGGTGGAGAATAGGGTTGTTTTCCCCCAC
 4201 ATAATTGGAAGGGAAGGAATATCATTAAAGCTATGGGAGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTA

HindIII (4337)

4301 AAACAGGCCAAAACTGAGTCCTTGGGTTGCATAGAAAAGCTTCATGTTGCTAAACCAATGTTAAGTGAATCTTTGGAAACAAAAATGTTTCCAAATTACTG
 4401 GGATGTGCATGTTGAAACGTGGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGGTGTGCGCCTTTTCCA
 4501 TAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGCTTCCCCCTGGA
 4601 AGCTCCCTCGTGCCTCTCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGCT
 4701 GTAGGTATCTCAGTTCGGTGTAGGTCTGCTCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCG
 4801 TCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC
 4901 TTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT
 5001 GATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTT
 5101 TTCTACGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCTAGAGATTATCAAAAAGGATCTTACCTAGATCCTTTAAATTA
 5201 AAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTAT
 5301 TTCGTTCCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTCTGCAATGATACCGCGAGACCC
 5401 ACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATT
 5501 AATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGC AACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTG
 5601 GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGT
 5701 TGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACT
 5801 GGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGGCCACATAGCAGAA
 5901 CTTTAAAAGTGCTCATATTGAAAAACGTTCTTCGGGGGAAAACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACC
 6001 CAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAAAGGAAAGGCAAAATGCCGCAAAAAAGGAATAAGGGCGACCGGAAA
 6101 TGTTGAATACTCATACTCTTCTTTTCAATATATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATA
 6201 AACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCAC
 6301 GAGGCCCTTTCGTCTCGCGCTTTCGGTGTGACGGTGAACCTCTGACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGG
 6401 AGCAGACAAGCCCGTCAGGGCGCTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATA

NotI (6511)

XhoI (6506)

6501 TGGATCTCGAGCGCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACA
 6601 AAACGAAACAAAAAACTAGCAAAATAGGCTGTCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA