

pORF39-Pac

An expression vector containing the Puromycin resistance gene (Pac)

Catalog # porf-pac

For research use only

Version # 03L24-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pORF39-Pac.
- GT100 genotype is: *F-*, *mcrA*, $\Delta(mrr-hsdRMS-mcrBC)$, $\emptyset 80lacZ\Delta M15$, *MacX74*, *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® 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* Fast-Media® Amp at room temperature. Fast-Media® 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.

Pac gene may be cut out by using *BspH I* and *Nhe I* enzymes

BspH I is compatible with *Nco I* and *BspLU11 I*.

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.

• **EM7** is a bacterial promoter that enables the constitutive expression of the Puromycin resistance gene in *E. coli*.

• **Pac Gene**

Puromycin resistance gene (intronless ORF) from the ATG to the stop codon.

Size: 597 bp

- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.³
- **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.
- 3- Carswell et al (1989). *Mol. Cell Biol.* 10: 4248-4258.

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* Fast-Media® 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 Fast-Media® Amp liquid provided (see below).
- 5- Extract the pORF plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* Fast-Media Amp:

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

E. coli Fast-Media® 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** Fast-Media®.
- 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 Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

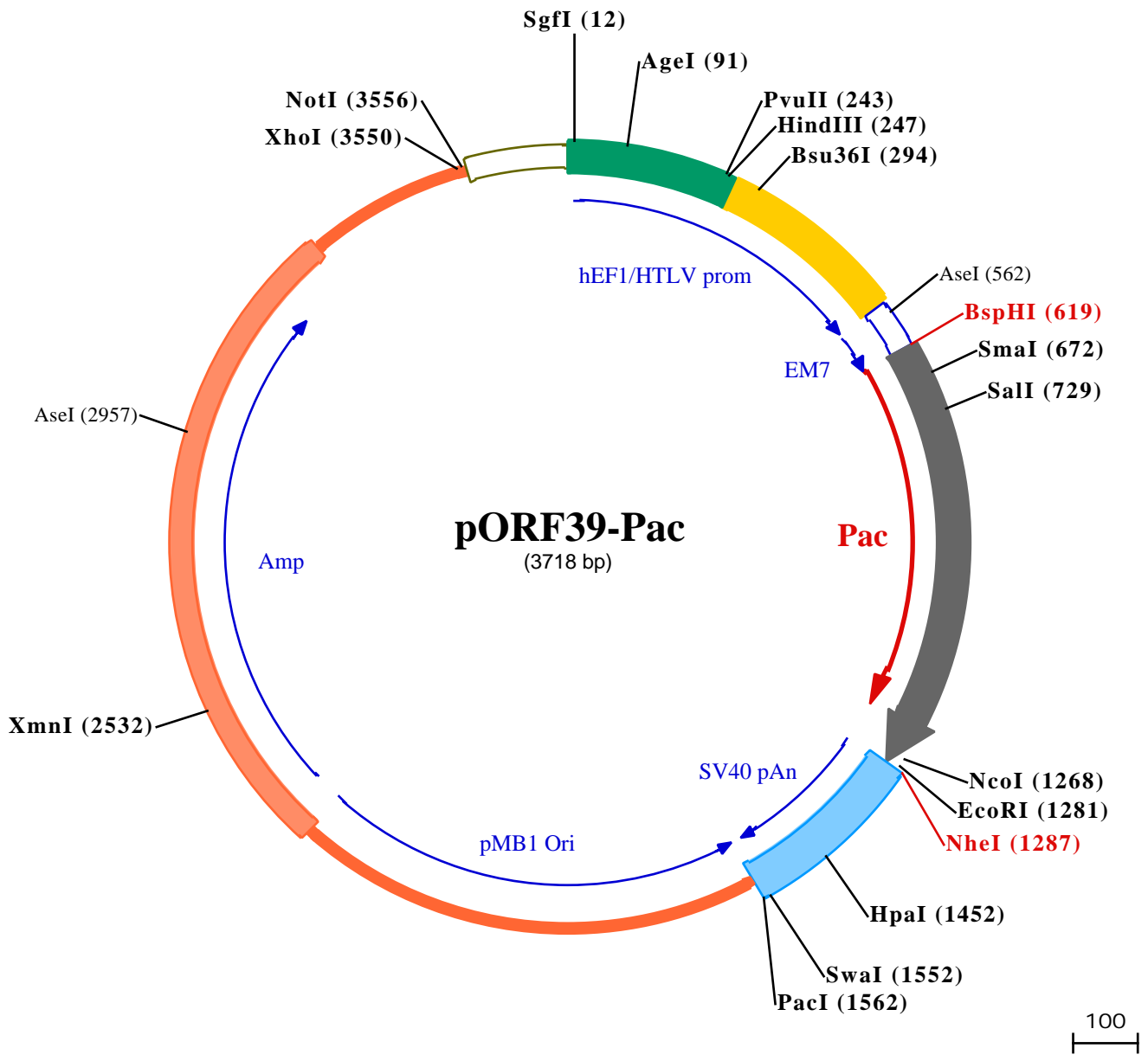
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SgfI (12) **AgeI (91)**
1 GGATCTCGCATCGCTCCGGTCCCCGTCAGTGGGCAGAGCCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACCGGTGCCTA
101 GAGAAGGTGGCGGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (247) **PvuII (243)** **Bsu36I (294)**
201 GTGAACGTTCTTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCGCTACCTGAGGCC
301 GCCATCCACGCGGTTGAGTCGCGTCTGCGCCTCCCGCTGTGGTGCTCTGAACTGCGTCCGCGCTAGGTAAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGCTTTTGTTCGTTT

AseI (562)
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCCTACAAACAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGAC

BspHI (619) **SmaI (672)**
601 TCACTATAGGAGGCCATCATGACCGAGTACAAGCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCCGGGGCCGTACGCACCTCGCCGCCGCTTC
1→MetThrGluTyrLysProThrValArgLeuAlaThrArgAspAspValProArgAlaValArgThrLeuAlaAlaAlaPhe
SalI (729)
701 GCCGACTACCCCGCCACGCGCCACACCGTCGACCCGACCCGACCCGACCTCGAGCGGGTCAACGAGTGCAGAAGTCTTCTCAGCGCGTCCGGCTCGACA
28▶AlaAspTyrProAlaThrArgHisThrValAspProAspArgHisIeGluArgValThrGluLeuGlnGluLeuPheLeuThrArgValGlyLeuAspI
801 TCGGCAAGGTGTGGTTCGCGGACGACGCGCGCGGTGGCGGTCTGACCACCGCGAGAGCGTGAAGCGGGGGCGGTGTTCCGCCGAGATCGGCCCGCG
61▶IeGlyLysValTrpValAlaAspAspGlyAlaAlaValAlaValTrpThrThrProGluSerValGluAlaGlyAlaValPheAlaGluIeGlyProAr
901 CATGGCCGAGTTGAGCGGTTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCCTCTGGCGCGCACCGGCCAAGGAGCCCGGTGTTCTGGCCACC
94▶gMetAlaGluLeuSerGlySerArgLeuAlaAlaGlnGlnGlnMetGluGlyLeuLeuAlaProHisArgProLysGluProAlaTrpPheLeuAlaThr
1001 GTCGGCGTCTCGCCCGACCAAGGCAAGGGTCTGGGACGCGCTCGTCCCGGAGTGGAGCGCGCCGAGCGCGCGGGTGGCCGCTTCTCGG
128▶ValGlyValSerProAspHisGlnGlyLysGlyLeuGlySerAlaValValLeuProGlyValGluAlaAlaGluArgAlaGlyValProAlaPheLeuG
1101 AGACTCCGCGCCCGCAACCTCCCTTCTACGAGCGGCTCGGCTTACCGTCAACCGGACGTCGAGGTGCCGAAAGGACCGCGCACCTGGTGCATGAC
161▶IuThrSerAlaProArgAsnLeuProPheTyrGluArgLeuGlyPheThrValThrAlaAspValGluValProGluGlyProArgThrTrpCysMetTh
NheI (1287)
NeoI (1268) **EcoRI (1281)**
1201 CCGCAAGCCCGTGCCTGACGCCCGCCACGACCCGACGCGCCGACCGAAAGGAGCGCACGACCCATGGCGCGCTGAATTCGTAGCTCGACATGA
194▶rArgLysProGlyAla•••
1301 TAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATT

HpaI (1452)
1401 TGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGT

PacI (1562) **SwaI (1552)**
1501 GGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAGATCCATTAAATGTTAATTAGAACATGTGAGCAAAAGGCCAGAAAAGGCCAGGAA
1601 CCGTAAAAAGGCCCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
1701 GGACTATAAAGATACCAGCGTTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTACCGGATACCTGTCCGCTTTCTCCCTT
1801 CGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGGTTCA
1901 GCCCGACCGCTGCGCCTTATCCGTAACCTATCGTCTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC
2001 AGAGCGAGGTATGTAGCGGTGTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGC
2101 CAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAG
2201 AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGCATGAGACA
2301 ATAACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCACATTTCCGTGTCGCCCTTATCCCTTTTTTGGCGCATTTCCTT
1▶MetSerIeGlnHisPheArgValAlaLeuIeProPhePheAlaAlaPheCysLeu
2401 CCTGTTTTTGTCTACCCAGAAACCGTGGTAAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCAGGTTACATCGAACTGGATCTCAACAGCGGTA
20▶ProValPheAlaHisProGluThrLeuValLysValLysAspAlaGluAspGlnLeuGlyAlaArgValGlyTyrIeGluLeuAspLeuAsnSerGlyL
XmnI (2532)
2501 AGATCCTTGAGAGTTTTCCGCCGGAAGACGTTTTCCAATGATGAGCACTTTTTAAAGTCTGCTATGTGGCGGGTATTATCCCGTATTGACGCGGGCA
53▶yslIeLeuGluSerPheArgProGluGluArgPheProMetMetSerThrPheLysValLeuLeuCysGlyAlaValLeuSerArgIeAspAlaGlyGI
2601 AGAGCAACTCGGTCCGCCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCCAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAA
86▶nGluGlnLeuGlyArgArgIeHisTyrSerGlnAsnAspLeuValGluTyrSerProValThrGluLysHisLeuThrAspGlyMetThrValArgGlu
2701 TTATGCAGTGTGCCATAACCATGAGTGATAACACTGCGGCAACTTACTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACA
120▶LeuCysSerAlaAlaIeThrMetSerAspAsnThrAlaAlaAsnLeuLeuLeuThrThrIeGlyGlyProLysGluLeuThrAlaPheLeuHisAsnM
2801 TGGGGATCATGTAACCTGCCTTGTATCGTTGGGAACCGGAGTGAATGAAGCCATACCAACGACGAGCGTGACACCAGATGCCTGTAGCAATGGCAAC
153▶etGlyAspHisValThrArgLeuAspArgTrpGluProGluLeuAsnGluAlaIeProAsnAspGluArgAspThrThrMetProValAlaMetAlaTh
AseI (2957)
2901 AACGTTGCGCAACTATTAACCTGCGCAACTACTTACTCTAGCTTCCCGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTG
186▶rThrLeuArgLysLeuLeuThrGlyGluLeuLeuThrLeuAlaSerArgGlnGlnLeuIeAspTrpMetGluAlaAspLysValAlaGlyProLeuLeu
3001 CGCTCGGCCCTCCGGCTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGGCGTGGGTCTCGCGGTATCATTGAGCACTGGGCCAGATGTAAGC
220▶ArgSerAlaLeuProAlaGlyTrpPheIeAlaAspLysSerGlyAlaGlyGluArgGlySerArgGlyIeIeAlaAlaLeuGlyProAspGlyLysP
3101 CCTCCGATCGTAGTTATCTACACGACGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTG
253▶roSerArgIeValValIeTyrThrThrGlySerGlnAlaThrMetAspGluArgAsnArgGlnIeAlaGluIeGlyAlaSerLeuIeLysHisTr

3201 GTAACGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACCTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTGATAATCTC
286▶ p...
3301 ATGCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG
3401 GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTTCGGGGCTGGCTTAACTATG

NotI (3556)

XhoI (3550)

3501 CGGCATCAGAGCAGATTGTAAGTGCACCATATGGTGACCGGATCTCGAGCGGCCGCAATAAAAATATCTTTATTTTCATTACATCTGTGTGTTGGT
3601 TTTTGTGTAATCGTAACTAACATAGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCA
3701 GAACATTCTCTATCGAA