

# pORF9-mcs

An expression vector containing a multiple cloning site.

Catalog # porf9-mcs

For research use only

Version # 05B04-SV

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pORF9-mcs.  
- GT100 genotype is: *F-*, *mcrA*,  $\Delta$ (*mrr-hsdRMS-mcrBC*),  $\Delta$ 80*lacZ* $\Delta$ M15,  $\Delta$ *lacX74*, *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® is 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

pORF9-mcs is a ready-made expression vector containing the hybrid EF1 $\alpha$ /HTLV promoter and a multiple cloning site.

pORF9-mcs may be used for:

**Cloning in a gene of interest.** Six unique restriction sites comprise the MCS facilitating cloning of genes. Cloned genes will be under the control of the EF1 $\alpha$ /HTLV promoter.

**As an "empty" control vector.** Since pORF9-mcs does not contain a therapeutic gene, it can be used in conjunction with other vectors of the pORF9 family to serve as an experimental control.

## PLASMID FEATURES

• **EF-1 $\alpha$  / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1 $\alpha$  (EF-1 $\alpha$ ) promoter<sup>1</sup> and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1 $\alpha$  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 Repeat<sup>2</sup> has been coupled to the EF-1 $\alpha$  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.

• **Multiple cloning site.**

The MCS contains the restriction sites *Sal I*, *BamH I*, *Eco47 III*, *Pst I*, *Nco I*, and *Nhe I*.

*Sal I* is compatible with *Ava I* and *Xho I*.

*BamH I* is compatible with *Bgl II*, *BstY I* and *Bcl I*.

*Eco47 III* is compatible with any other blunt-end restriction enzyme.

*Pst I* is compatible with *Bsp1286 I*, *Nsi I*, and *Sbf I*.

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

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

• **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.<sup>3</sup>

• **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.

**Note:** For long-term storage of the pORF-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

### 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-l, 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

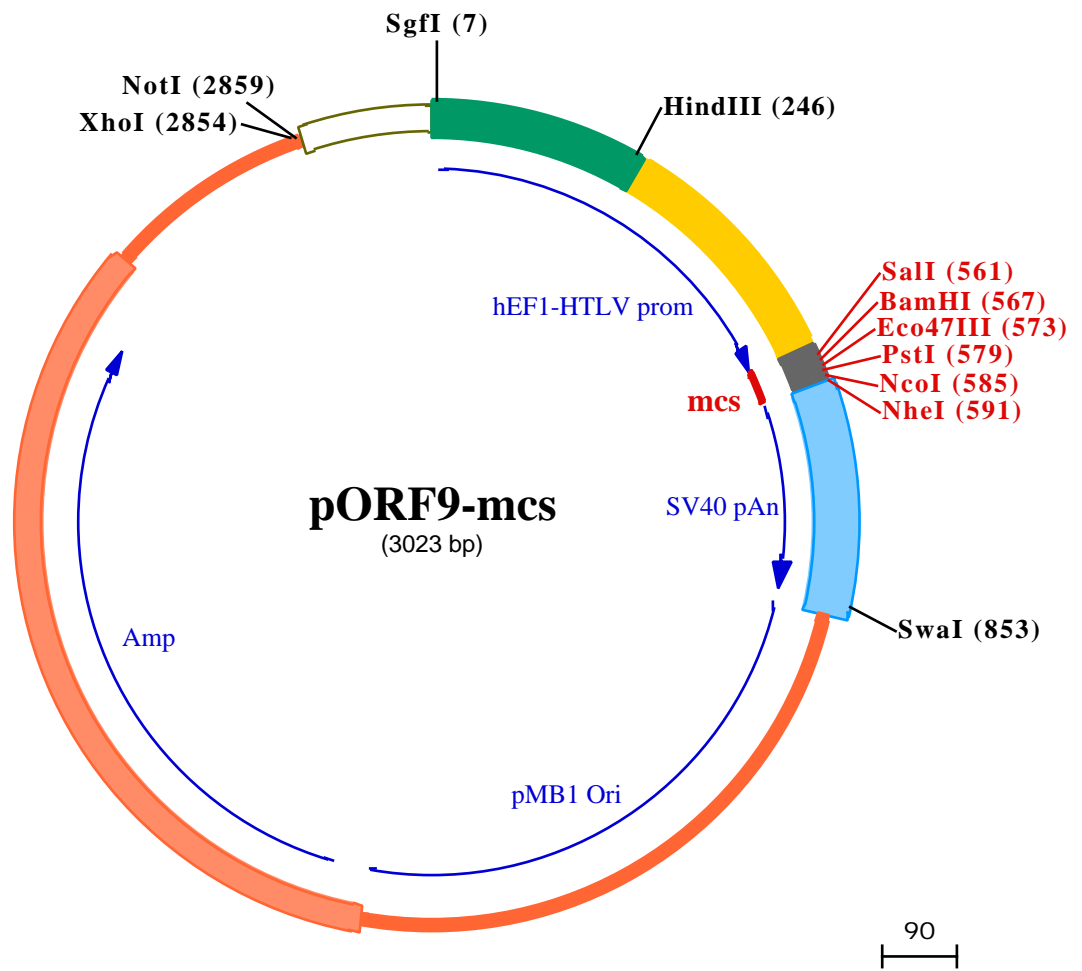
Outside US: (+1) 858-457-5873

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

Website: [www.invivogen.com](http://www.invivogen.com)



3950 Sorrento Valley Blvd. Suite A  
San Diego, CA 92121 - USA



SgfI (7)

1 GGATCTGCGATCGCTCCGGTCCCGTCCAGTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTTCGGCAATTGAACCGGTCCCTA

101 GAGAAGGTGGCGCGGGTAAACTGGGAAAAGTATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)

201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGGCTTCTGCCGCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTCCTGACCCTGCTTGCTCAACTCTACGCTTTTGTTCGTTT

BamHI (567) PstI (579) NheI (591)
SalI (561) Eco4VII (573) NcoI (585)

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCCCTACCTGAGATCACCGCGTGTGACGGATCCAGCGCTCTGCAGCCATGGCTAGCTCGA

601 CATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTGTG

701 AAATTTGTGATGCTATTGCTTTATTGTAAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTACAGGGGA

SwaI (853)

801 GGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAGAACATGTGAGCAAAAAGCCAGCAAAAAGGCC

901 AGGAACCGTAAAAAGGCCGCTGTGCTGGCGTTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC

1001 CGACAGGACTATAAAGATACCAGCGTTTCCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTTCT

1101 CCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGTTCGCTCAAGTCGGGTGTGTGCACGAACCCCC

1201 GTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA

1301 TTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT

1401 GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCAGCGTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACG

1501 CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTGGTCATGCATG

1601 AGACAATAACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGATATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTGGCGCATTTT

1 MetSerI leGlnHisPheArgValAlaLeuI leProPhePheAlaAlaPheC

1701 GCCTTCCTGTTTTTGTCAACCCAGAAACGCTGGTGAAGTAAAGATGCTGAAGATCAGTTGGGTGCAGAGTGGGTACATCGAAGTGGATCTCAACAG

1801 CGGTAAGATCCTTGAGAGTTTTTCGCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGGGTATTATCCCGTATTGACGCC

1901 GGGAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCCAGTACAGAAAAAGCATCTTACGGATGGCATGACAGTAA

2001 GAGAATTATGCAGTGTGCCATAACCATGAGTGATAAACAAGTCCGCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGGCA

2101 CAACATGGGGATCATGTAACCTGCCTTGATCGTTGGGAACCGAGCTGAATGAAGCCATACCAACGACGAGCGTGACACCAGATGCCTGTAGCAATG

2201 GCAACAACGTTGCGCAAACTATTAAGTGGCAACTACTTACTCTAGCTTCCCGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCAC

2301 TTCTGCGCTCGGCCCTTCCGGCTGGCTGTTTTATTGCTGATAAATCTGGAGCCGGTGGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG

2401 TAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAG

2501 CATTTGTAAGTGCAGCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTAAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATA

2601 ATCTCATGCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCTTTCGGTGTGACGGTGAACCTCTGACACATGCAGC

2701 TCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAAGGCGCTCAGCGGTGTTGGCGGGTGTGCGGGCTGGCTTAA

NotI (2859)

XhoI (2854)

2801 CTATCGGCATCAGAGCAGATTGACTGAGAGTGACCATATGGTGACCGGATCTCGAGCGGCCGCAATAAAAATATCTTTATTTTTCATTACATCTGTGTG

2901 TTGGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGG

3001 TGCCAGAACATTTCTCTATCGAA