# 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 , Δ(mrr-hsdRMS-mcrBC) , Ø80lacZΔM15 , ΔlacX74, 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 EF1α/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 EF1α/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-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter[1] and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α 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[2] has been coupled to the EF-1α 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, *Bam*H I, *Eco*47 III, *Pst* I, *Nco* I,** and ***Nhe* I**.

*Sal* I is compatible with *Ava* I and *Xho* I.
*Bam*H I is compatible with *Bgl* II, *Bst*Y I and *Bcl* I.
*Eco*47 III is compatible with any other blunt-end restriction enzyme.
*Pst* I is compatible with *Bsp*1286 I, *Nsi* I, and *Sbf* I.
*Nco* I is compatible with *Bsp*H I and *Bsp*LU11 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.[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.

***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.*

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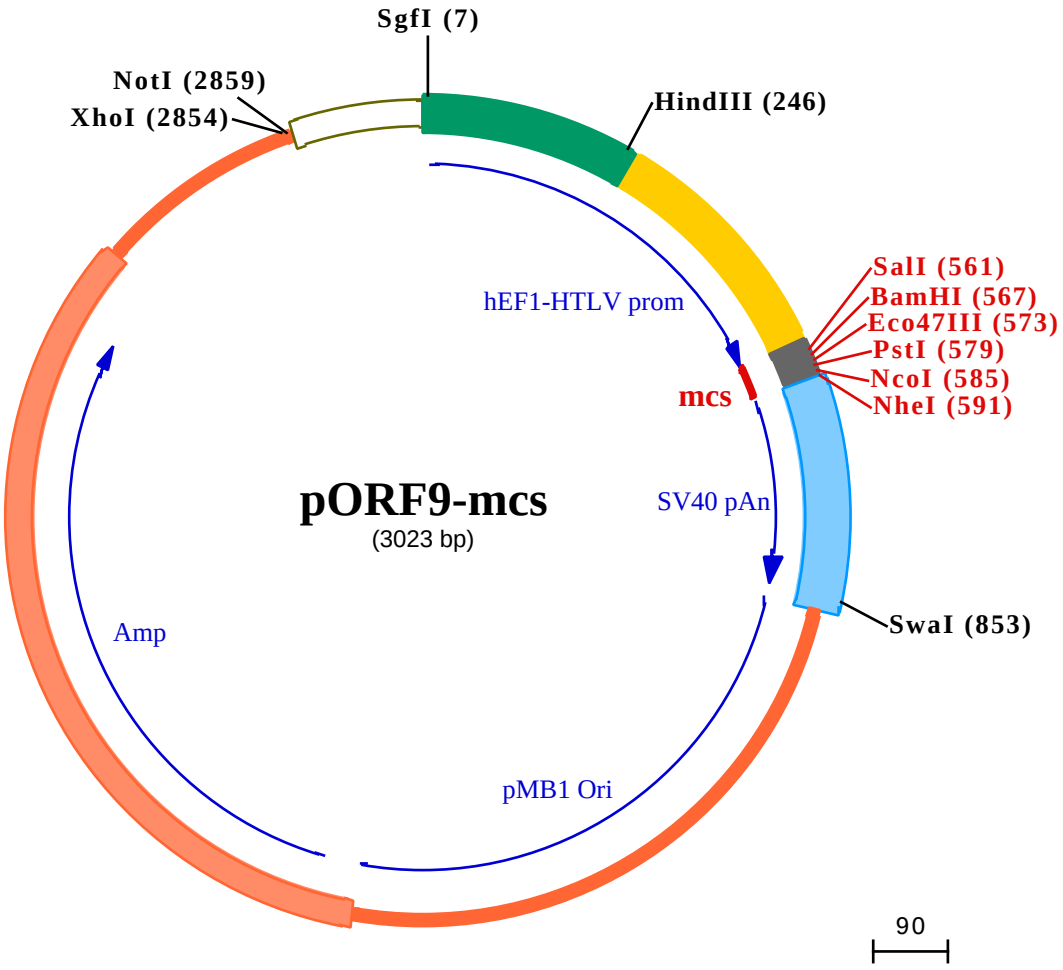
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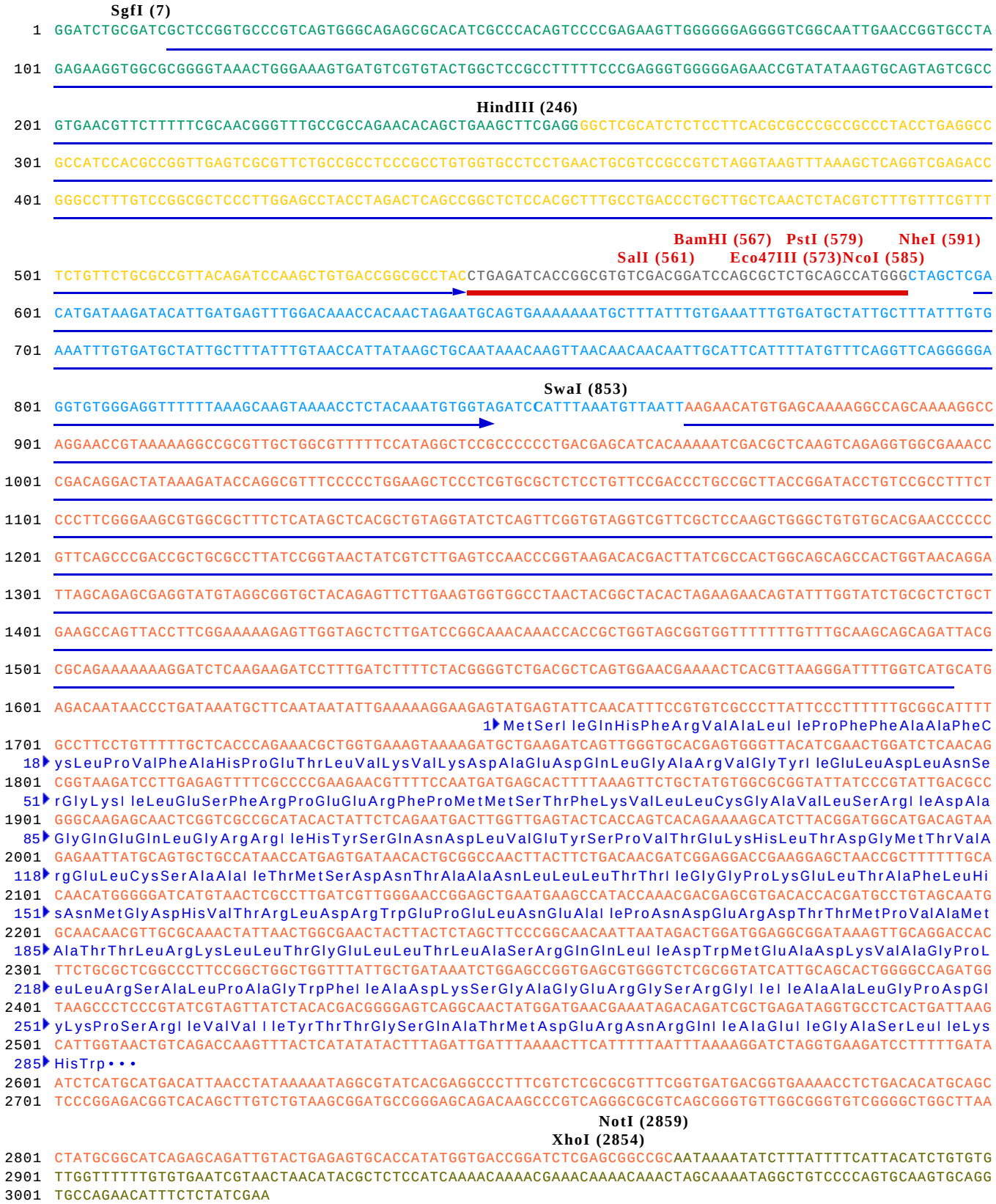
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