

pUNO1-mcs

A plasmid containing a multiple cloning site and the blasticidin resistance gene

Catalog code: puno1-mcs

<https://www.invivogen.com/puno-mcs>

For research use only

Version 19L11-MM

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and Stability

- Product is shipped at room temperature.
 - Lyophilized DNA should be stored at -20°C.
 - Resuspended DNA should be stored at -20°C and is stable at least for 1 year.
 - Store blasticidin at 4°C or -20°C. *
- *The expiry date is specified on the product label.

Quality control

- Plasmid construct has been confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

pUNO1-mcs is a ready-made expression vector containing the blasticidin resistance gene, the hybrid EF1α/HTLV promoter and a multiple cloning site.

pUNO1-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. pUNO1-mcs plasmids were designed to serve as experimental control vectors for the pUNO1 plasmid family that feature a wide choice of native and fusion genes.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. For *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α, we recommend using Blasticidin at 100 µg/ml. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

PLASMID FEATURES

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter¹ and the 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. It 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-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.

• **MCS:** The multiple cloning site contains the following restriction sites:

5' - SgrAI, Sall, BamHI, Eco47III, NcoI, NheI - 3'

Each restriction site is compatible with many other enzymes, increasing the cloning options.

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA³.

• **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **CMV promoter & enhancer** drives the expression of the blasticidin resistance in mammalian cells.

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

• **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference⁴ and possible recombination events with the SV40 polyadenylation signal.

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2):217-23. 2. Takebe Y. *et al.*, 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol Cell Biol.* 8(1):466-72. 3. Carswell S. & Alwine JC., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol Cell Biol.* 9(10):4248-58. 4. Yu J. & Russell JE., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. *Mol Cell Biol.* 21(17):5879-88.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

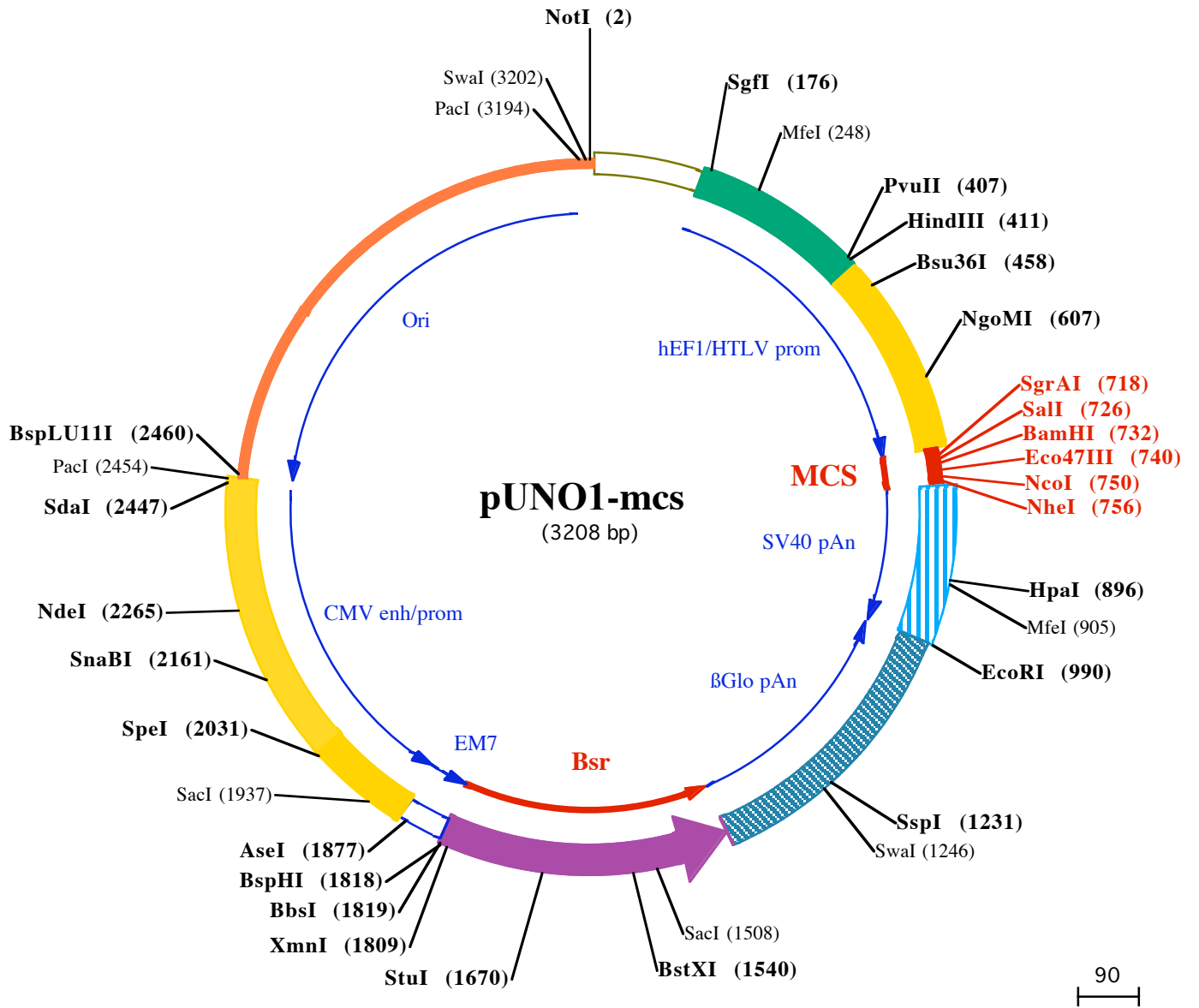
InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

 **InvivoGen**
www.invivogen.com



NotI (2)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA

SgfI (176)
101 AAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCATCGCTCCGGTGCCCGTCAGTGGGCA

MfeI (248)
201 GAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCC TAGAGAAGGTGGCGGGGTAAACTGGGAAAGTGATG

301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (411)
PvuII (407)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACCGCGCCCGCCCTACCTGAGGCCGCATCCACGCCGGTTGAGTCGCCTTCTGCCGCTT

Bsu36I (458)
501 CCGCGCTGTGGTGCCTCCTGAACTGCGTCCGCGTCTAGTAAAGTTAAAGTCAAGTGCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

NgoMI (607)
601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTCTCAACTCTACGTTCTTTGTTTCTGTTTCTGTTCTGGCGGTTACAGATCCAAGCTGTGACC

SgrAI (718) **SalI (726)** **Eco47III (740)** **NheI (756)**
701 GCGCGCTACCTGAGATCaccggtgtcgcagcggatccagcgtctgcagCCATGGCTAGCTGCCAGACATGATAAGATACATTGATGAGTTTGGACAA

HpaI (896)
801 ACCACAAC TAGAATGCAGTGA AAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAACA

MfeI (905) **EcoRI (990)**
901 ACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATCTAAATA

1001 TACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC

1101 ATTAGCTGTTTGACGCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCAAAGTTTGAAC TAGCTCTTCATTTCTTTATGTTTTAAATGCA

SspI (1231) **SwaI (1246)**
1201 CTGACCTCCCACATTCCTTTTTAGTAAAATATTGAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGC

1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTTGGACAGCAAGAAGCGAGCTTCTAGCTTT

1401 AGTTCCTGGTACTTTCAGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCATTCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGA
1404 AsnArgThr TyrLysLeuProl IeLeuGI uGI uI IeThr Thr LysVal IeLeuLysGI yAsnMetuGI uI IeLeuVal PheCysAspP roAI aTyrAspSer

SacI (1508) **BstXI (1540)**
1501 GATGAGCTCTTCGCACATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCC

1074 I IeLeuGI uArgCysMe tGI yCysP roSer Val IeVal ArgI IeSer ArgAspVal GI uAspSer TyrProHi sArgValAI aVal I IeThrAspPheAspL

StuI (1670)
1601 TTCTGCCGTTGCTCACAGCAGACCAATGGCAATGGCTTACGACAGACAGTACCCTGCCAATGTAGGCCTCAATGTGGCAGCAGAGATGATCTCCC

734 ysGI nGI yAsnSer ValAI aSer GI yI IeAI aI IeAI aGI uAI aCysVal Thr ValArgGI yI IeTyrAl aGI uI IeHi sValAI aSer I IeI IeGI uGI

1701 CAGTCTTGGTCTGATGGCCGCCGACATGGTGTGTTGTCTCATAGAGCATGGTGTCTTCTCAGTGCCGACCTCCACCAGCTCCAGATCTCGCTG

404 yThr LysThr ArgI IeAI aAI aGI yVal Hi sLysAsnAspGI uTyrLeuMe tThr I IeLysGI uThr AI aVal GI uVal IeLeuGI uLeuAspGI nGI n

BspHI (1818)
BbsI (1819)
XmnI (1809) **AseI (1877)**
1801 AGAGATGTTGAAGGCTTTCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATG

74 Ser I IeAsnPheThr LysMet

SacI (1937)
1901 GCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCAATTTGCGTCAATGGGGCGGAGTTG

SpeI (2031)
2001 TTACGACATTTTGGAAAGTCCCCTGATTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCTGAGTCAAACCGCT

SnaBI (2161)
2101 ATCCACGCCATTGATGACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCAAT

NdeI (2265)
2201 GTA CTGGGCATAATGCCAGGCGGGCATTACCCTGATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCAAGTGGGCGAGTT

2301 TACCGTAAATACTCCACCCATTGACGTCAATGAAAGTCCCTATTGGCGTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGCTGTTGG

PacI (2454) **SdaI (2447)** **BspLU11I (2460)**
2401 GCGGTCAGCCAGCGGGCCATTTACCCTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAA

2501 AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCTTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT

2601 AAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTCGGGAAG

2701 CGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCGCTCCAAGCTGGGCTGTGTGCAGAACCCCGCTTACGCCGAC

2801 CGCTGCGCTTATCCGTAACATCTGCTTGTAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA

2901 GGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCTAACTACGGTCACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC

3001 CTTGGA AAAAGAGTTGGTAGCTTGTATCCGGCAAAACCAACCCTGGTAGCGGTGTTTTTTGTTTGAAGCAGCAGATTACGCCGAGAAAAA

PaeI (3194) **SwaI (3202)**
3101 GGATCTCAAGAAGATCCTTTGATCTTTTACGGGCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACAT

3201 TTAATCA