

# pVITRO1-neo-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro1-nmcs

<https://www.invivogen.com/pvitro1-mcs>

For research use only

Version 19L17-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVITRO1-neo-mcs provided as lyophilized DNA

### Storage and stability

- Products are shipped at room temperature.

- Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable for at least one year at -20°C.

### Quality control

- Plasmid construct has been confirmed by restriction analysis and sequencing.

- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

## GENERAL PRODUCT USE

pVITRO is a family of plasmids developed mainly for *in vitro* studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

pVITRO1-neo-mcs plasmid is selectable with kanamycin in *E. coli* and G418 in mammalian cells. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

## PLASMID FEATURES

• **rEF1 and mEF1 prom:** pVITRO1-neo-mcs plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart<sup>1</sup>, both promoters display a strong activity that yield similar levels of expression. EF-1α promoters are expressed at high levels in all cell cycles and lower levels during G0 phase. EF-1α promoters are also non-tissue specific; they are highly expressed in all cell types.

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range<sup>2</sup>. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer<sup>3</sup>.

• **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>4</sup>

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

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression<sup>5</sup>.

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

• **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In bacteria, *neo* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *neo* is transcribed from the rat EF-1α promoter as a polycistronic mRNA and translated via the FMDV IRES.

• **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.

• **MCS1 and MCS2:** To facilitate cloning each multiple cloning site contains several restriction sites that are compatible with many other enzymes.

**MCS1** contains **Bsp EI, Bst 1107I, Bam HI, Bsi WI and Avr II.**

- *Bsp EI* is compatible with *Age I* and *Sgr AI*.

- *Bst 1107I* (blunt-end restriction enzyme)

- *Bam HI* is compatible with *Bgl II, Bst YI* and *Bcl I*.

- *Bsi WI* is compatible with *Acc 65I, Ban I* and *Bsr GI*.

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

**MCS2** contains **Age I, Eco RV, Bgl II, Bsr GI, and Nhe I.**

- *Age I* is compatible with *Bsp EI* and *Sgr AI*.

- *Eco RV* (blunt-end restriction enzyme)

- *Bgl II* is compatible with *Bam HI, Bst YI* and *Bcl I*.

- *Bsr GI* is compatible with *Acc 65I, Ban I* and *Bsi WI*.

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

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 91(2):217-23. 2. Moreau P. *et al.*, 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. *Nucleic Acids Res.* 9(22):6047-68. 3. Boshart M. *et al.*, 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141(2):521-30. 4. Carswell S. & Alwine J.C. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258. 5. Ramesh N *et al.*, 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.

## 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 H<sub>2</sub>O. 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.

### Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

### Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

## RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent <i>E. coli</i> cells	gt116-11
G418	Selection antibiotic	ant-gn-1

### TECHNICAL SUPPORT

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

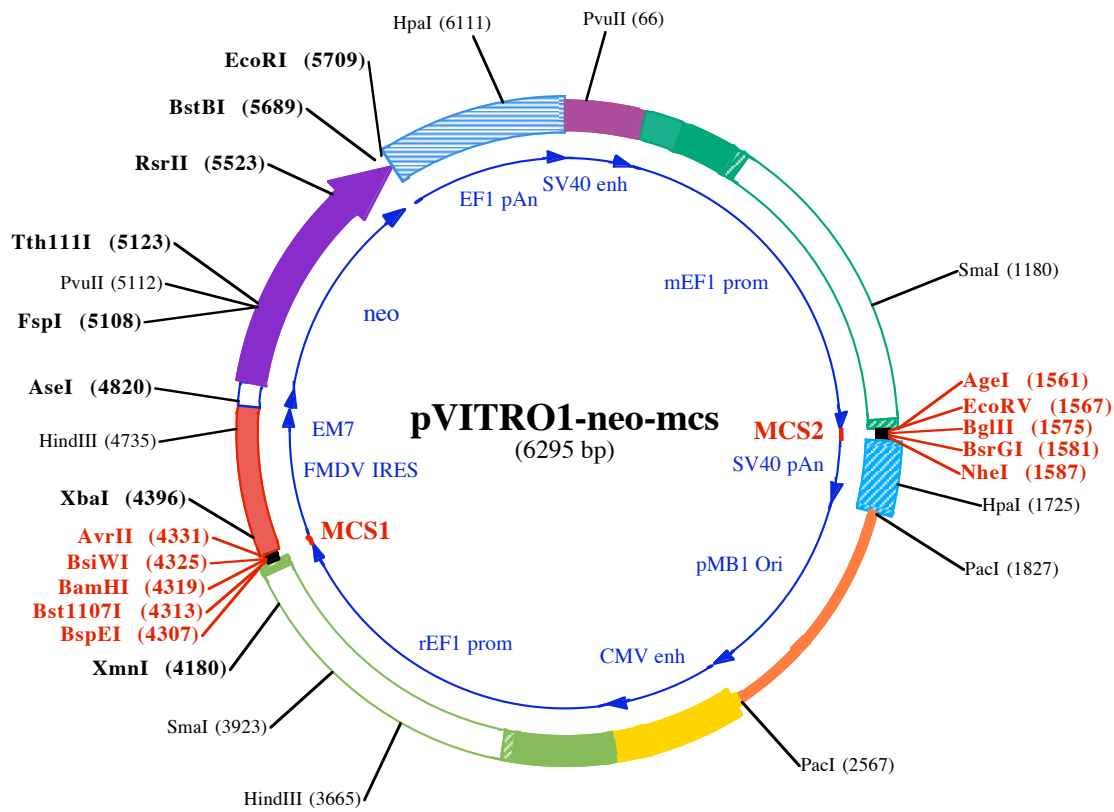
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PvuII (66)

1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA  
 101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG  
 201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCTTCGCAAGGGGAGAG  
 301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA  
 401 GCACACAAGCTCATAGACCCTGGTCGTGGGGGGAGGACCGGGGAGCTGGCGGGGGCAAACCTGGGAAAGCGGTGTCGTGTGCTGGCTCCGCCCTCTTCC  
 501 CGAGGGTGGGGGAGAACGGTATATAAGTGCAGCAGTCGCTTGGACGTTCTTTTTCGCAACGGGTTTCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGC  
 601 TTCCGCGGGCCCGGAGCTGGAGTCTGCTCCGAGCGGGCCGGCCCCGCTGTCGTGCGGGGATTAGTCTGAGCATTCCCGCTCGAGTTGCGGGC  
 701 GCGCGGGAGGCAGAGTGCAGGCTAGCGGCAACCCCTAGCCTCGCCTCGTGTCCGGCTTAGGGCTAGCGTGGTGTCCGCGCCCGCCGCGTGCTA  
 801 CTCCGCGCGCACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCGGTTTCAGCAATAGGGGCTAACAAAGGAGGGTGCGGGGCT  
 901 TGCTCGCCCGAGCCCGGAGAGGTATGGTTGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCGCTTCGGAGCACATGTCCGACGCCAC  
 1001 CTGGATGGGGCGAGGCTGGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCG

SmaI (1180)

1101 GCGCCCGTTGCCCTGCCTCCCTAACTAGGGTGAGGCCATCCCGTCCGGCACCAGTTGCGTGCCTGAAAGATGGCCCTCCCGGGCCCTGTTGCAAGGA  
 1201 GCTCAAAATGGAGGACGCGCAGCCCGTGGAGCGGGGGTGGAGTACCCACACAAAGGAAGAGGGCTGGTCCCTACCGGCTGCTGCTTCTGTGAC  
 1301 CCCGTGGTCTATCGCCGCAATAGTCACCTCGGCTTTTGGACACGGTAGTCGCGGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATT  
 1401 GGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGTGAAGTCATTTTTGAATTTGTCCCTTGTGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGG

EcoRV (1567) BsrGI (1581)  
 AgeI (1561) BglIII (1575) NheI (1587)

1501 TTCAAAGGTATCTTTTAAACCTTTTTTAGGTGTTGTGAAAACCCGTAATTCAAAGCAACCGGTGATATCAAAGATCTGTACAGCTAGCTGGCCAG  
 1601 ACATGATAAGATACATTGATGAGTTTGGACAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTGT

HpaI (1725)

1701 AACCATTATAAGTCGAATAAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAA

PacI (1827)

1801 AACCTCTACAAATGTGGTATGAAATGTTAATTAAGTACCCATGACCAAAATCCCTAACGTGAGTTTTGTTCCACTGAGCGTCAGACCCCGTAGAAAA  
 1901 GATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGAACAACAAAAAACCCGCTACCAGCGGTGGTTTTGTTTCCGGATCAA  
 2001 GAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTTCAAGA  
 2101 ACTCTGTAGCACCGCTACATACCTCGCTGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCAGGTTGGACTCAAGACG  
 2201 ATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGAGGCGAACGACCTACACCGAACTGAGATACCTACAG  
 2301 CGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGGGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTTC  
 2401 CAGGGGGAAACGCTGGTATCTTTATAGTCTGTGGGTTTCGCCACCTCTGACTTGAAGTGTGATTTTGTGATGCTGTACAGGGGGCGGAGCCTATG

PacI (2567)

2501 GAAAAACGCCAGCAACCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCTTTTGTGCTCACATGTTCTTAATTAACCTGCAGGCCTTACATAACTTACGG  
 2601 TAAATGGCCCCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACG  
 2701 TCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTTATTGACGTCAATGACGGTAAATGG  
 2801 CCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTT  
 2901 GGCAGTACATCAATGGGCGTGATAGCGGTTTGAAGTCTCACCCCATTTGACGTCAATGGGAGTTTGTGTTTACTAGTGGAGCC  
 3001 GAGAGTAATTCATACAAAAGGAGGGATCGCTTCGCAAGGGGAGAGCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCCCACG  
 3101 ACAGCGCAGGAGCATGCGCCAGGGCTGAGCGCGGTAGATCAGAGCACAAAGCTCACAGTCCCGCGGTGGGGGAGGGGCGCGCTGAGCGGGGGC  
 3201 CAGGGAGCTGGCGGGGGCAAACCTGGGAAAGTGGTGTGCTGTGCTGCTCCGCCCTTCCCGAGGGTGGGGGAGAAGCGGTATATAAGTGGGTAGTCGC  
 3301 CTTGGACGTTCTTTTTCGCAACGGGTTTCCGTCAGAACGCAgtagtgagggggtgtggtctccgccccggagctggagccctgctctgagcggg

3401 ccgggctgatatgagtgctgcccaggggttagctgtgagcattcccacttcgagtgggcgggcggtgcgggggtgagagtgcgaggcctagcggcaa  
3501 ccccgtagcctcgcctcgtgtccggcttgaggcctagcgtggtgtccgcccgcgctgccactccggccgcactatgcgttttttgccttgctgcctc  
HindIII (3665)  
3601 gattgccttcagcagcatgggctaacaaagggaggggtgtggggctcactcttaagagcccatgaagcttacgttgataggaatggaagggcaggagg  
3701 ggcgactggggcccgcccgccttcggagcacatgtccgacgccacctggatggggcgaggcctgtggctttccgaagcaatcgggcgtgagtttagccta  
3801 cctgggccatgtggccctagcactgggcacggtctggcctggcggtgccggttccttgctcccaacaagggtagggcgtcccgcggcaccagtt  
SmaI (3923)  
3901 gcttgcgcggaagatggccgctcccggggccctgttgcaaggagctcaaatggaggacgcgccagcccgggtggagcgggcgggtgagtcacccacaca  
4001 aaggaagagggccttgcctcgcggccgctgtcttctgtgaccccgtggtctatcgccgcgatagtcacctcgggcttctcttgagaccgctcgtcg  
XmnI (4180)  
4101 cggcggggggaggggactaattggcgttgagtttggttcacatttgggtgggtggagactagtcaggccagcctggcgctggaagtcatcttggatttg  
4201 cccctttgagtttgagcagagctaattctcaagcctcttagcggttcaaggtatcttctaaacccgtttccagGTGTTGTGAAAGCCACCGCTAATTC  
BsiWI (4325)  
Bst1107I (4313)  
BspEI (4307) BamHI (4319) AvrII (4331) XbaI (439)  
4301 AAAGCAATCCGGAGTATACGGATCCCGTACGCCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTAAACTCCGCTGGTCTTCCAGGTCTA  
4401 GAGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGG  
4501 AACTCCTCCTTGGTAACAAGACCCACGGGGCCAAAAGCCACGCCACAGGGCCGTCATGTGTGCAACCCAGCACGGGACTTTACTGCGAAACCA  
4601 CTTTAAAGTGACATTGAAACTGGTACCCACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACTCGGGATCTGAGAAG  
HindIII (4735)  
4701 GGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGTGGCACCTTTCCTTTGCAATTAAGTACCCATG  
AseI (4820)  
4801 AATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGATTGAACAAGATGGATT  
4901 GCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTTCGGCTGTCA  
5001 CGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGCTATCGTGGCTGGCCACGACGG  
PvuII (5112)  
FspI (5108) Tth111I (5123)  
5101 CGGTTCTTGGCAGCTGTGCTCGACGTTGCTACTGAAGCGGAAGGGACTGGCTGCTATTGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT  
5201 TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGTGCATACGTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAACATCGC  
5301 ATCGAGCGAGCACGTAAGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTGCCAGGC  
5401 TCAAGGCGAGCATGCCGACGGCAGGATCTCGTGTGACACATGGCGATGCTGCTTGCCTGAATATCATGGTGAAAATGGCCGCTTTCTGGATTCA  
RsrII (5523)  
5501 CGACTGTGGCCGGCTGGGTGTGGCGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGGAATGGGCTGACCGCTTC  
BstBI (5689)  
5601 CTCGTCTTACGGTATCGCCGCTCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTACGAGTCTTCTGAGCGGGACTCTGGGGTTGAAATGAC  
EcoRI (5709)  
5701 CGACCAAGCGAATTCGCTAGGATTATCCCTAATACCTGCCACCCACTTAAATCAGTGGTGAAGAACGGTCTCAGAAGTGTGTTTCAATTGGCCAT  
5801 TTAAGTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAAGAAGAAAGGAGAATGTTTGGGACCACTTTGGTTTTCTTTTT  
5901 GCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAACAACCTTGACCAAAAATTTGTCACAGAATTTTGGACCCATTA  
6001 AGTTAAATGAGAACTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAGACATCTAATTCTGGTTTTACGAATCTGAAACTTCTTGA  
HpaI (6111)  
6101 GTAATCTTGTAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTTGC  
6201 TTTGATTACAACACTGGAGAGAAATGAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACCTGAGTCTTGGGTTGCATAGAAAGCTG