

pVITRO2-blasti-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro2-bmcs

<https://www.invivogen.com/pvitro2-mcs>

For research use only

Version 20H17-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-blasti-mcs provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- 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 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.

pVITRO2-blasti-mcs plasmid is selectable with blasticidin in both *E. coli* and mammalian cells. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

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. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

PLASMID FEATURES

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein¹. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range². 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 enhancer³.

- **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.*⁴

- **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 expression⁵.

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

- **Blasti:** Resistance to blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the hFerH/mEF1α 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:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

Age I, Eco RV, Bam HI, Sal I and Avr II

- Age I is compatible with Bsp EI and Sgr AI.
- Eco RV (blunt-end restriction enzyme).
- Bam HI is compatible with Bgl II, Bst YI and Bcl I.
- Sal I is compatible with Ava I and Xho I.
- Avr II is compatible with Xba I, Spe I and Nhe I.

MCS2 contains the following restriction sites:

Sgr AI, Bgl II, Xho I and Nhe I

- *Sgr AI is compatible with Bsp EI and Age I.*
- *Bgl II is compatible with Bam HI, Bst YI and Bcl I.*
- *Xho I is compatible with Ava I and Sal I.*
- *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* 91:217-23. 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.* 9:6047-68. 3. Boshart M. *et al.*, 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141:521-30. 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: 4248-58. 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.

TECHNICAL SUPPORT

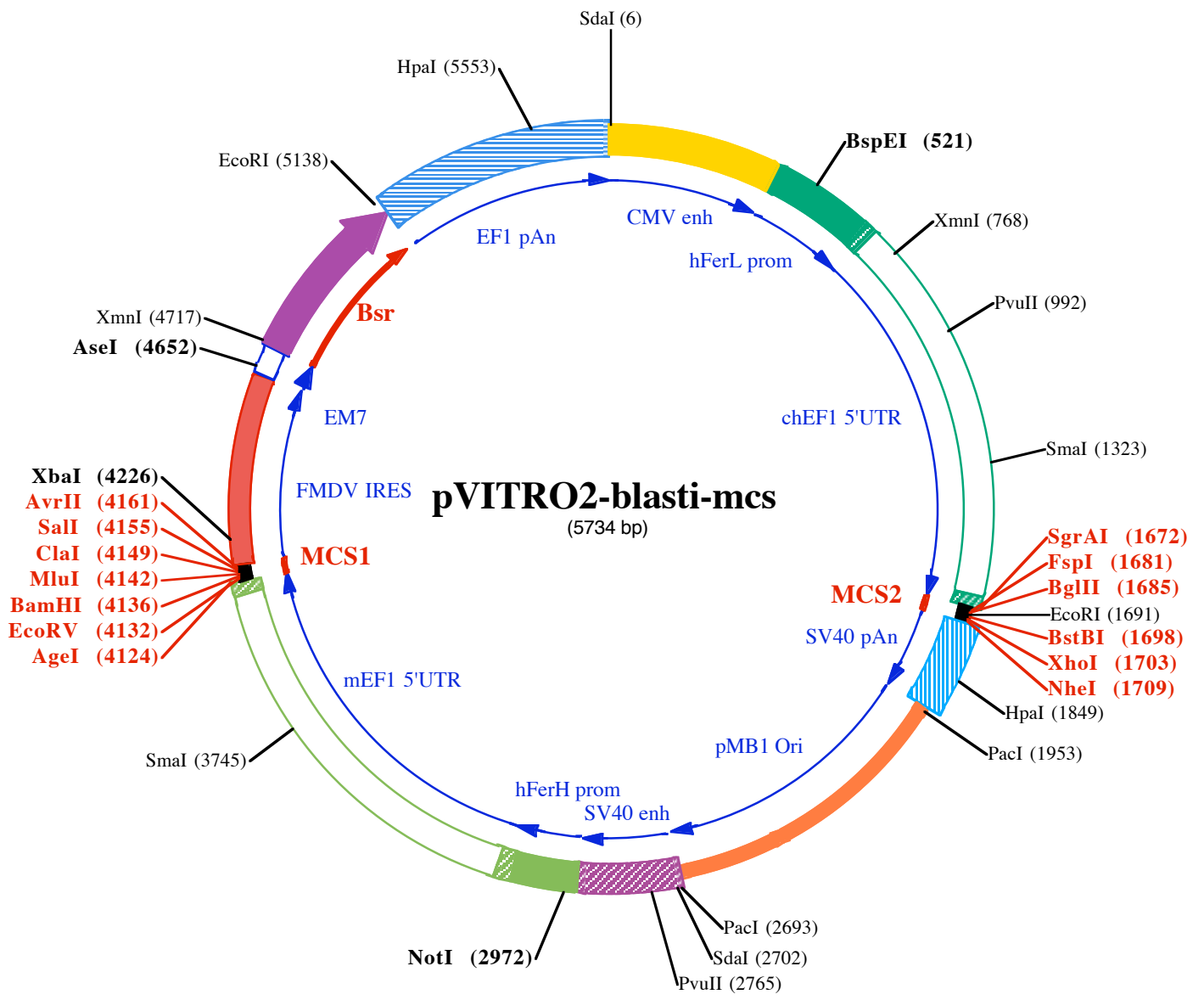
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150

SdaI (6)
1 CCTGCAGGCGTTACATAA...
101 CGCCAATAGGACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC
201 TATTGACGTCAATGACGTTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATG
401 GGAGTTTGT...
BspEI (521)
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCTTCGGCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCCCTCCCGAGCGCCCTGCC
601 TCCGAGGCGCGGCACCATAAAAGAAGCCGCTAGCCACGTCCCTCGCAGTTGCGCGGTCCGCGGGTCTGTCTCAAGTTGCCGCCAGAACACAGg
XmnI (768)
701 taagtgcgctgtgtggttccgcgcccctggcctctttacgggttatggccttgctgccttgaattacttccatgcccctggctgcagtacgtgattc
801 ttgatcccgagcttcgggttgaagtgggtgggagagttcgagccttgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttggggc
PvuII (992)
901 ctggggccgcccgtgctaactcgtggcaccttcgcgctgtctcgtgctttcgttaagtctctagccattaaaatTTTTgataaccagctgcgacg
1001 cttttttctggcagatagcttctaagtgcggccagagctgcacactggtatcttgggttttggggccgcccggcggcggcaggggcccgtgcctcc
1101 agcgcacatgttcggcagggcggggcctgcgagcgcggccaccgagaatcgacggggtagtctcaaacggcggcctgctcgtgctgctgctgc
1201 gccgcccgtgatcggccgcccggcggcaggctggcccggctggccaccagttgctgtagcggaaagatggccgcttcccggcctgctcagggagc
SmaI (1323)
1301 tcaaaatggaggacgcccggcgggagagcgggcccgggtgagtcaccacacaaaggaaaaggccttctcctcatccgtcgttcatgtgactcca
1401 cggagtagcgggcccgtccaggcacctcattagttgtcgagctttggagtagctgctttagttgggggaggggtttatgcatggagtttcc
1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgaattctccttgganttgccctttttagtttgatcttgcctcattc
FspI (1681) EcoRI (1691)
SgrAI (1672) BglIII (1685) BstBI (1698)
1601 tcaagcctcagacagtggttcaagtttttttcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGCGTGCAGATCTGAATCTTCTCG
NheI (1709)
XhoI (1703)
1701 AACTCGAGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG
HpaI (1849)
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGTGG
PacI (1953)
1901 GAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGAAATGTAATTAAGTACCATGACCAAAATCCCTTAACGTGAGTTTTCTGTCCACTG
2001 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGAACAACAAAAACACCGCTACCAGCG
2101 GTGGTTTTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGT
2201 AGTTAGGCCACCCTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCGAGTGGCGATAAGTCTGTCT
2301 TACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTAC
2401 ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCCACGCTTCCGAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGAGGGTCGGAACAG
2501 GAGAGCGCACGAGGAGCTTCCAGGGGAAACGCTGGTATCTTATAGTCTGTGCGGTTTTGCCACCTCTGACTTGAGCGTGCATTTTTGTGATGCTC
PacI (2693) SdaI (2702)
2601 GTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCCATGTTCTTAATTAACCTG
PvuII (2765)
2701 CAGGGCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTC
2801 CCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAAGGTGTGAAAGTCCCAAGCTCCCCAGCAGGCAGAAGTATG
NotI (2972)
2901 CAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCCCGCAGAGCGCGAGGGCCTCCAGCGGCCGCCCTCCCCACAGCAGGGGCGG
3001 GGTCCCGCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCTGATTGGCCGGGCGGGCTGACGCCGACGCGGCTATAAGAGACCAAGCGACCC
3101 GCAGGGCCAGAGCTTCTTCGCCAAGCTTCCGTCAGAACGCAgtagggggggggtggtgcttccgcccggcggcagctggaggtcctgctccgagcg
3201 ggccgggcccgcctgctcgtcggcgggattagctgcgagcattccgccttcgagttgcggcggcggcggagggcagagtgagggcctagcggcaacccc
3301 gtagcctcgcctcgtcggccttagggcctagcgtggtgctcgcgcccggcggcggcgtgctactcggccgcaactctggtcttttttttttggttggtg
3401 ttgcccctgctgcttccgattgcccgttcagcaataggggctaacaaggagggtgcggggcttgcctcgcggagcccggagaggtcatggttggggagg
3501 aatggagggacaggagtgccggctggggcccgccttcggagcacatgtccgacccacctggatggggcagggcctgggtttttccgaagcaac

3601 caggctggggttagcgtgccgagccatgtggccccagcaccggcagcatctggcttggcggcgccgcttccctgcctccctaaactagggtgagggc

SmaI (3745)

3701 atcccgctccggcaccagttgctgctggaagatggccgctcccgggcccctgttgcaaggagctcaaaatggaggacgcgagcccgggtggagcgggc

3801 gggtgagtcacccacacaaaggaagagggcctggctccctaccggctgctgcttctgtgaccccggtgctcctatcggcgcgcaatagtcacctcgggctt

3901 ttgagcacggctagtcgcgccgggggggggaggtgtaatggcgttgaggtttgttcaatttgggtgggtggagactagtcaggccagcctggcgtggaa

4001 gtcatttttggaaatttgtccccttgagttttgagcggagctaatctcgggcttcttagcggttcaaggtatcttttaaaccttttttagGTGTTGTG

EcoRV (4132) MluI (4142) SalI (4155)
AgeI (4124) BamHI (4136) ClaI (4149) AvrII (4161)

4101 AAAACCACCGCTAATTCAAAGCAAACCGGTGATATCGGATCCACGCTATCGATTGTCGACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT

XbaI (4226)

4201 TGAAACTCCGCTGGTCTTCCAGGCTAGAGGGTAACACTTTGACTGCGTTTGGCTCCACGCTCGATCCACTGGCAGTGTTAGTAACAGCACTGTT

4301 GCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGGCCACGGGGCCAAAAGCCACGCCACACGGGCCCTCATGTGTGCAACC

4401 CCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAG

4501 GTAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTGCGC

AseI (4652)

4601 ACCTTTCCTTTGCAATTAAGTACCTATGAATACAAGTACTGTTTGAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAG

XmnI (4717)

4701 GAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGTCCGCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCAC

4801 CATGTCGGGGCGGCATCAGGACCAAGACTGGGAGATCATCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTG

4901 CCATTGGGTCTGCTGTGAGCAACGGGCAGAAAGACTTTGACACCATTGGCTGTGAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGT

5001 CAGCCCCGTGGCATGTGCAGAGAGCTCATCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCCACTTGGAG

5101 GAACTCATCCCCCTCAAGTACACCAGGAACCTAAACCTGAATTCGCTAGGATATTAGCTAGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGT

EcoRI (5138)

5201 GGAAGAACGGTCTCAGAAGCTGTTTGTTC AATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAAGGA

5301 AAGGAGAATGTTTTGTGGACCATTGGTTTTCTTTTTTTCGCTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAACAACCTTG

5401 ACCAAAAATTTGTACAGAAATTTGAGACCATTAAAAAGTTAAATGAGAAACCTGTGTGTTCCCTTGGTCAACCCGAGACATTTAGTGAAAGACAT

HpaI (5553)

5501 CTAATTCTGGTTTTACGAATCTGAAACTTCTTGAAAATGTAATCTTGAGTTAACACTTCTGGGTGGAGAATAGGTTGTTTTCCCCACATAATTGG

5601 AAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCTCACTAAAACAGGC

5701 CAAAAAGTACTGCTTGGTTGCATAGAAAGCTG