

pVITRO1-hygro-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro1-mcs

For research use only

Version 18J06-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO1-hygro-mcs provided as lyophilized DNA
- 1 ml Hygromycin B Gold at 100 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 Hygromycin B Gold 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 new family of vectors with improved features. pVITRO1 and pVITRO2 allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels.

pVITRO1-hygro-mcs plasmid is selectable with hygromycin 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.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-mcs plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart¹, 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 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*.
- **hph gene** confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* 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:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

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 the following restriction sites:

Age I, Eco RV, Bgl II, Bsr GI, and Nhe I

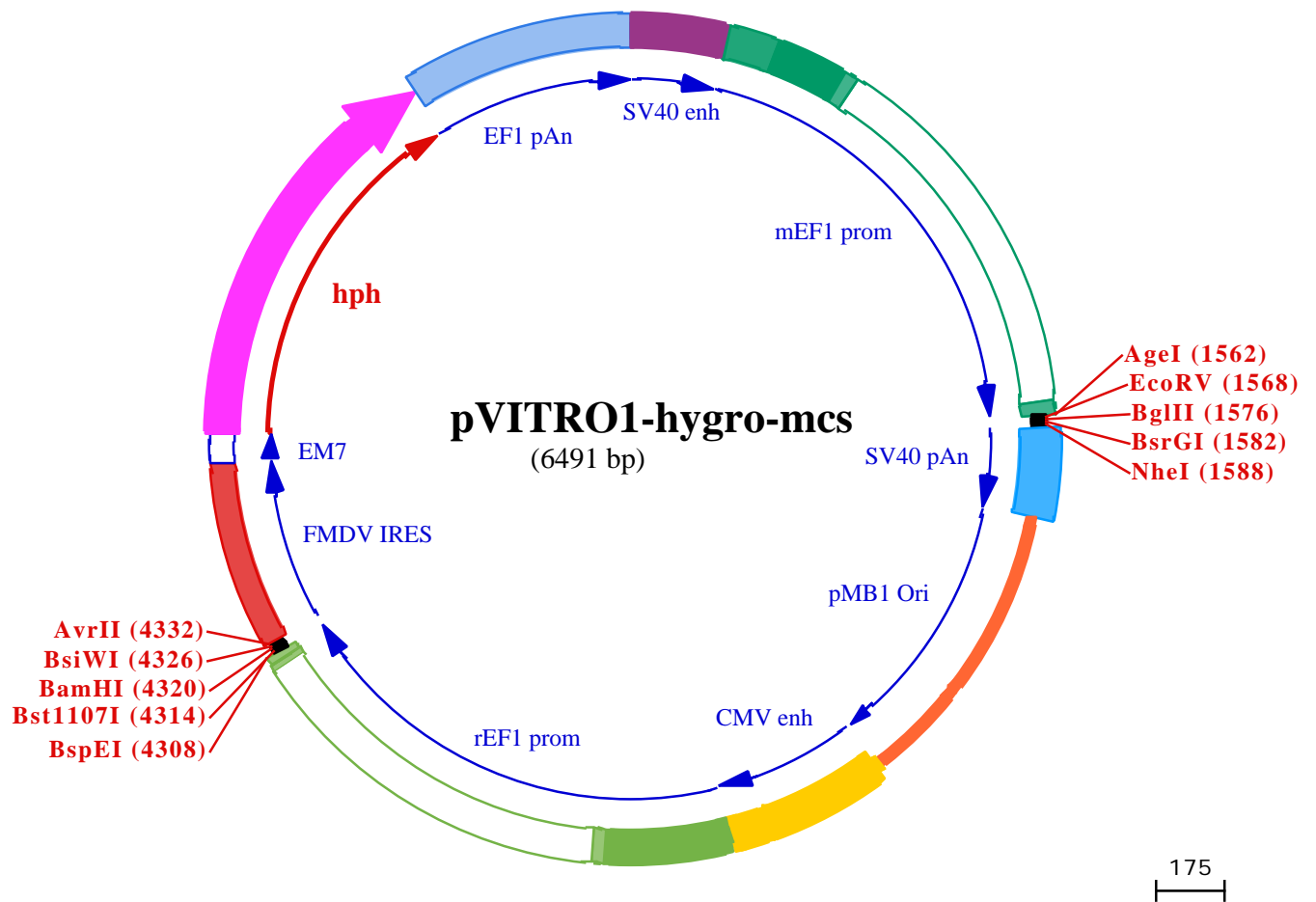
- *Age I* is compatible with *Bsp EI* and *Sgr AI*.
- *Eco RV* (blunt-end restriction enzyme)
- *Bgl II* is compatible with *Bam HI*, *Bst YI* and *Bcl I*.
- *Bsr GI* is compatible with *Acc 65I*, *Ban I* and *Bsi WI*.
- *Nhe I* is compatible with *Xba I*, *Spe I* and *Avr II*.

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., and Alwine JC. 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.

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





1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTGAGTTAGGGTGTGGAA
101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG
201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG
301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGGGGAGAGCAGA
401 GCACACAAGCTCATAGACCCTGGTCGTGGGGGGAGGACCGGGGAGCTGGCCGGGGGCAAACCTGGGAAAGCGGTGTCGTGTGCTGGCTCCGCCCTCTTC
501 CGAGGGTGGGGAGAACGGTATATAAGTGGCGCAGTCGCCTTGGACGTTCTTTTTTCGCAACGGGTTTGGCCTCAGAACGCAGGTGAGGGCGGGTGTGGC
601 TTCCGGGGCCGCGAGCTGGAGGTCCTGCTCCGAGCGGGCCGGCCCGCTGTCGTGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGC
701 GCGCGGGAGGCAGAGTGCAGGGCTAGCGGCAACCCCTAGCCTCGCCTCGTGTCCGGCTTGGAGCCTAGCGTGGTGTCCGGCCGCGCCCGCTGCTA
801 CTCGGCCGCACTCTGGTCTTTTTTTTTTTTGTGTGTGTGCCCTGCTGCCTTCGATTGCGCTTCAGCAATAGGGCTAACAAAGGGAGGGTGCGGGGCT
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1001 CTGGATGGGGCGAGGCTGGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCACGACCCGGCAGCATCTGGCTTGGCG
1101 GCGCCGCTTGCCTGCCTCCCTAAGTGGGTGAGGCCATCCGTCGCGCACAGTGTGGTGGTGGAAAGATGGCCGCTCCCGGCCCTGTTGCAAGGA
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1501 TTCAAAGGTATCTTTTAAACCCTTTTTAGGTGTTGTGAAAACCACCGCTAATTCAAAAGCAACCGGTGATATCAAAGATCTGTACAGCTAGCTGGCCAG
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1801 AACCTCTACAAATGTGGTATGGAAATGTAATTAAGTACCATGACAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAA
1901 GATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGTTGCAACAACAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGATCAA
2001 GAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCCTCAAGA
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2301 CGTGAGCTATGAGAAAGCCACGCTTCCGAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAGGGAGCTTC
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BsiWI (4326)

Bst1107I (4314)

BspEI (4308) BamHI (4320) AvrII (4332)

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7▶ rAlaThrSerValGluLysPheLeuI leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArgAlaPheSerPheAsp
5001 GTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATCTTGTGCTGATGGTTTTTACAAAGACAGATATGTTTACAGACACTTTGCTCTGCTGCTGCCAA
41▶ ValGlyGlyArgGlyTyrValLeuArgValAsnSerCysAlaAspGlyPheTyrLysAspArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProI
5101 TTCCAGAAGTTCTGGACATTGGAGAATTTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGA
74▶ leProGluValLeuAspI leGlyGluPheSerGluSerLeuThrTyrCysI leSerArgArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGI
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5301 ATTTGTCAGTACCCACTTGGAGGGATTCATTTGTGCCATTGCTGCTCATGTCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTG
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174▶ aIAlaGlnAlaLeuAspGluLeuMetLeuTrpAlaGluAspCysProGluValArgHisLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAs
5501 CAATGGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTGGAGACCTGGCTGGCT
207▶ pAsnGlyArgI leThrAlaVal I leAspTrpSerGluAlaMetPheGlyAspSerGlnTyrGluValAlaAsnI lePhePheTrpArgProTrpLeuAla
5601 TGCATGGAACAACAACAAGATATTTTGAAGAAGACCCAGAAGTGGCTGGTTCCTCCAGACTGAGAGCCTACATGCTCAGAATTGGCTGGACCAAC
241▶ CysMetGluGlnGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArgI leGlyLeuAspGlnL
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274▶ euTyrGlnSerLeuValAspGlyAsnPheAspAspAlaAlaTrpAlaGlnGlyArgCysAspAlaI leValArgSerGlyAlaGlyThrValGlyArgTh
5801 TCAAATTGCAAGAAGGTCTGCTGCTGTTGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAACAGGAGACCCCTCCACAAGACCCAGAGCCAAG
307▶ rGlnI leAlaArgArgSerAlaAlaValTrpThrAspGlyCysValGluValLeuAlaAspSerGlyAsnArgArgProSerThrArgProArgAlaLys
5901 GAATGAATATTAGCTAGATTATCCCTAATACCTGCCACCCACTTAAATCAGTGGTGAAGAACGGTCTCAGAAGCTGTTTGTTCATTTGGCCATTTAA
341▶ Glu•••
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