

# pVITRO1-hygro-mcs

An innovative multigenic plasmid for high levels of expression

Catalog # pvitro1-mcs

For research use only

Version # 05G06-MT

## PRODUCT INFORMATION

### Content:

- 20  $\mu$ g of pVITRO1-hygro-mcs provided as lyophilized DNA
- 4 pouches of *E. coli* FastMedia™ Hygro

### Storage and Stability:

- Product is shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at -20°C (see Methods).
- Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* FastMedia™ Hygro at room temperature. FastMedia™ pouches are stable 18 months when stored properly.

### 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.

## PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-mcs plasmid carries two elongation factor 1 alpha (EF-1 $\alpha$ ) 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-1 $\alpha$  promoters are expressed at high levels in all cell cycles and lower levels during G0 phase. EF-1 $\alpha$  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*.
- **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-1 $\alpha$  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*.

### References:

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

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## METHODS

### **Plasmid resuspension:**

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1  $\mu\text{g}/\mu\text{l}$ , resuspend the DNA in 20  $\mu\text{l}$  of sterile  $\text{H}_2\text{O}$ . Store resuspended plasmid at  $-20^\circ\text{C}$ .

### **Selection of bacteria with *E. coli* FastMedia™ Hygro:**

*E. coli* FastMedia™ Hygro is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Hygro is a TB (liquid) or LB (solid) based medium with hygromycin B.

*E. coli* FastMedia™ Hygro can be ordered separately (catalog code # fas-hg-l, fas-hg-s).

### **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 FastMedia™.**

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  $45^\circ\text{C}$  before pouring plates. Let liquid media cool to  $37^\circ\text{C}$  before seeding bacteria.

**Note:** Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.

## RELATED PRODUCTS

Product	Quantity	Catalog Code
pVITRO1-GFP/LacZ	20 $\mu\text{g}$	pvitro1-gfplacz
Hygromycin	1 g	ant-hm-1
	5 g	ant-hm-5
HygroGold™	1 g	ant-hg-1
	5 g	ant-hg-5
Fast-Media™ Hygro Agar	30 pouches	fas-hg-s
Fast-Media™ Hygro TB	30 pouches	fas-hg-l

### **TECHNICAL SUPPORT**

Toll free (US): 888-457-5873

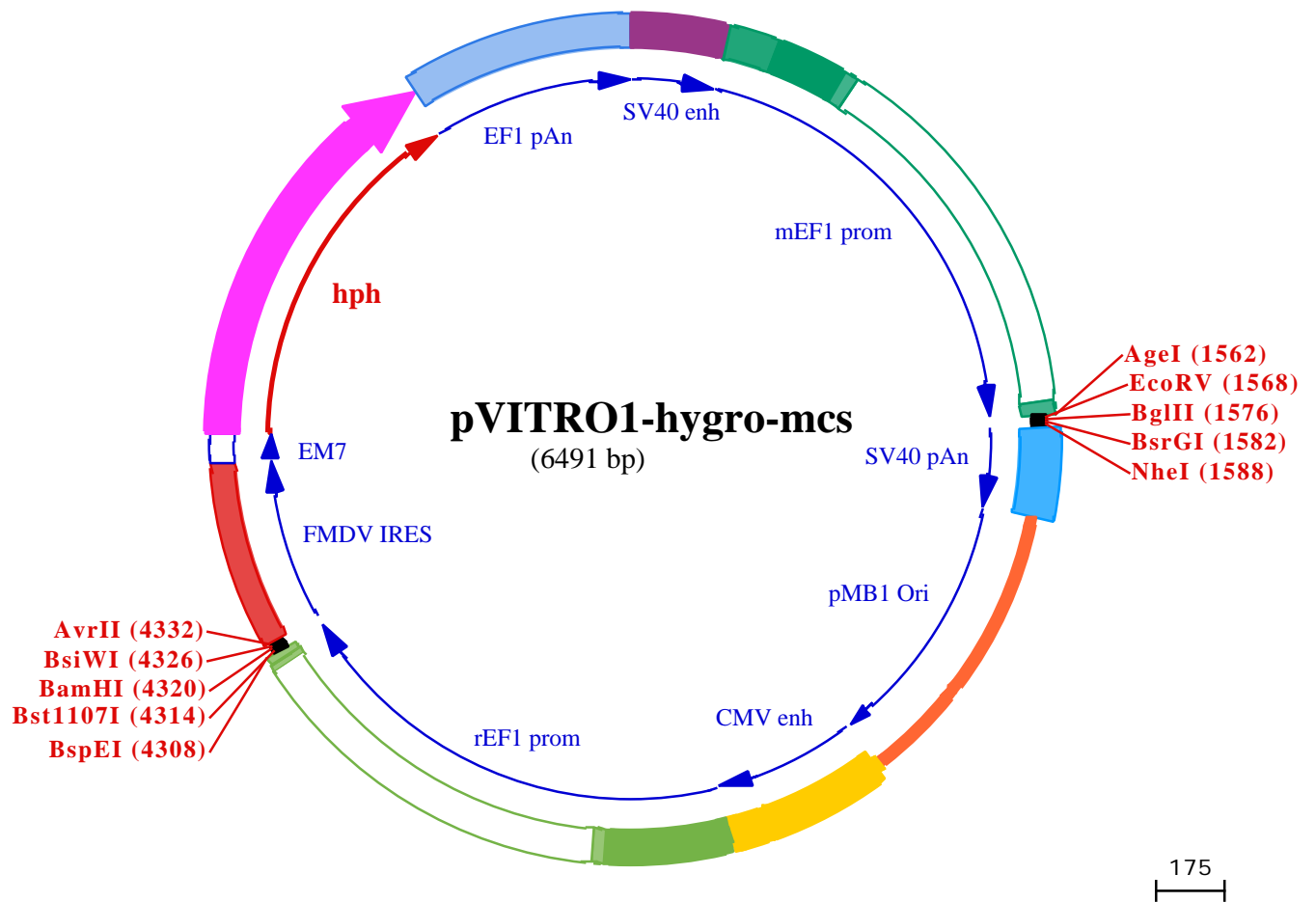
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1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTGAGTTAGGGTGTGGAA  
101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG  
201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG  
301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCTCAGGGCTGAGCGGGGAGAGCAGA  
401 GCACACAAGCTCATAGACCCTGGTCGTGGGGGGAGGACCGGGGAGCTGGCGGGGGCAAACCTGGGAAAGCGGTGTCGTGTGCTGGCTCCGCCCTCTTCC  
501 CGAGGGTGGGGAGAACGGTATATAAGTGGCGCAGTCGCCTTGGACGTTCTTTTTTCGCAACGGGTTTGGCGTCAGAACGCAGGTGAGGGCGGGTGTGGC  
601 TTCCGGGGCCGCGAGCTGGAGGTCCTGCTCCGAGCGGGCCGGCCCCGCTGTCGTGGCGGGGATTAGCTGGAGCATTCCCGCTTCGAGTTGCGGGC  
701 GCGCGGGAGGCAGAGTGCAGGGCTAGCGGCAACCCCTAGCCTCGCCTCGTGTCCGGCTTGGAGCCTAGCGTGGTGTCCGGCCGCGCCCGCTGCTA  
801 CTCGGCCGCACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCGCTTCAGCAATAGGGCTAACAAAGGGAGGGTGCGGGGCT  
901 TGCTCGCCCGGAGCCCGGAGAGGTATGTTGGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCGCTTCGGAGCAGATGTCGACGCCAC  
1001 CTGGATGGGGCGAGGCTGGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCAGATCTGGCTTGGCG  
1101 GCGCCGCTTGCCTGCCTCCCTAAGTGGGTGAGGCCATCCGTCGCGCACAGTGTGGTGGTGGAAAGATGGCCGCTCCCGGCCCTGTTGCAAGGA  
1201 GCTCAAAATGGAGGACGCGGAGCCCGGTGGAGCGGGGGGTGAGTCAACCACAAAAGGAAGAGGGCTGGTCCCTCACCAGGCTGCTGTTCTGTGAC  
1301 CCCGTGGTCTATCGCCGCAATAGTCACCTCGGGCTTTTGGACAGGCTAGTCGGCGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTT  
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1801 AACCTCTACAAATGTGGTATGGAAATGTAATTAAGTACCATGACAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAA  
1901 GATCAAAGGATCTTCTTGAGATCCTTTTTTCTGGCGTAATCTGCTGTTGCAACAACAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAA  
2001 GAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCCTCAAGA  
2101 ACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGGATAAGTGTGCTTACCAGGTTGGACTCAAGACG  
2201 ATAGTTACCGGATAAGGCGCAGCGTCCGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAG  
2301 CGTGAGCTATGAGAAAGCCACGCTTCCGAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAGGGAGCTTC  
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2701 TCAATGGGTGGAGTATTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG  
2801 CCCGCTGGCATTATGCCAGTACATGACCTTATGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTT  
2901 GGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTGGAGCC  
3001 GAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAGCCAGGACCCCTAAATTCACAGACCCAAATCCCTGTAGCCGCCACG  
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4101 cggcggggggaggggatctaatggcgttggagtttggttcacatttgggggggagactagtcaggccagcctggcgctggaagtcttctggaatttg  
4201 cccctttgagtttggagcggagcctaattctcaagcctcttagcggttcaaaagtattttctaaccggtttcagGTGTTGTGAAAAGCCACCGTAATTC

**BsiWI (4326)**

**Bst1107I (4314)**

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

4301 **AAAGCAAT**TCGGAGTATACGGATCCCGTACGCCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACTCCGCTGGTCTTCCAGGTCTA  
4401 GAGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGG  
4501 AACTCCTCCTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACAGGGCCCGTCATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCA  
4601 CTTTAAAGTGACATTGAAACTGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGTACCCGAGGTAACACGGCACACTCGGGATCTGAGAAG  
4701 GGGACTGGGCTTCTATAAAAGCGCTCGGTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCTATG  
4801 **AATACACTGACTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTACTATAGGAGGGCCACC**ATGAAGAAACCTGAACTGAC  
4901 AGCAACTTCTGTTGAGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTTGTG  
7▶ rAlaThrSerValGluLysPheLeuI leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArgAlaPheSerPheAsp  
5001 GTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATCTTGTGCTGATGGTTTTTACAAAGACAGATATGTTTACAGACACTTTGCTCTGCTGCTGCCAA  
41▶ ValGlyGlyArgGlyTyrValLeuArgValAsnSerCysAlaAspGlyPheTyrLysAspArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProl  
5101 TTCCAGAAGTTCTGGACATTGGAGAATTTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGA  
74▶ leProGluValLeuAspI leGlyGluPheSerGluSerLeuThrTyrCysI leSerArgArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGl  
5201 GCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTGGATTGGTCTTTTGGTCCCAAGGC  
107▶ uLeuProAlaValLeuGlnProValAlaGluAlaMetAspAlaI leAlaAlaAlaAspLeuSerGlnThrSerGlyPheGlyProPheGlyProGlnGly  
5301 ATTTGGTCAGTACCCACTTGGAGGGATTCATTTGGCCATTGCTGCTCATGTCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTG  
141▶ l leGlyGlnTyrThrThrTrpArgAspPheI leCysAlaI leAlaAspProHisValTyrHisTrpGlnThrValMetAspAspThrValSerAlaSerV  
5401 TTGCTCAGGCACTGGATGAACCTCATGCTGTGGGCAGAAGATGTCTGAAAGTCAGACACCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGA  
174▶ aIAlaGlnAlaLeuAspGluLeuMetLeuTrpAlaGluAspCysProGluValArgHisLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAs  
5501 CAATGGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTGGAGACCTGGCTGGCT  
207▶ pAsnGlyArgI leThrAlaVal l leAspTrpSerGluAlaMetPheGlyAspSerGlnTyrGluValAlaAsnI lePhePheTrpArgProTrpLeuAla  
5601 TGCATGGAACAACAACAAGATATTTTGAAGAAGACCCAGAAGTGGCTGGTTCCTCCAGACTGAGAGCCTACATGCTCAGAATTGGCTGGACCAAC  
241▶ CysMetGluGlnGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArgI leGlyLeuAspGlnL  
5701 TGTATCAATCTCTGGTTGATGGAACCTTTGATGATGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGCTGCTGGTCTGGAACCTTTGGAAGAAC  
274▶ euTyrGlnSerLeuValAspGlyAsnPheAspAspAlaAlaTrpAlaGlnGlyArgCysAspAlaI leValArgSerGlyAlaGlyThrValGlyArgTh  
5801 TCAAATTGCAAGAAGGTCTGCTGCTGTTGGACTGATGGATGTTGAAAGTTCTGGCTGACTCTGGAACAGGAGACCCCTCCACAAGACCCAGAGCCAAG  
307▶ rGlnI leAlaArgArgSerAlaAlaValTrpThrAspGlyCysValGluValLeuAlaAspSerGlyAsnArgArgProSerThrArgProArgAlaLys  
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341▶ Glu•••  
6001 GTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACCTTTGTTTTCTTTTTGCGT  
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6201 AAATGAGAAAACCTGTGTCTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTTCTGTTTTACGAATCTGGAACCTTCTGAAAAATGTA  
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