# 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 counterpart[1], 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[2]. 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[3].

• **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 expression[5].

• **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 mammalin 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
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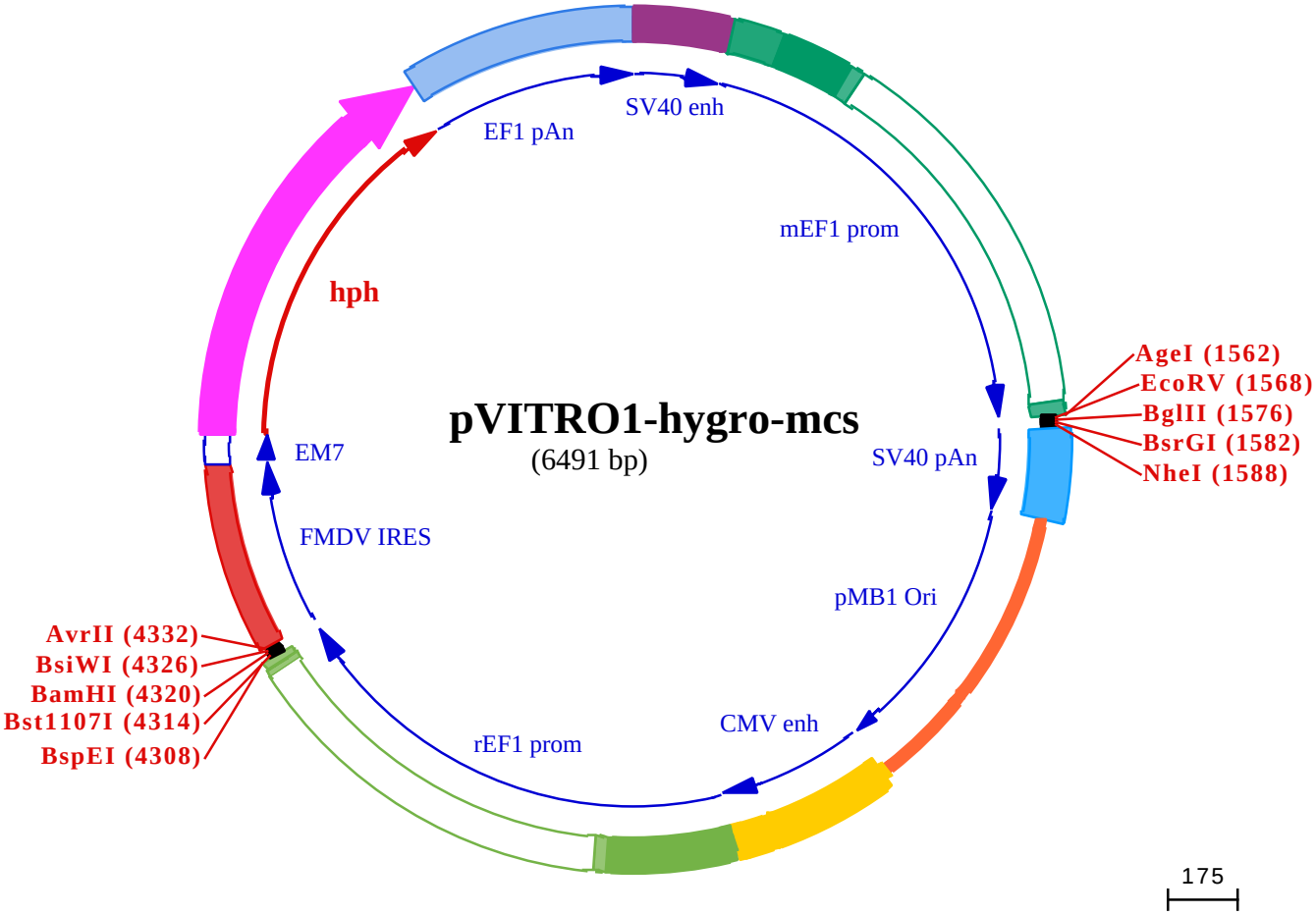
pVITRO1-hygro-mcs
(6491 bp)
SV40 enh
EF1 pAn
mEF1 prom
hph
EM7
FMDV IRES
SV40 pAn
pMB1 Ori
CMV enh
rEF1 prom
AgeI (1562)
EcoRV (1568)
BglII (1576)
BsrGI (1582)
NheI (1588)
AvrII (4332)
BsiWI (4326)
BamHI (4320)
Bst1107I (4314)
BspEI (4308)
175

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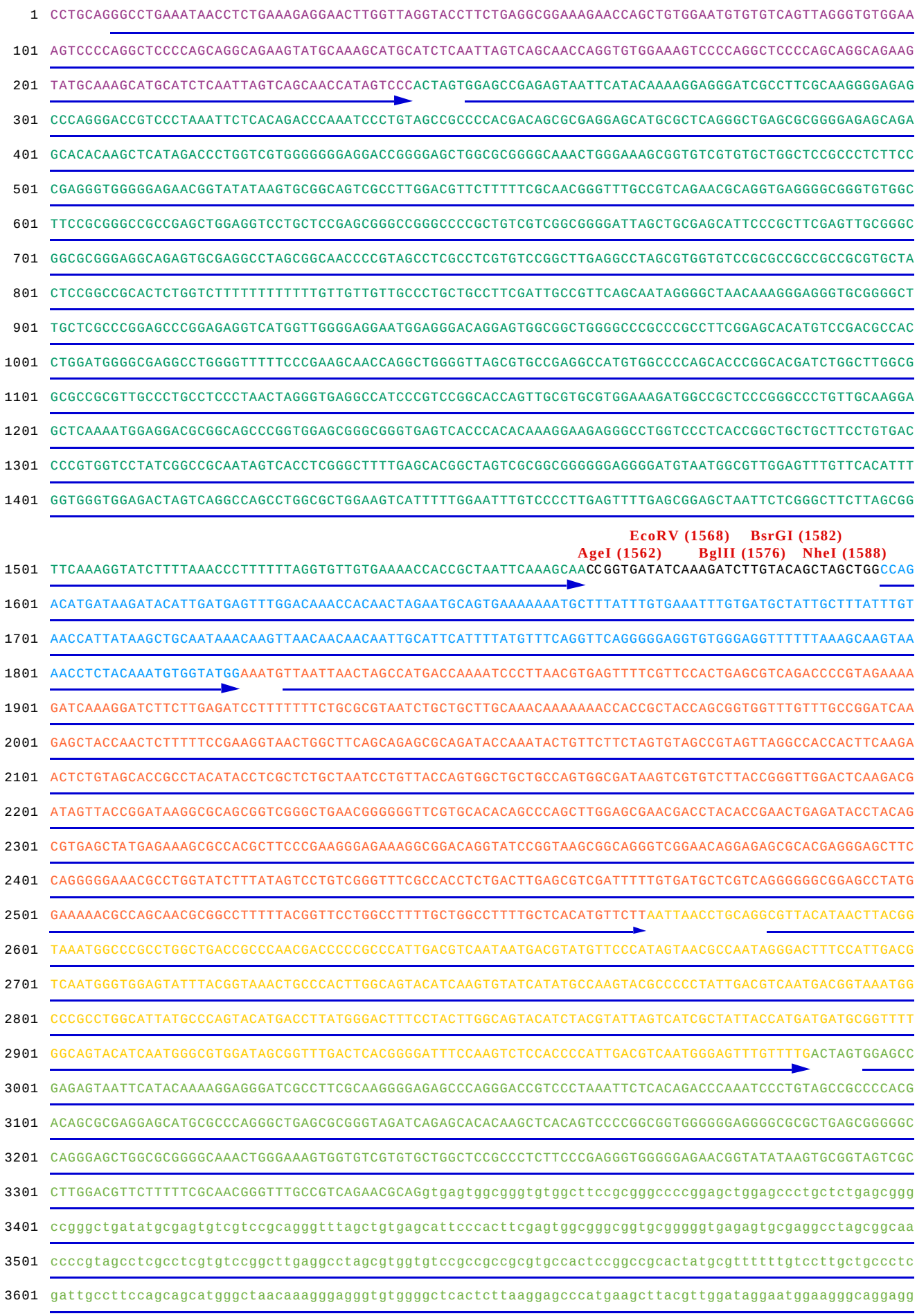
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