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 µg 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-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.

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.

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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 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20°C.

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

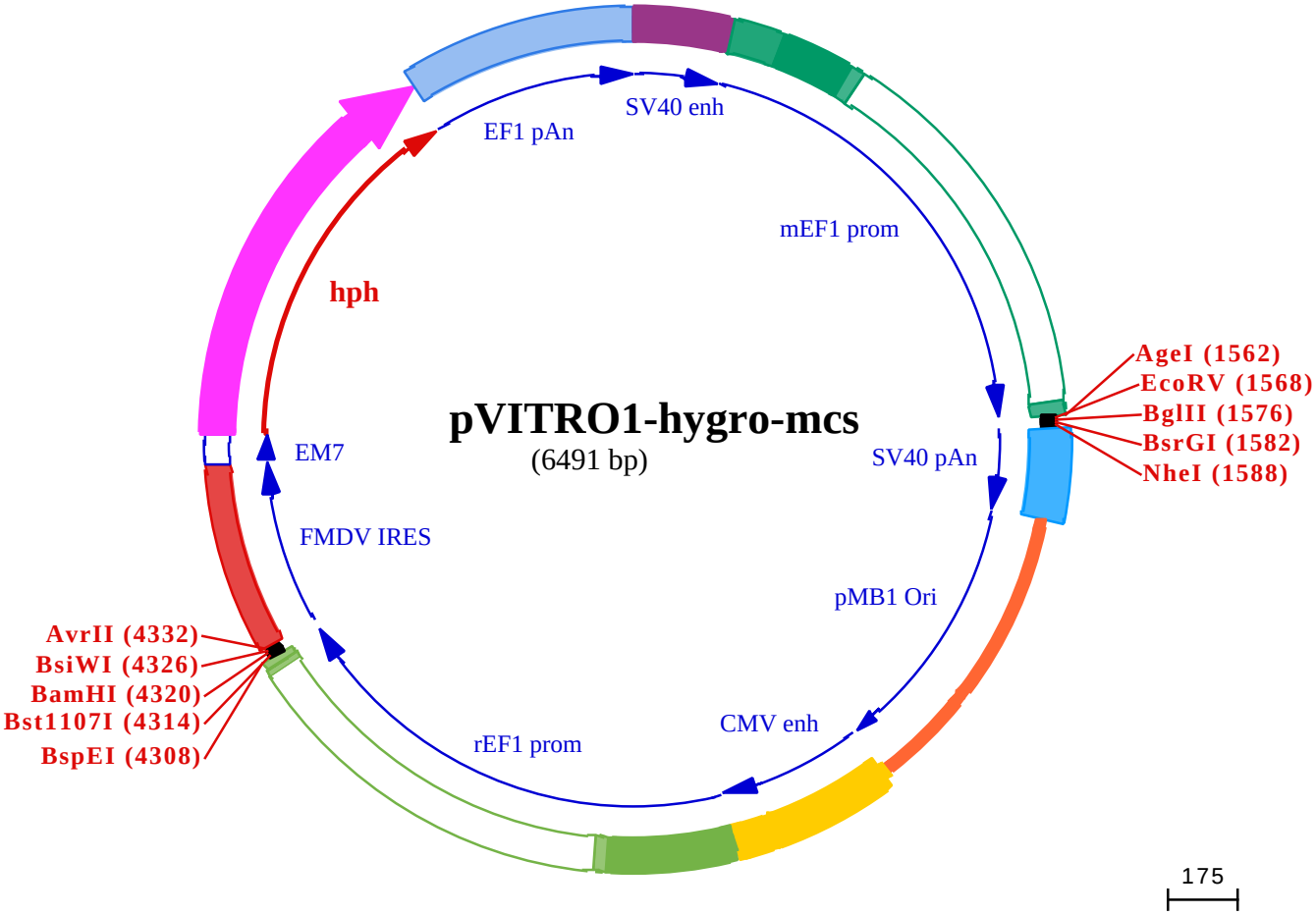
E. coli FastMedia™ Hygro is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Hygro is a TB (liquid) or LB (solid) based medium with hygromycin B.
E. coli FastMedia™ Hygro can be ordered separately (catalog code # fas-hg-l, fas-hg-s).

Method:

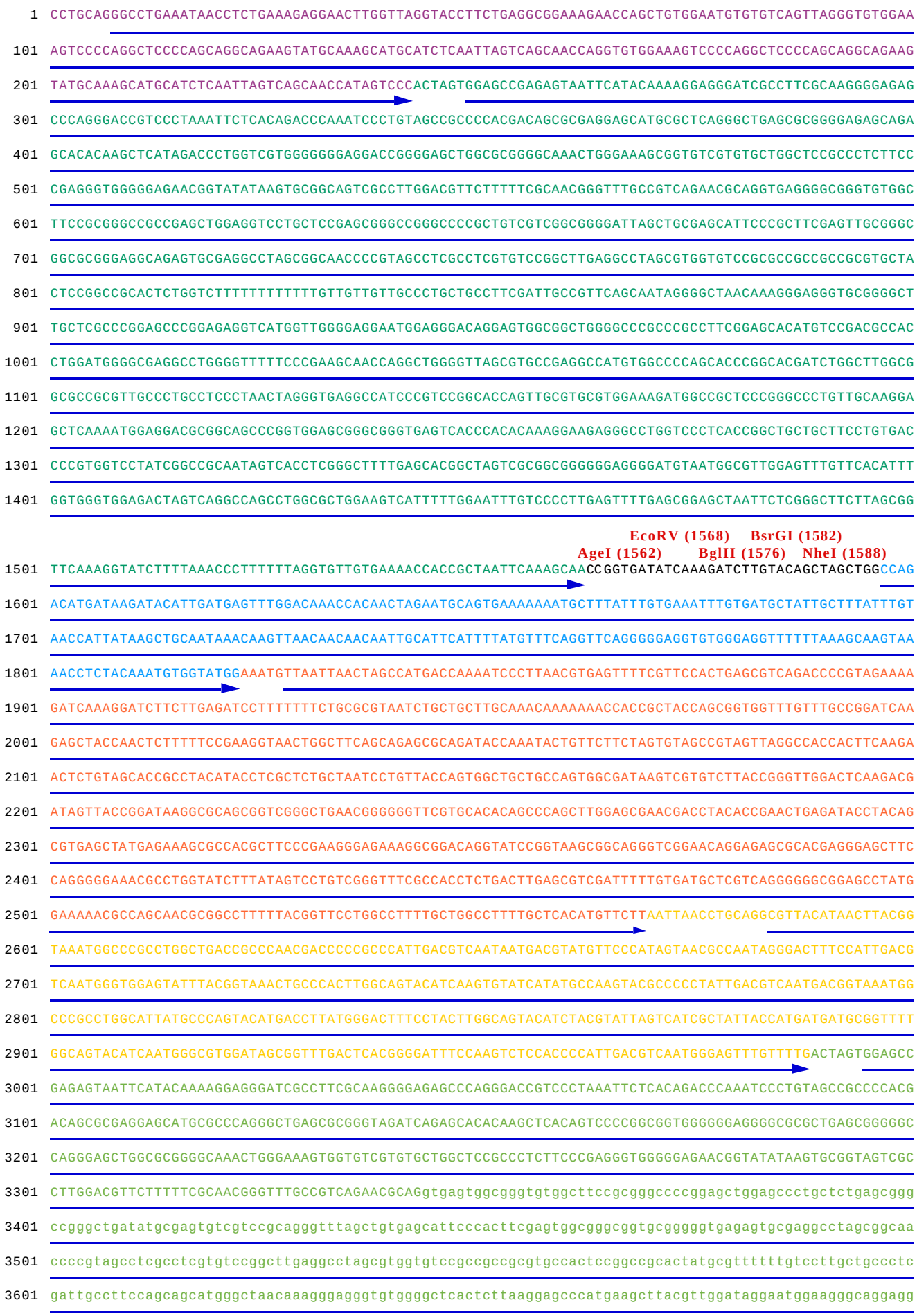
1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave FastMedia™.**
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°C before pouring plates. Let liquid media cool to 37°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 µg	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


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

InvivoGen


EcoRV (1568)
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