pVIVO1-mcs

A multigenic cloning plasmid for strong and sustained expression in tumors

Catalog code: pvivo1-mcs

https://www.invivogen.com/pvivo-mcs

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO1-mcs provided as lyophilized DNA
- 1 ml of Hygromycin B Gold (ultrapure Hygromycin B; 100 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Resuspended DNA is stable for 12 months when stored at -20 °C. Avoid repeated freeze-thaw cycles.
- Store Hygromycin B Gold at 4 °C or at -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

pVIVO1 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector. pVIVO1-mcs may be used for:

- **Cloning two genes of interest.** pVIVO1-mcs contains two multiple cloning sites (MCS) for convenient insertion of two genes.
- **Co-expression of two genes** *in vivo*. Both multiple cloning sites are located downstream of the glucose regulated protein (GRP) 78 and 94 promoters, allowing high levels of expression of the cloned transgenes in tumors. Expression may be achieved *in vivo* and *in vitro* in transient transfection experiments.

PLASMID FEATURES

• **haGRP78 and hGRP94 prom:** The hamster GRP78 and human GRP94 promoters drive weak levels of expression in normal conditions and are induced in stress conditions prevailing inside tumors, such as glucose deprivation and hypoxia[1]. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly[2].
• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[3].
• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), 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[4].
• **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.*[5]
• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
• **Hygro-ΔCpG** is a new allele of the *hph* gene conferring resistance to hygromycin B. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Hygro-ΔCpG* gene allows the selection of *E. coli* clones transformed with a pVIVO plasmid.
Note: Stable transfection of mammalian clones cannot be performed due to the absence of a eukaryotic promoter upstream of the Hygro-ΔCpG gene.
• **Term:** The *E. coli rps O* terminator allows efficient transcription termination of the *Hygro-ΔCpG* gene.
• **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.
• **MCS:** 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, *Bsp* HI, *Xba* I, *Hind* III, *Bst* 1107I, and *Avr* II
Age I is compatible with *Ava*I, *Xma*I, *Bsa*WI, *Bsp*EI, *Bsr*FI, *Sgr*AI and *Ngo*MIV
Bsp HI is compatible with *Nco* I and *Bsp* LU11I
Xba I is compatible with *Nhe* I, *Spe* I and *Avr* II
Bst 1107I is compatible with any other blunt-end restriction enzymes
Avr II is compatible with *Xba* I, *Spe* I and *Nhe* I

MCS2 contains the following restriction sites:
*Ngo*M I, *Nco* I, *Bgl* II, *Eco*R I, *and Nhe* I
Nco I is compatible with *Bsp*H I and *Bsp*LU11 I
Bgl II is compatible with *Bam*H I, *Bst*Y I and *Bcl* I
*Eco*R I is compatible with *Apo* I, *Mfe* I and *Tsp*509 I
Nhe I is compatible with *Xba* I, *Spe* I and *Avr* II

Important: *Bsp* HI and *Nco* I restriction sites, located at the 5' end of MCS1 and MCS2 respectively, contain an ATG in their sequence.

1. Little E. *et al.*, 1994. The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. Crit. Rev. Eukaryot. Gene Expr. 4:1-18. **2. Gazit G. *et al.* 1999.** Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. Cancer Res 59: 3100-6. **3. Dean DA. *et al.*, 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **4. Boshart M. *et al.*, 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41(2):521-30. **5. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258.

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

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

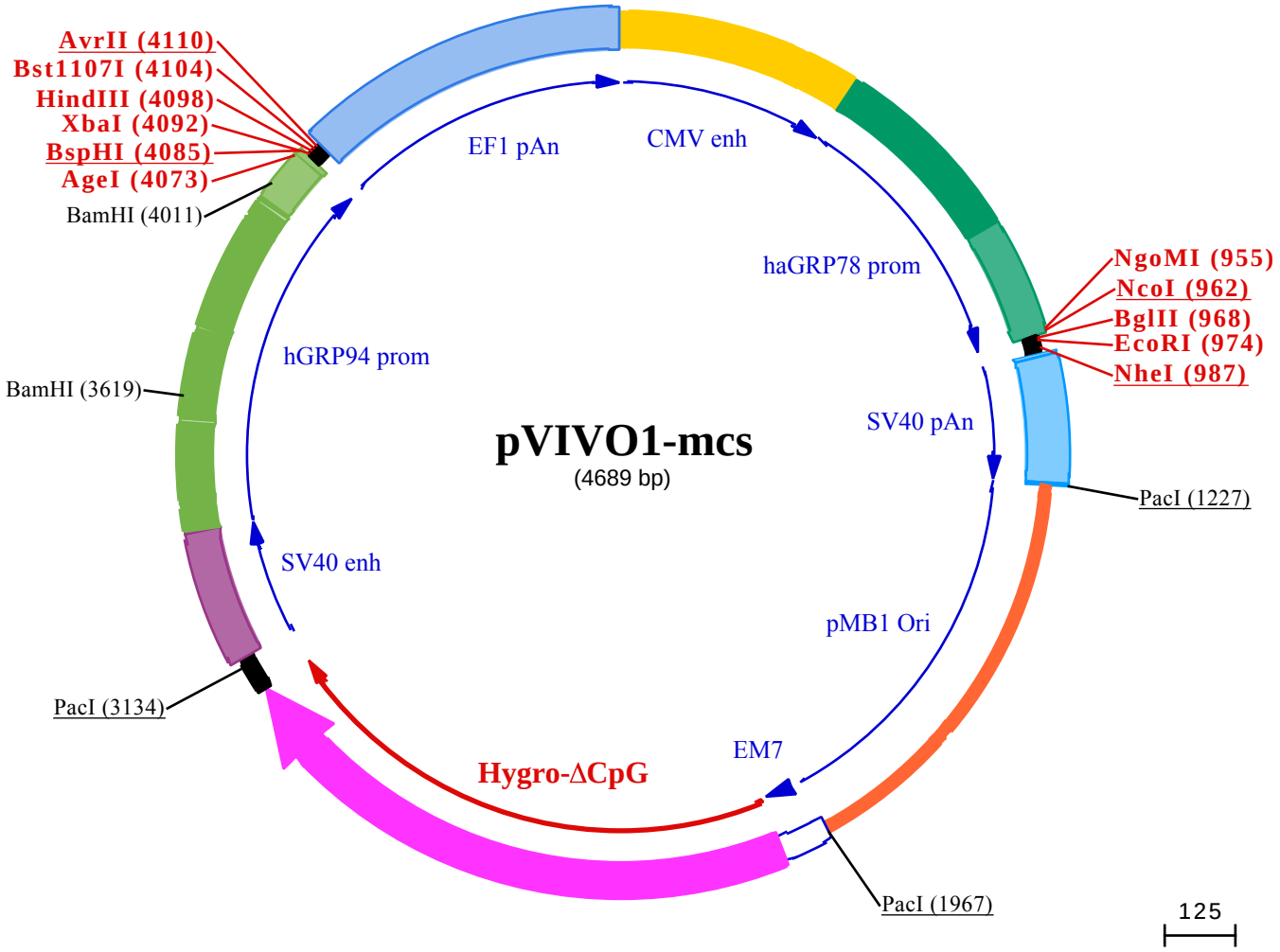
Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

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.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent *E. coli*	gt116-11
Hygromycin B Gold	Selection antibiotic	ant-hg-1
pVIVO1-GFP/LacZ	Dual reporter plasmid	pvivo1-gfplacz
pVIVO1-GFP/SEAP	Dual reporter plasmid	pvivo1-gfpsp
pVIVO1-Lucia/SEAP	Dual reporter plasmid	pvivo1-lucsp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Bst1107I (4104)
HindIII (4098)
XbaI (4092)
BspHI (4085)
AgeI (4073)
BamHI (4011)
BamHI (3619)
PacI (3134)
PacI (1227)
PacI (1967)
NgoMI (955)
NcoI (962)
BglII (968)
EcoRI (974)
NheI (987)
EF1 pAn
CMV enh
haGRP78 prom
hGRP94 prom
SV40 pAn
SV40 enh
pMB1 Ori
EM7
Hygro-ΔCpG
pVIVO1-mcs
(4689 bp)
125

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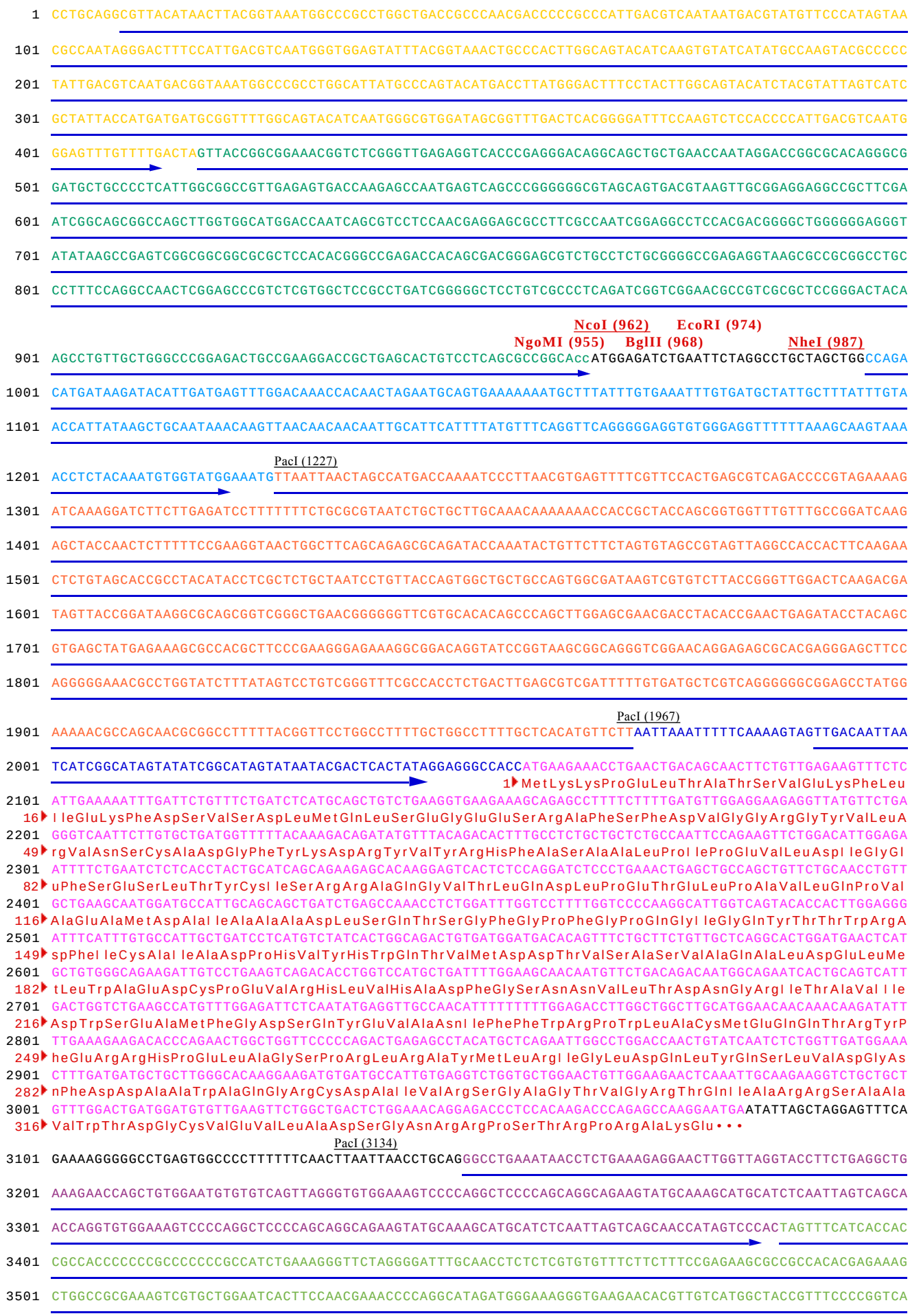
NcoI (962)
EcoRI (974)
NgoMI (955)
BglII (968)
NheI (987)
PacI (1227)
PacI (1967)
PacI (3134)


BamHI (3619)
3601 CGGAATAAACGCTCTCTAGGATCCGGAAGTAGTTCCGCCGCGACCTCTCTAAAAGGATGGATGTGTTCTCTGCTTACATTCATTGGACGTTTTCCCTTAG
3701 AGGCCAAGGCCGCCCAGGCAAAGGGGCGGTCCCACGCGTGAGGGGCCCGCGGAGCCATTTGATTGGAGAAAAGCTGCAAACCCTGACCAATCGGAAGGAG
3801 CCACGCTTCGGGCATCGGTCACCGCACCTGGACAGCTCCGATTGGTGGACTTCCGCCCCCCCTCACGAATCCTCATTGGGTGCCGTGGGTGCGTGGTGCG
3901 GCGCGATTGGTGGGTTCATGTTTCCCGTCCCCCGCCCGCGAGAAGTGGGGGTGAAAAGCGGCCCGACCTGCTTGGGGTGTAGTGGGCGGACCGCGCGGCT
XbaI (4092)
BamHI (4011)
AgeI (4073)
BspHI (4085)
HindIII (4098)
4001 GGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGGTGGAGGCGGCTCCTGCGATCGAAGGGGACTTGAGACTCACCGGTCGCACGTCATGAATCTAGAAAG
AvrII (4110)
Bst1107I (4104)
4101 CTTGTATACCCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGT
4201 TTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGT
4301 GGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAAACAACTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAAGTTAA
4401 ATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATT
4501 CTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTTCTTTGAT
4601 TACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG