pVITRO1-hygro-GFP/SEAP

An expression plasmid coding for the GFP and SEAP reporter genes

Catalog code: pvitro1-gfpsp

https://www.invivogen.com/pvitro1-gfpseap

For research use only

Version 18J06-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-hygro-GFP/SEAP 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 family of vectors that 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-GFP/SEAP contains the reporter genes GFP and SEAP and can be used as a control vector. pVITRO1-GFP/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (NcoI/AvrII for GFP and BspHI/NheI for SEAP) that allow for convenient cloning of ORFs.

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-hygro-GFP/SEAP 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. 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[2].
- 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 several-fold 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 is 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-1αpromoter 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.
- GFP gene: This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- SEAP is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophometer at 405 nm. SEAP activity that can be readily assessed qualitatively and quantitatively using HEK-Blue™ Detection or QUANTI-Blue™.

1. Kim DW. *et al.*, **1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system Gene 91(2):217-23. **2. Dean DA.** *et al.*, **1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **3. 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. **4. Carswell S. & Alwine JC. ,1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 9(10): 4248-58. **5. Ramesh N. et al., 1996.** High-riter 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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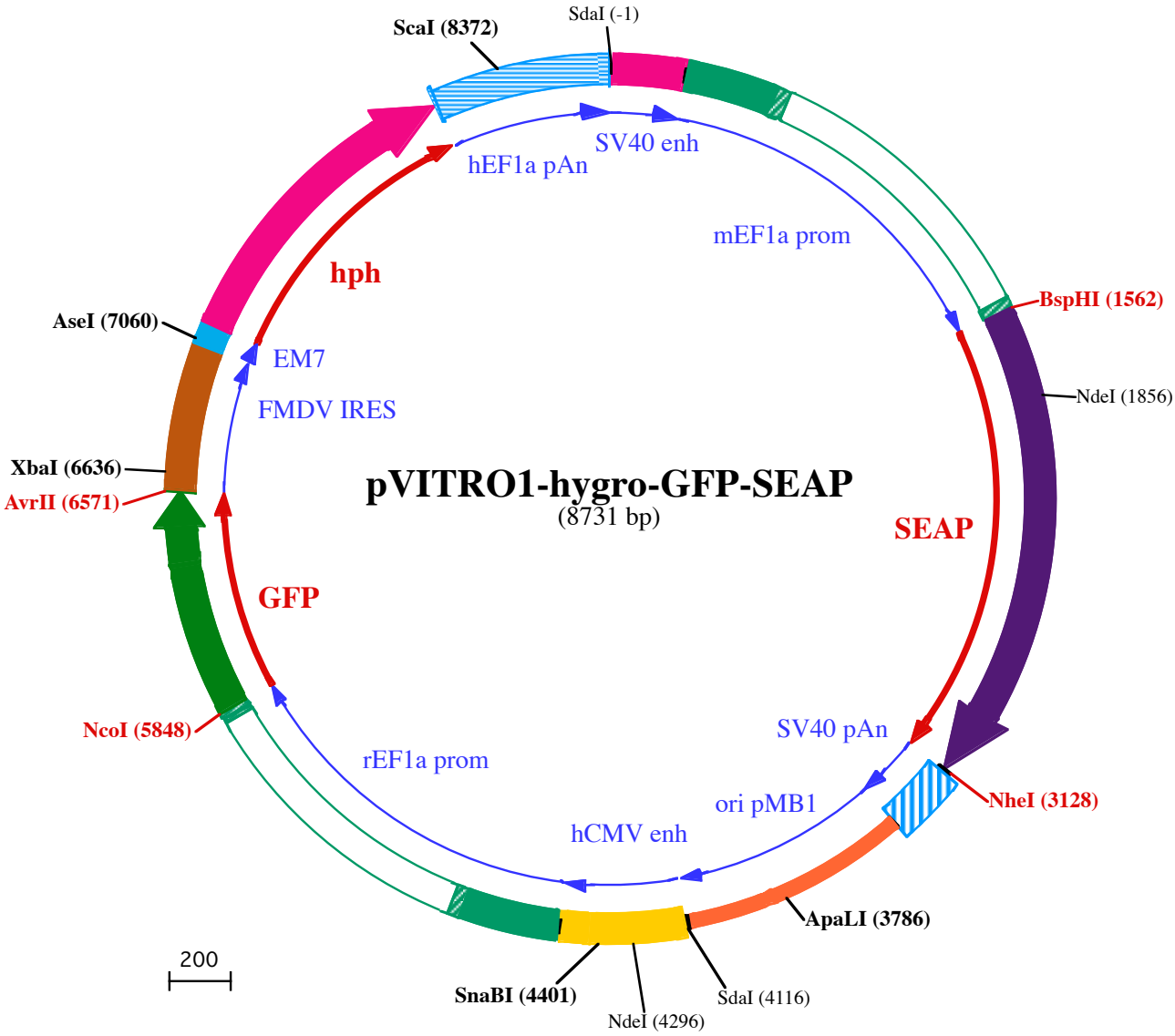
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mEF1a prom
hph
BspHI (1562)
AseI (7060)
NdeI (1856)
EM7
FMDV IRES
XbaI (6636)
AvrII (6571)
pVITRO1-hygro-GFP-SEAP
(8731 bp)
SEAP
GFP
NcoI (5848)
SV40 pAn
rEF1a prom
ori pMB1
NheI (3128)
hCMV enh
ApaLI (3786)
200
SnaBI (4401)
NdeI (4296)
SdaI (4116)


SdaI (-1)
BspHI (1562)
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NheI (3128)
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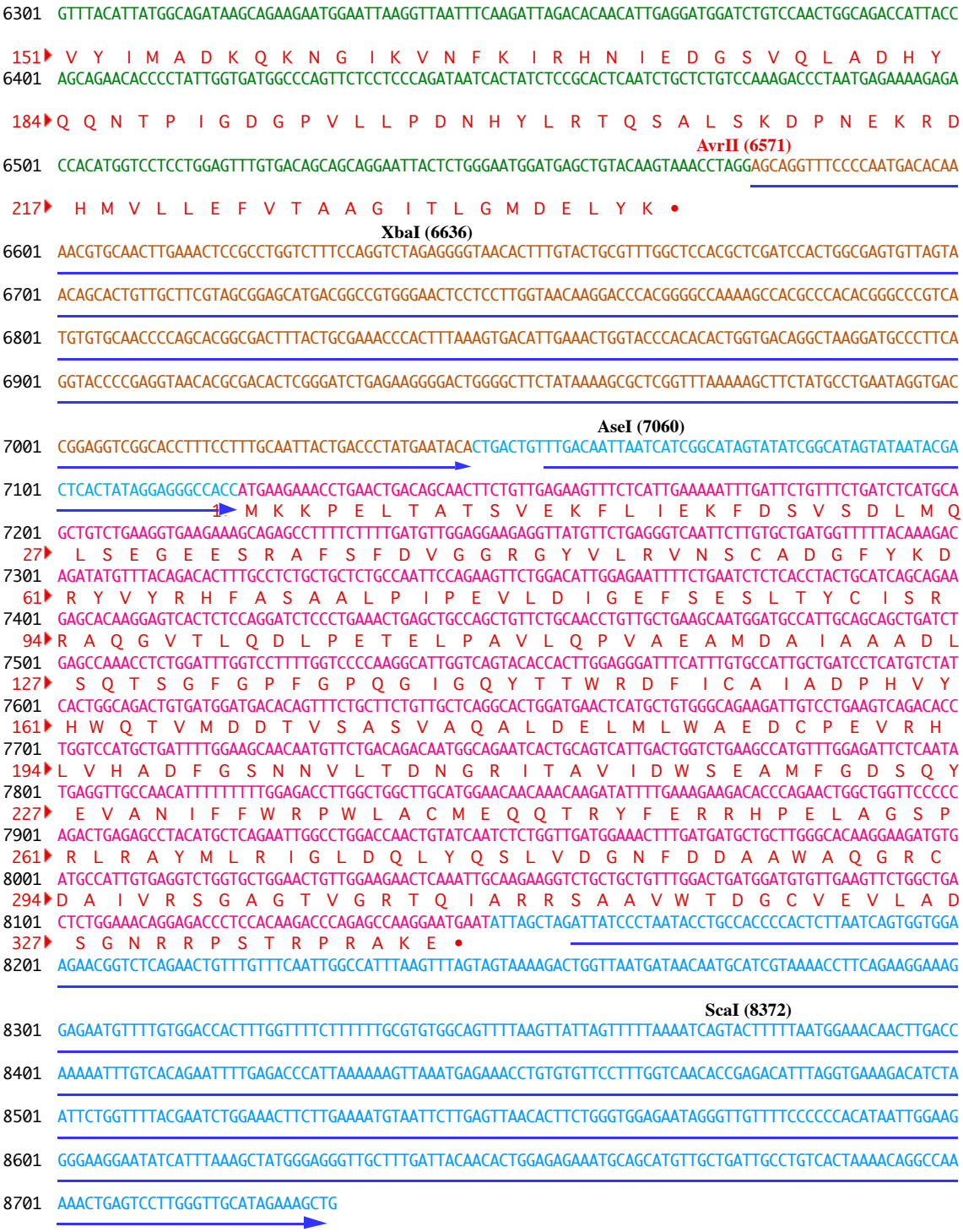
AvrII (6571)
XbaI (6636)
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