pVITRO1-neo-GFP/LacZ

An innovative multigenic plasmid for high levels of expression of the GFP and LacZ reporter genes

Catalog # pvitro1-ngfplacz

For research use only

Version # 05L08-SV

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-neo-GFP/LacZ provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Kan

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cyles.
- Store *E. coli* Fast-Media® Kan pouches at room temperature. Fast-Media® 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. pVITRO plasmids 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-neo-GFP/LacZ contains the reporter genes GFP and LacZ and can be used as a control vector.

pVITRO1-neo-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Nco* I/*Avr* II for GFP and *Bsp*H I/*Nhe* I for LacZ) that allow for convenient cloning of ORF's which can be selected from InvivoGen's extensive pORF and pBLAST lists

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. 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), 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 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*.

• **Neo:** The neo gene from Tn5 encodes an aminoglycoside 3'-phospho-transferase (3' APH II) that confers resistance to the antibiotics kanamycin in bacteria and G418 in mammalian cells. In bacteria, *neo* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *neo* 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.

• **LacZ gene:** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope.

• **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).

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* Fast-Media Kan:

E. coli Fast-Media® Kan is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Kan is a TB (liquid) or LB (solid) based medium with kanamycin, and contains stabilizers.

E. coli Fast-Media® Kan can be ordered separately (catalog code # fas-kn-l, fas-kn-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 Fast-Media®.**
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 Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

References:

1. Kim DW. *et al.* 1990. Gene 91(2):217-23.
2. Dean DA. *et al.* 1999. Exp. Cell. Res. 253:713-22
3. Boshart M. *et al.* 1985. Cell 141(2):521-30
4. Carswell S., and Alwine JC. 1989. Mol. Cell Biol. 10: 4248-4258.
5. Ramesh N *et al.* 1996. Nucleic Acids Res. 24(14):2697-700.

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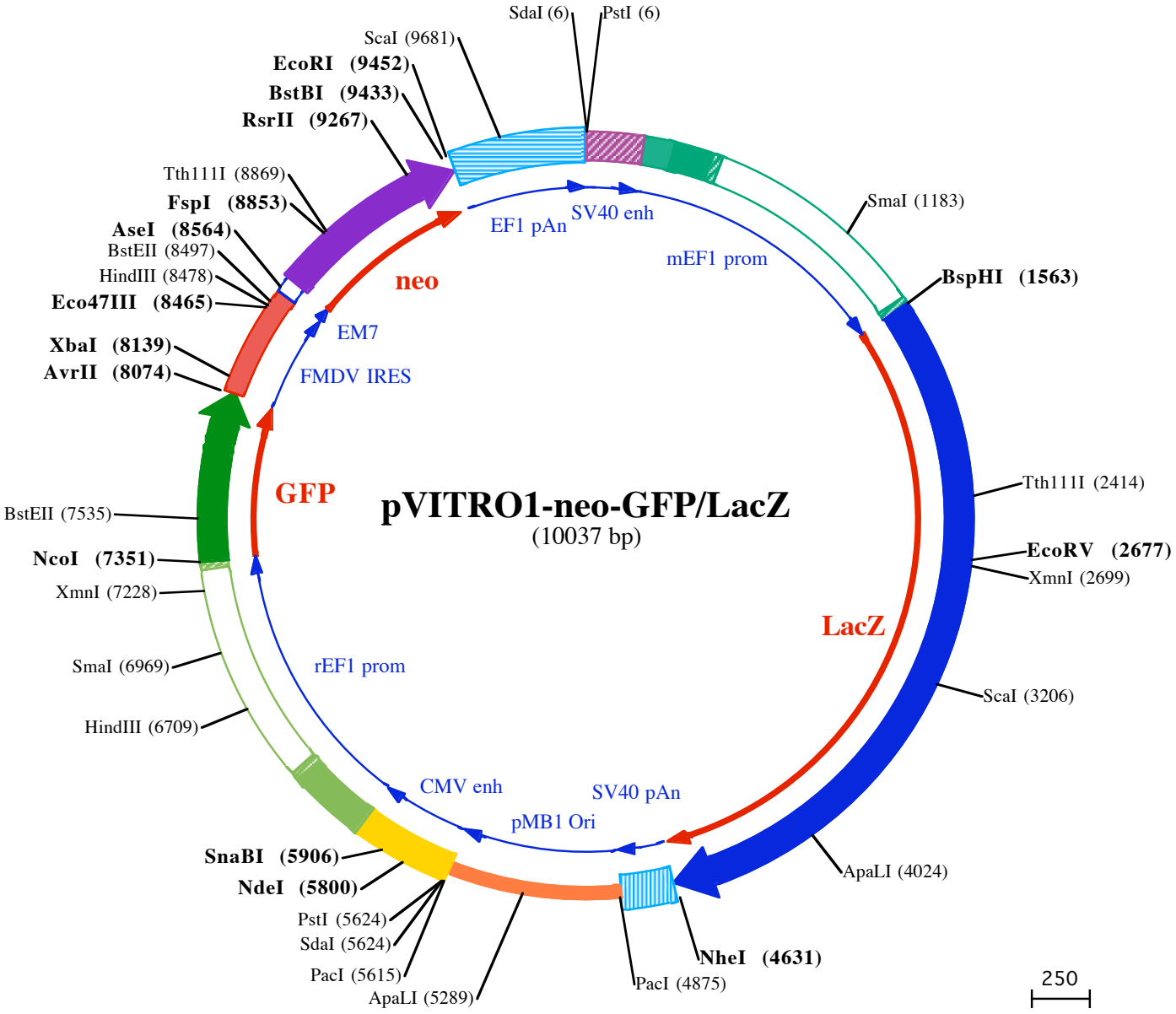
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mEF1 prom
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rEF1 prom
CMV enh
SV40 pAn
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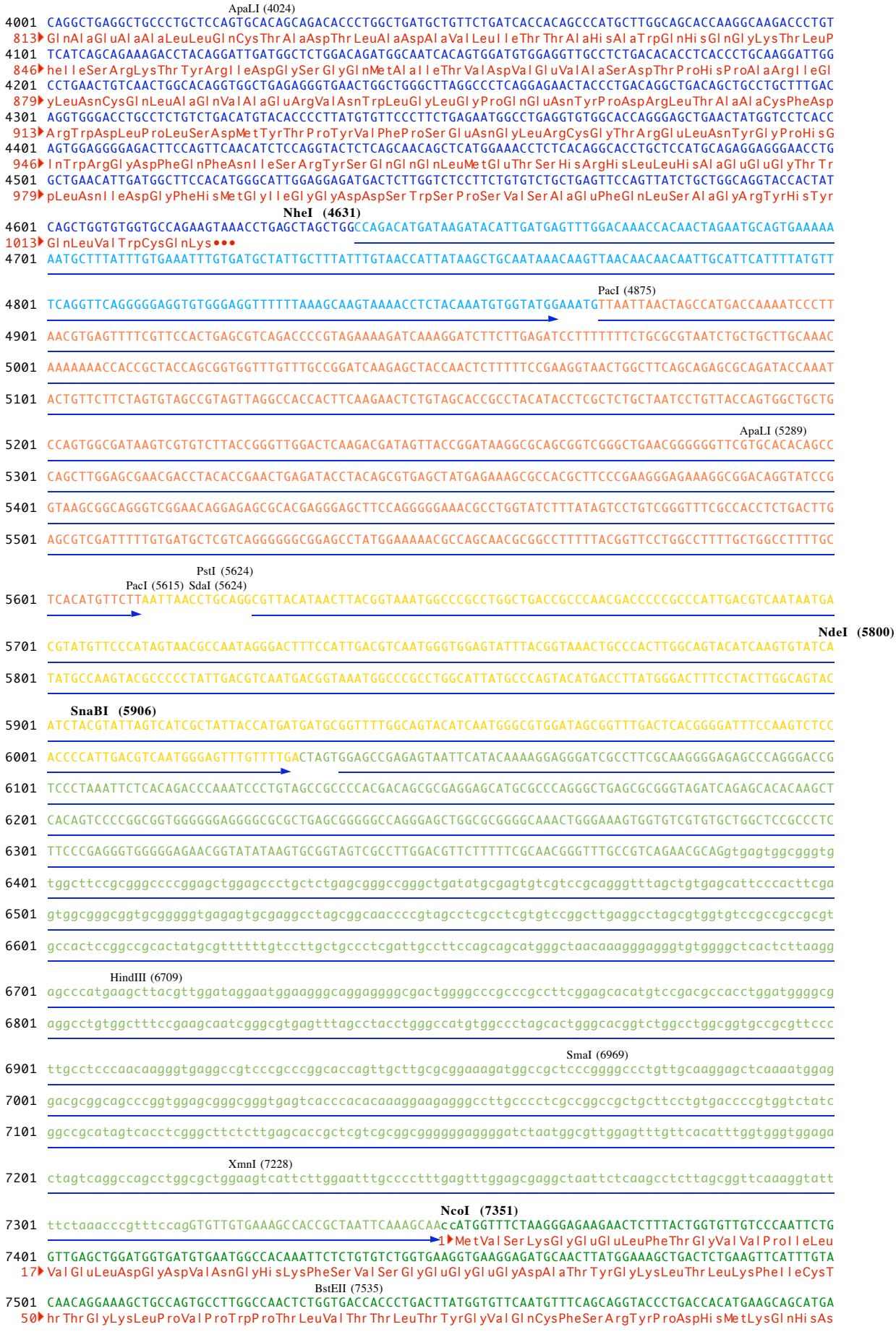
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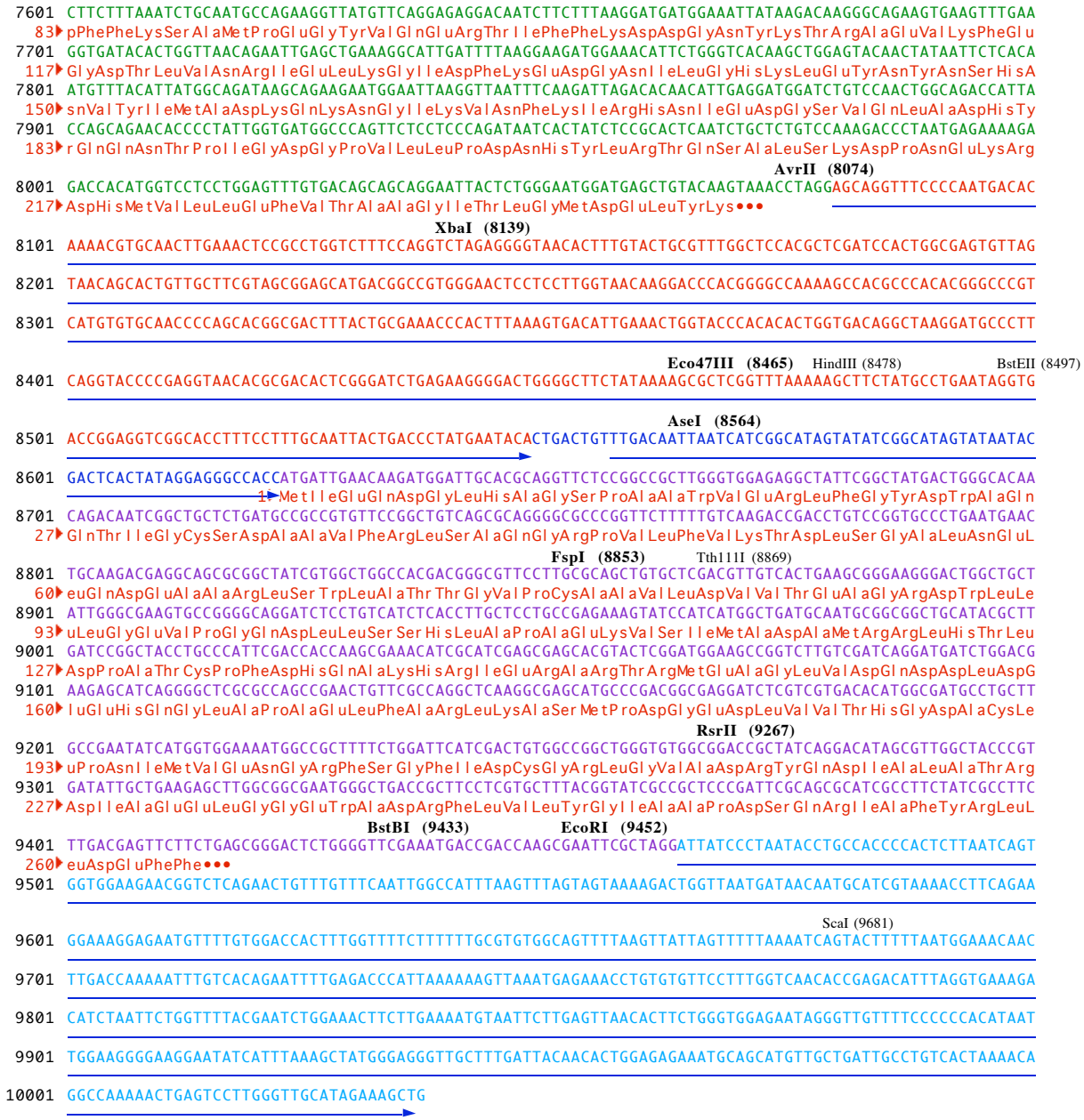
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