# pVIVO2-GFP/LacZ

A multigenic plasmid for high levels of expression of GFP and LacZ reporter genes

Catalog code: pvivo2-gfplacz

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

For research use only

Version 19J02-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVIVO2-GFP/LacZ is provided as lyophilized DNA.
- 1 ml of 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

pVIVO2 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector.

- **pVIVO2-GFP/LacZ** contains the reporter genes GFP and LacZ and can be used as a control vector.

- **pVIVO2-GFP/LacZ** also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Bsp*HI/*Avr* II for GFP and *Nco* I/*Nhe* I for LacZ) that allow for convenient cloning of ORFs which can be selected from InvivoGen's extensive list of genes. For more information, visit: https://www.invivogen.com/genes.

## PLASMID FEATURES

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein[1]. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- **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 severalfold more active than the SV40 enhancer[3].
- **LacZ-ΔCpG 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. In order to reduce the immunogenicity of this bacterial gene the 298 CpG motifs from the wildtype have been removed by chemically synthesizing the gene.
- **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.
- **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.
- **LGFP** is a new allele of the green fluorescent protein. The gene has been chemically synthesized to remove the CpGs to ensure long-lasting expression.
- **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.

## 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.

## References

**1. Eisenstein RS. & Munro HN. 1990.** Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **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. 10: 4248-4258.

TECHNICAL SUPPORT
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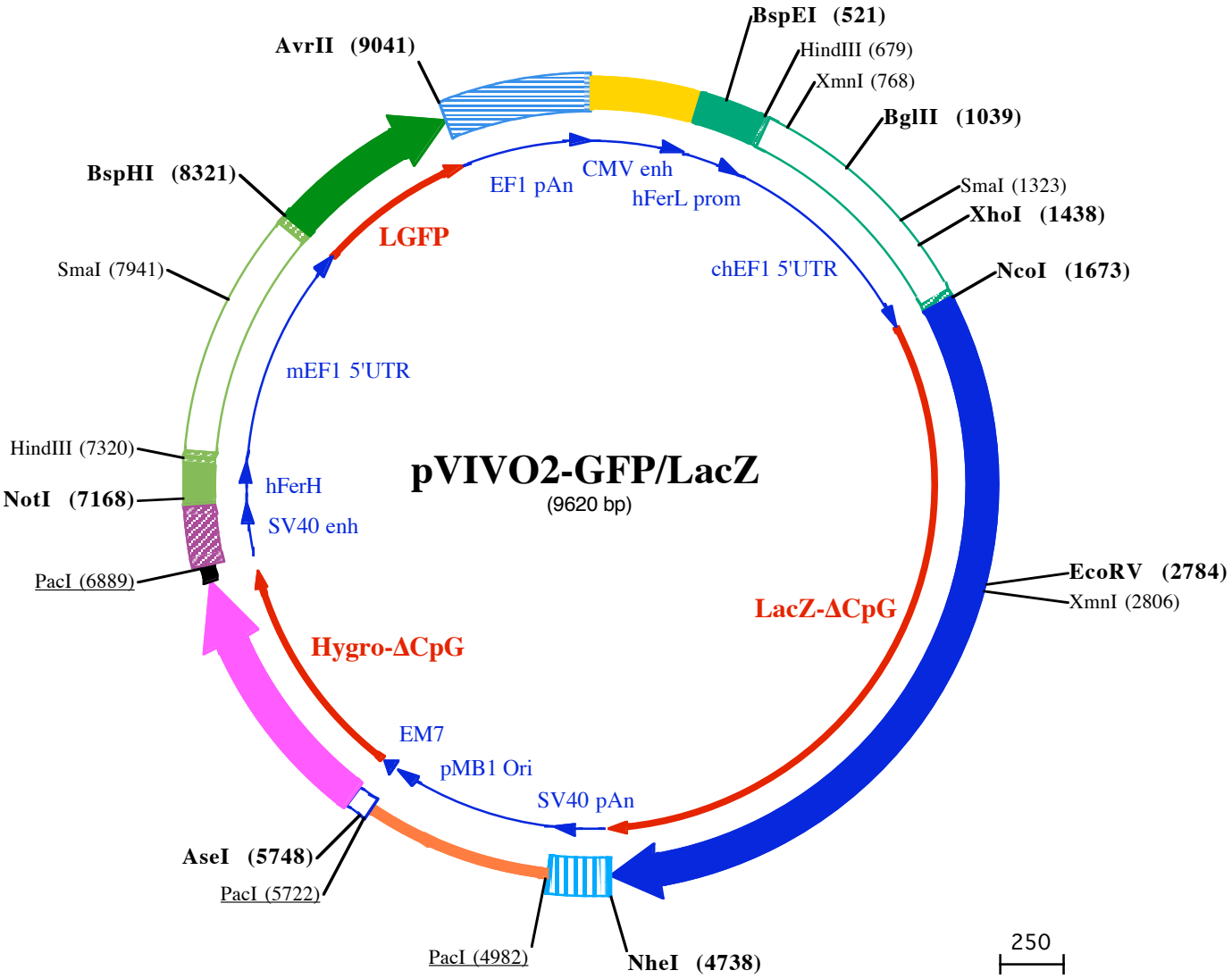
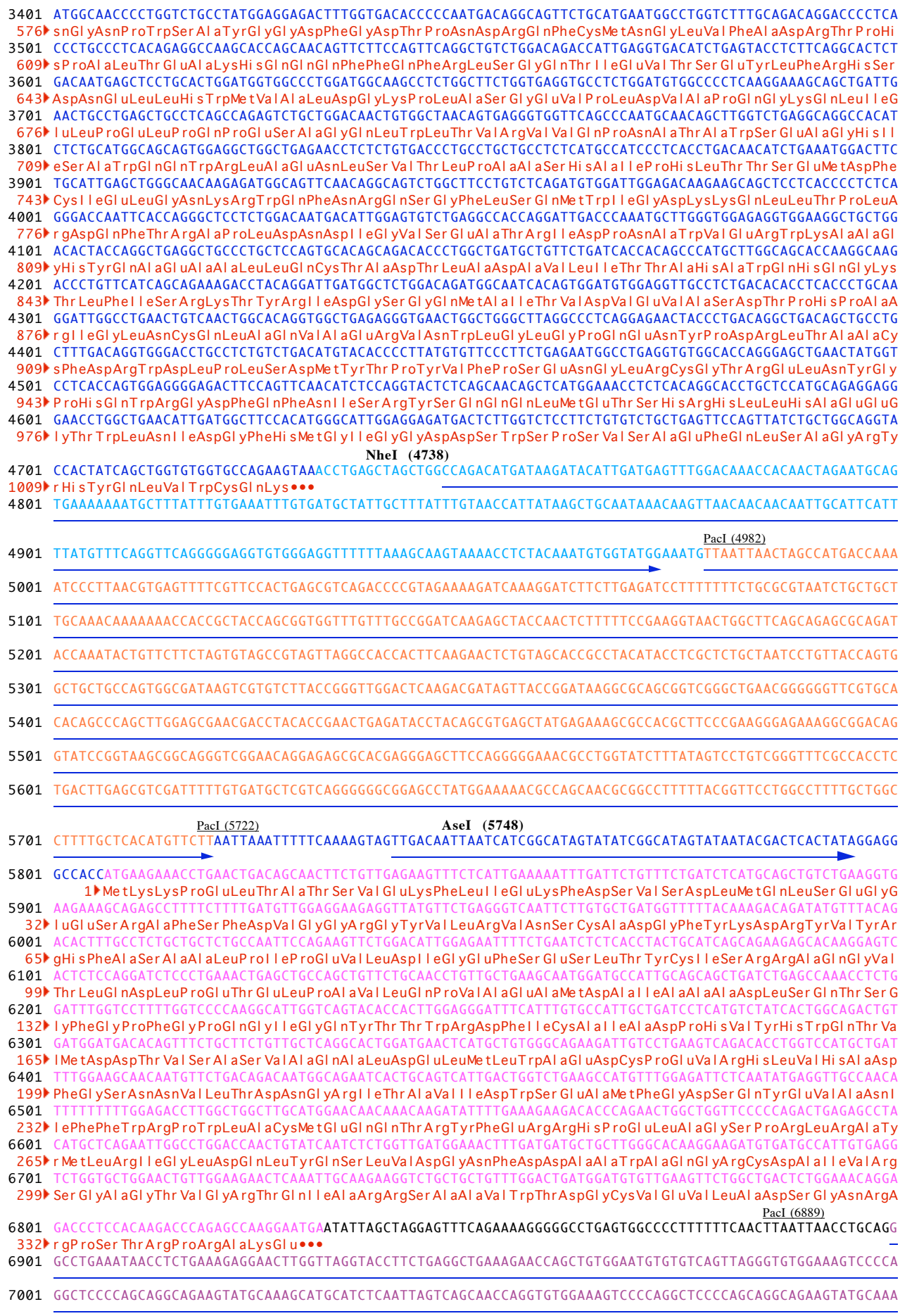

pVIVO2-GFP/LacZ
(9620 bp)
BspEI (521)
HindIII (679)
XmnI (768)
BglII (1039)
SmaI (1323)
XhoI (1438)
NcoI (1673)
EcoRV (2784)
XmnI (2806)
LacZ-ΔCpG
chEF1 5'UTR
hFerL prom
CMV enh
EF1 pAn
LGFP
AvrII (9041)
BspHI (8321)
SmaI (7941)
mEF1 5'UTR
HindIII (7320)
NotI (7168)
hFerH
SV40 enh
PacI (6889)
Hygro-ΔCpG
EM7
pMB1 Ori
SV40 pAn
AseI (5748)
PacI (5722)
PacI (4982)
NheI (4738)
250

InvivoGen


BspEI (521)
HindIII (679)
XmnI (768)
BglII (1039)
SmaI (1323)
XhoI (1438)
NcoI (1673)
EcoRV (2784)
XmnI (2806)


NheI (4738)
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