pVIVO2-mcs

A multigenic cloning plasmid for strong and sustained expression in normal tissues

Catalog code: pvivo2-mcs

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO2-mcs 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-mcs may be used for:

- **Cloning two genes of interest.** pVIVO2-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 strong, ubiquitous Ferritin composite promoters, allowing high levels of expression of the cloned transgenes. Expression may be achieved *in vivo* and *in vitro* in transient transfection experiments.

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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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].
- **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.
- **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:
Bsp HI, *Xba* I, *Bsr*G I, *Bst* 1107I, and *Avr* II.
Bsp HI is compatible with *Nco* I and *Bsp* LU11I.
Xba I is compatible with *Nhe* I, *Spe* I and *Avr* II.
*Bsr*G I iscompatible with *Acc*65 I, *Ban* I and *Bsi*W I.
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:
Nco I, *Bam*H I, *Eco*R I, *Eco*R V, *and Nhe* I
Nco I is compatible with *Bsp*H I and *Bsp*LU11 I.
*Bam*H I is compatible with *Bgl* II, *Bst*Y I, *Dpn* II, and *Bcl* I.
*Eco*R I is compatible with *Apo* I, *Mfe* I and *Tsp*509 I.
*Eco*R V is compatible with any other blunt-end restriction enzymes.
Nhe I is compatible with *Xba* I, *Spe* I and *Avr* II.

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.

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.

TECHNICAL SUPPORT
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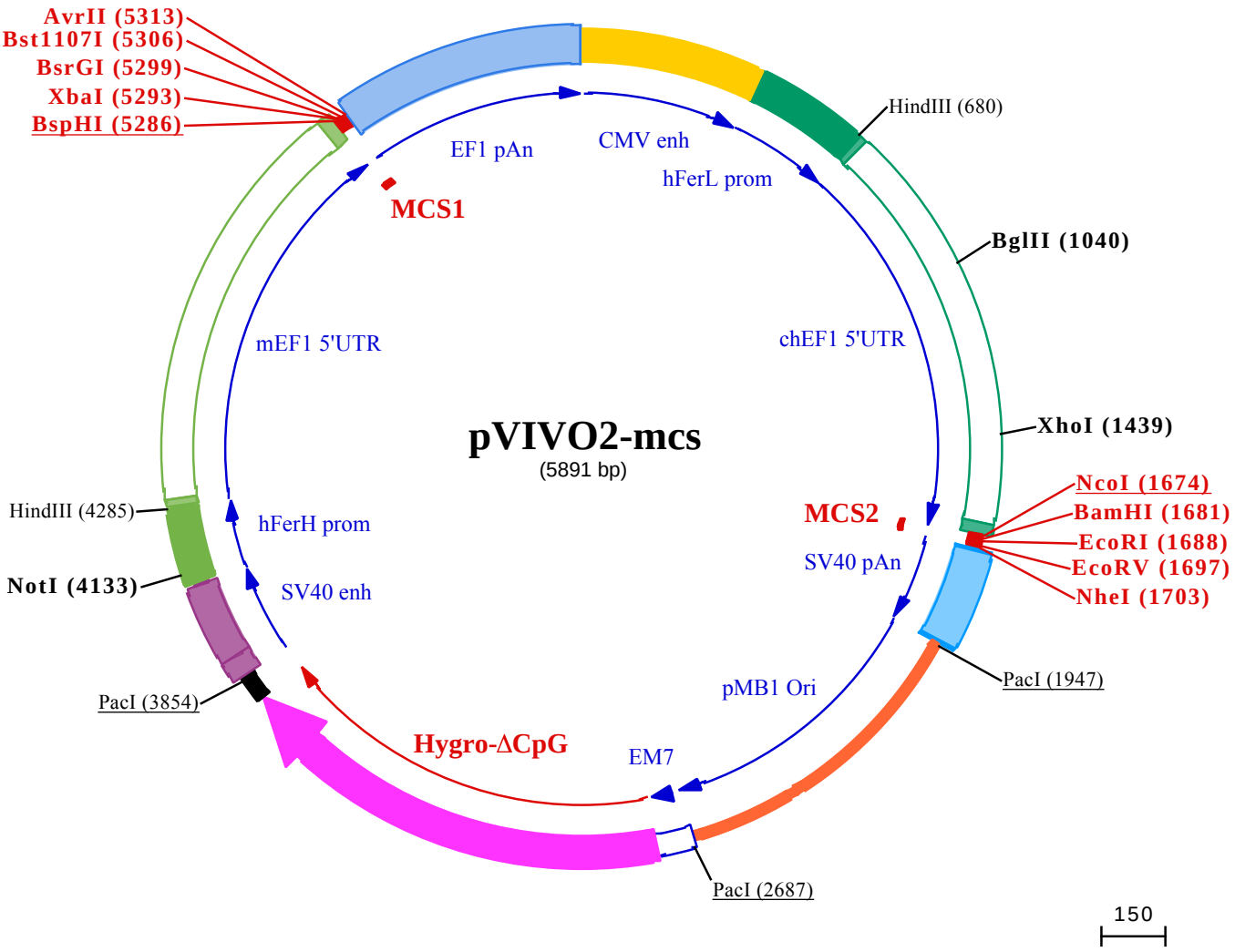
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BsrGI (5299)
XbaI (5293)
BspHI (5286)
HindIII (680)
BglII (1040)
XhoI (1439)
NcoI (1674)
BamHI (1681)
EcoRI (1688)
EcoRV (1697)
NheI (1703)
PacI (1947)
PacI (2687)
HindIII (4285)
NotI (4133)
PacI (3854)
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CMV enh
hFerL prom
MCS1
mEF1 5'UTR
chEF1 5'UTR
pVIVO2-mcs
(5891 bp)
hFerH prom
SV40 enh
MCS2
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pMB1 Ori
Hygro-ΔCpG
EM7
150

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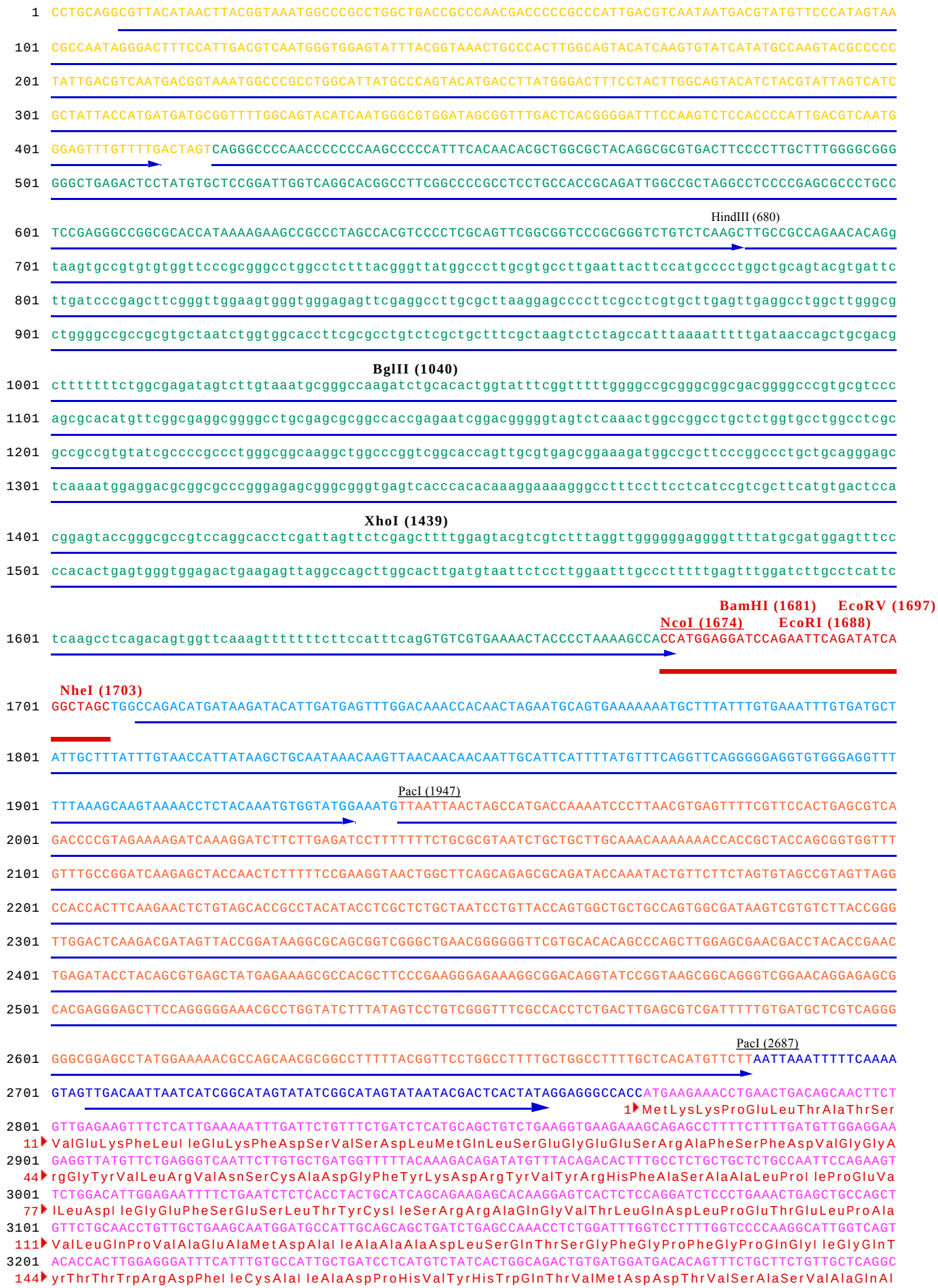
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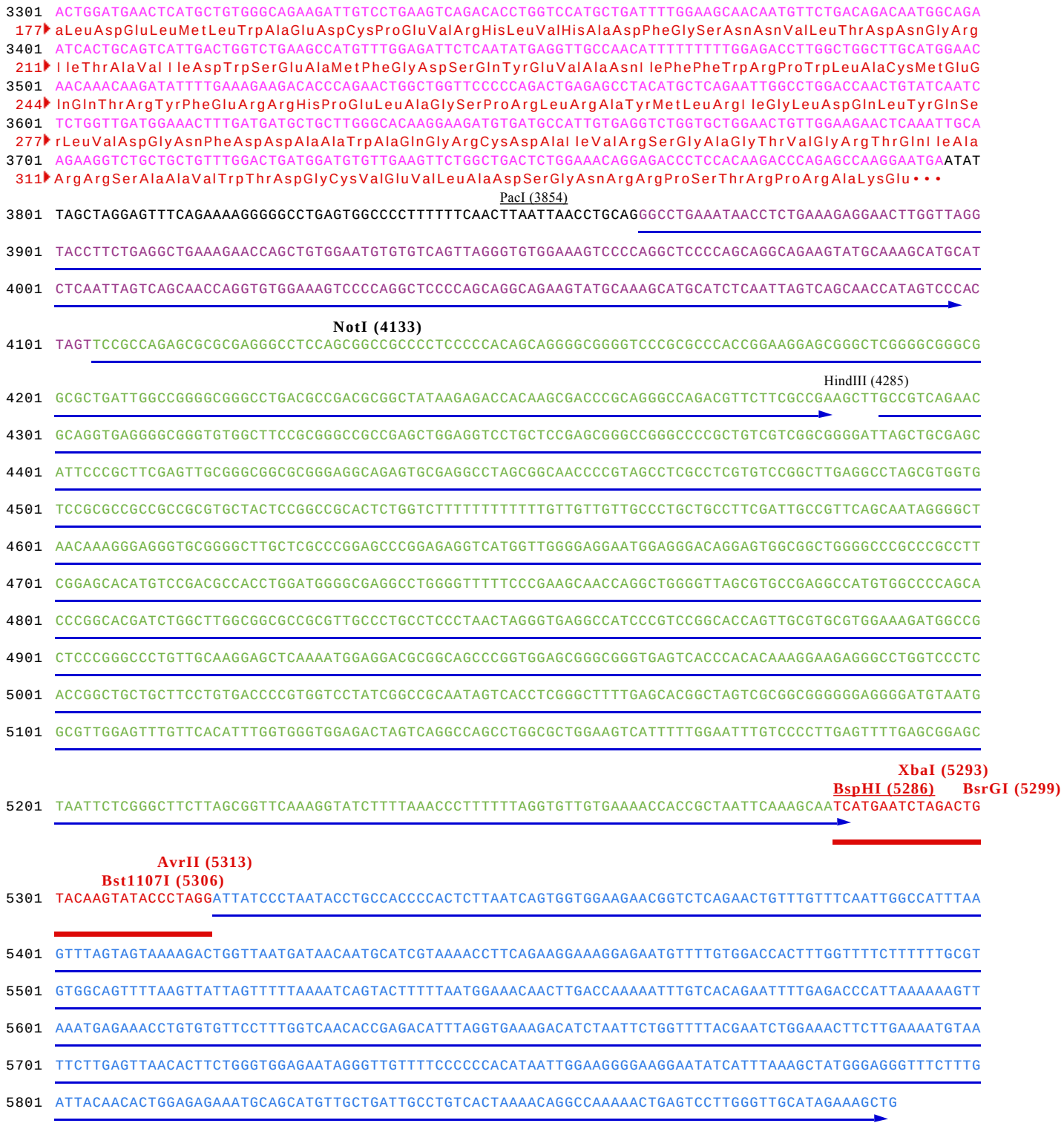
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