pVIVO2-mcs

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

Catalog # pvivo2-mcs

For research use only

Version # 04F30-SV

PRODUCT INFORMATION

Content:

pVIVO2-mcs is provided as 20 µg of lyophilized DNA.
- 4 pouches of *E. coli* Fast-Media® Hygro

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA is stable 1 year when stored at -20°C. Resuspended DNA is stable 12 months when stored at -20°. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Hygro 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

pVIVO2 is a new generation 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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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.

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H_2O. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* Fast-Media:

E. coli Fast-Media® Hygro 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® Hygro is a TB (liquid) or LB (solid) based medium with hygromycin B, and contains stabilizers.

E. coli Fast-Media® Hygro can be ordered separately (catalog code # fas-hg-l, fas-hg-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 over-boiling 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. Eisenstein RS. and Munro HN. 1990. Enzyme 44(1-4):42-58
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

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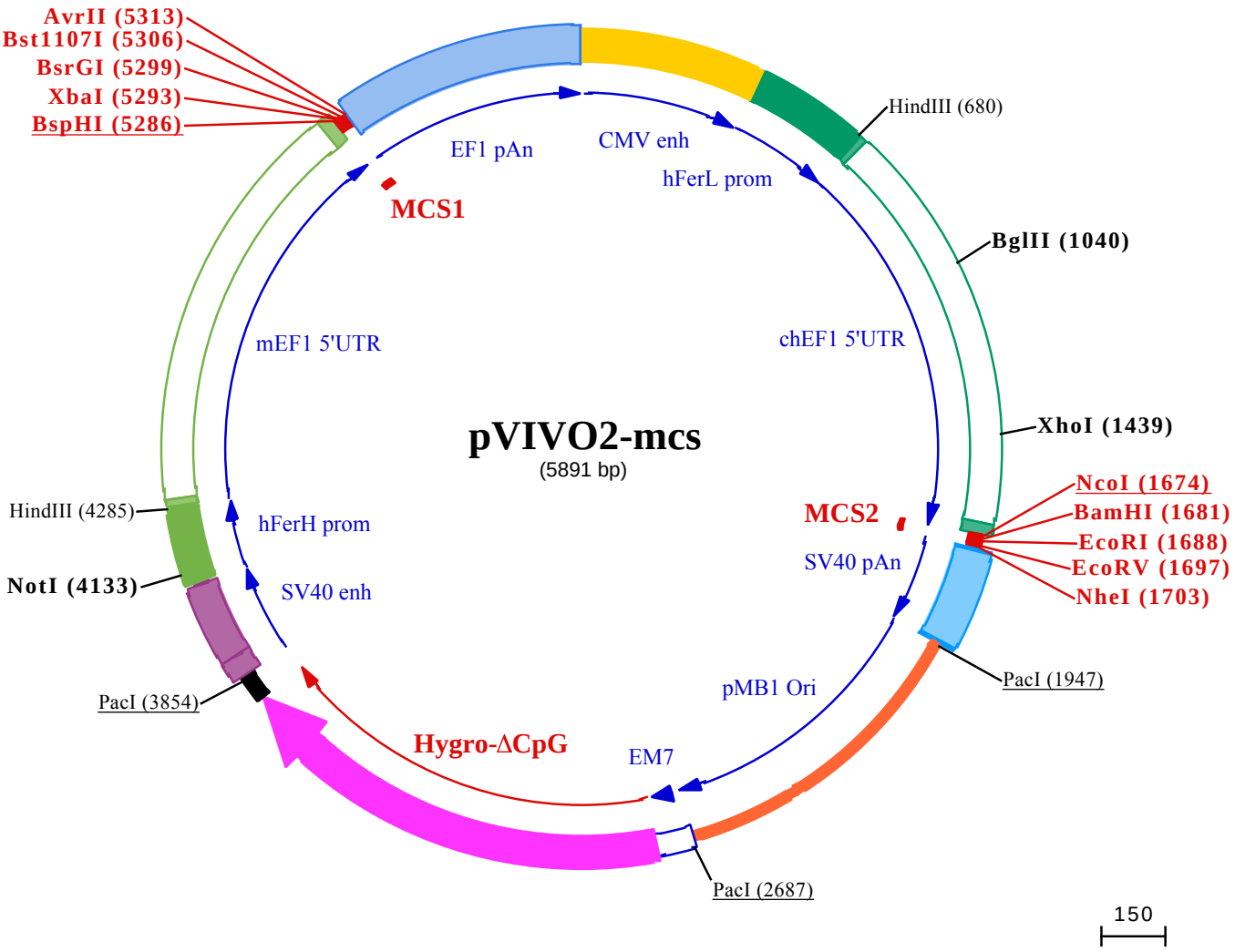
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hFerL prom
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chEF1 5'UTR
pVIVO2-mcs
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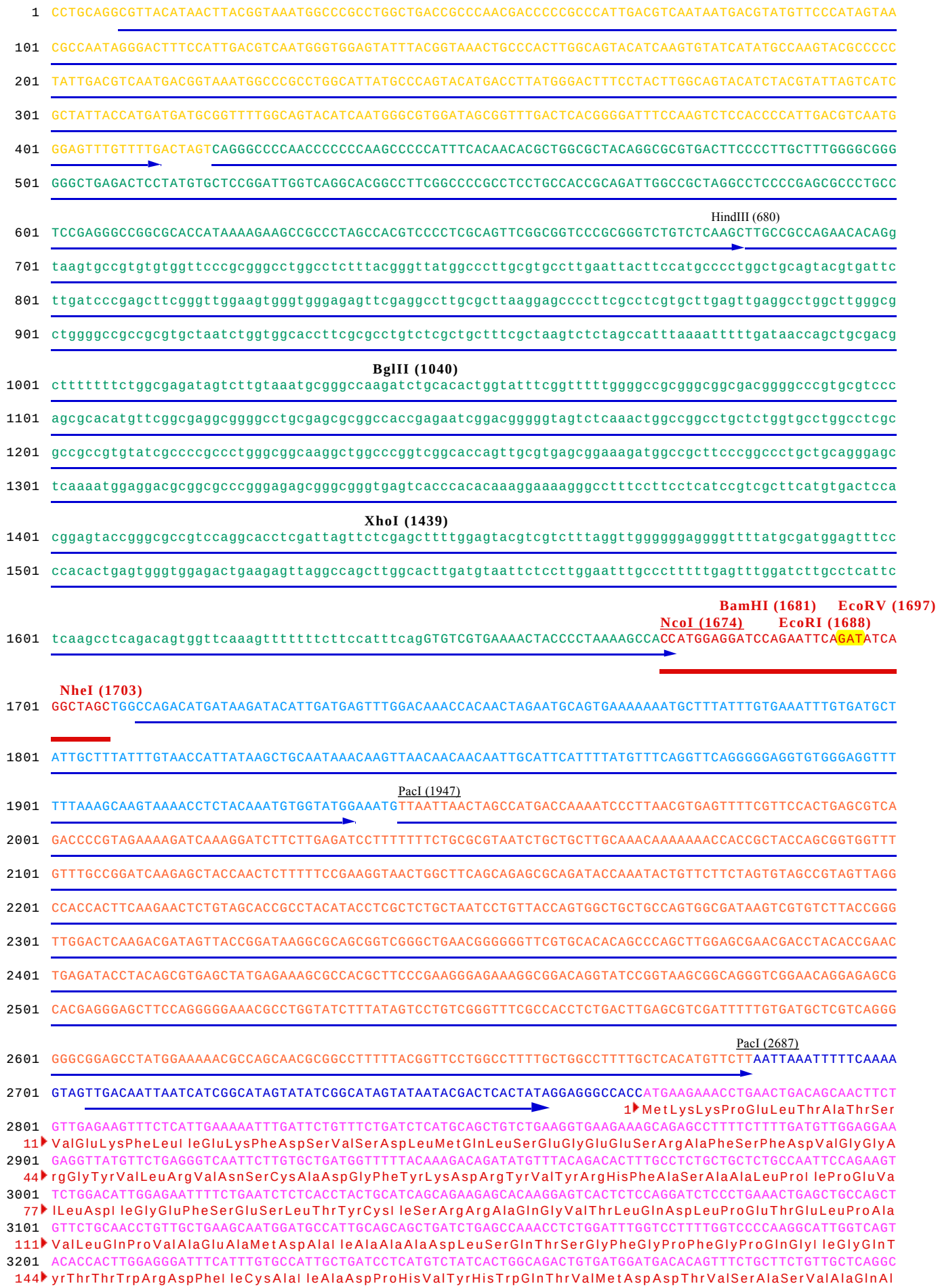
InvivoGen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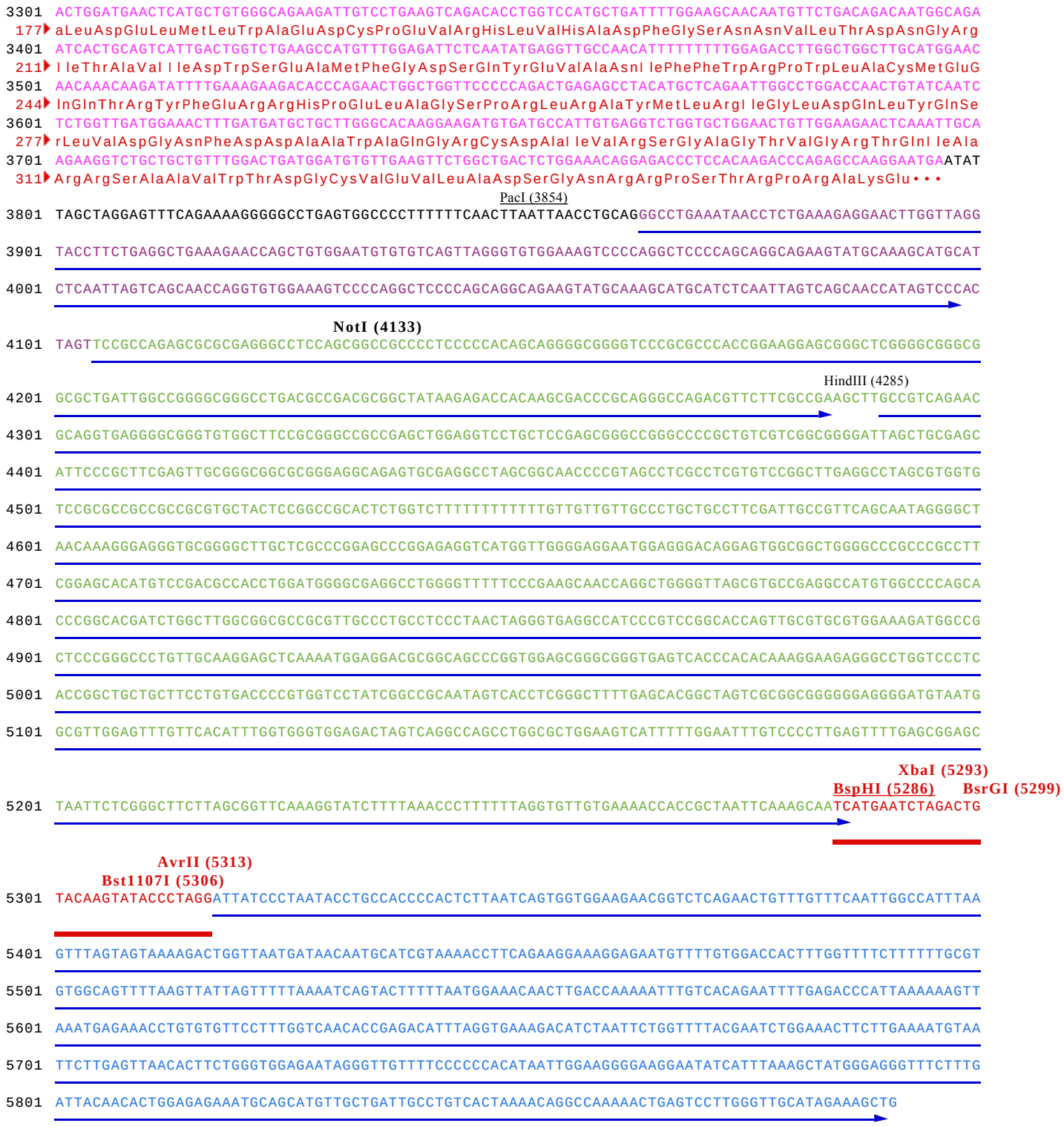
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