pUNO-hTLR4-GFP

A plasmid expressing the human TLR4 gene fused to a GFP gene

Catalog # phtlr4-gfp

For research use only

Version # 10K03-MM

PRODUCT INFORMATION

Content:

• 1 disk of lyophilized *E. coli* transformed with pUNO-hTLR4-GFP.
E. coli strain is GT116: *F- mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZM15 ΔlacX74 recA1 rpsL (StrA) endA1 ΔsbcC-sbcD.*
• 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- hTLR4::GFP fusion gene has been fully sequenced, its fluorescence confirmed and its function tested in HEK293 cells coexpressing MD2 and CD14 genes (pDUO-hMD2-CD14, cat.code #pduo2-hmd2cd14) and an NF-κB reporter plasmid (pNiFty-SEAP, cat. code #pnifty-seap).
- Plasmid construct has been confirmed by restriction analysis.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pUNO-TLR-GFP plasmids express high-levels of transient or stable TLR-GFP fusion proteins in a wide range of mammalian cells. These fusion proteins can be used to study the localization of the TLRs. Transfected cells can be analyzed for GFP expression by flow cytometry.
pUNO-TLR-GFP plasmids can be used directly for *in vitro* or *in vivo* transfection experiments. They are selectable with blasticidin, an antibiotic that allows the selection of stable mammalian clones in only a few days.
TLR::GFP fusion genes are under the control of a strong and ubiquitous promoter, called EF1α/HTLV, comprised of the elongation factor 1 alpha (EF-1α) core promoter and the R-U5' of the human T cell leukemia virus (HTLV).

PLASMID FEATURES

• Human TLR4::GFP fusion gene (3369 bp)
TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS)[1]. TLR4 alone is not sufficient to confer LPS responsiveness. It forms a complex with the lipopolysaccharide-binding protein (LBP) and the coreceptors CD14 and MD2. LPS-stimulated TLR4 signals through through different adaptor proteins: MyD88, TIRAP/Mal[2] , TRIF/TICAM1 and TRAM/TICAM2[3].
The hTLR4::GFP fusion gene was generated by fusing at the C terminus of the human TLR4 gene to a GFP variant. A synthetic intron was added between both moieties to increase the activity of GFP. This hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
The hTLR4::GFP fusion gene is under the control of the strong and ubiquitous hEF1/HTLV promoter. This composite promoter comprises the Elongation Factor-1α (EF-1α) core promoter[4] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[5]. The SV40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

• Blasticidin resistance (bsr) gene: The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. The *bsr* expression cassette is formed by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter, to allow blasticidin selection in both mammalian cells and *E. coli* bacteria, and the human beta globin polyadenylation signal (hβGlo pAn).

References

1. Poltorak A. *et al*., 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 282(5396): 2085-8.
2. Horng T,. GM. Barton, and R. Medzhitov, 2001. TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol, 2(9):835-41.
3. Fitzgerald KA. *et al*., 2003. LPS-TLR4 Signaling to IRF-3/7 and NF-{kappa}B Involves the Toll Adapters TRAM and TRIF. J Exp Med. 198(7):1043-1055.
4. Kim *et al*. (1990). Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 2: 217-223.
5. Takebe *et al*. (1988). SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol. Cell Biol. 1: 466-472.

METHODS

Growth of pUNO-transformed bacteria:

Use sterile conditions to do the following:
1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
2- Streak bacteria taken from this suspension on an blasticidin LB agar plate prepared with the *E. coli* Fast-Media® Blas agar provided (see below).
3- Place the plate in an incubator at 37°C overnight.
4- Isolate a single colony and grow the bacteria in TB supplemented with blasticidin using the Fast-Media® Blas liquid provided (see below).
5- Extract the pUNO plasmid DNA using the method of your choice.

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

E. coli Fast-Media® Blas is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave.

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.*

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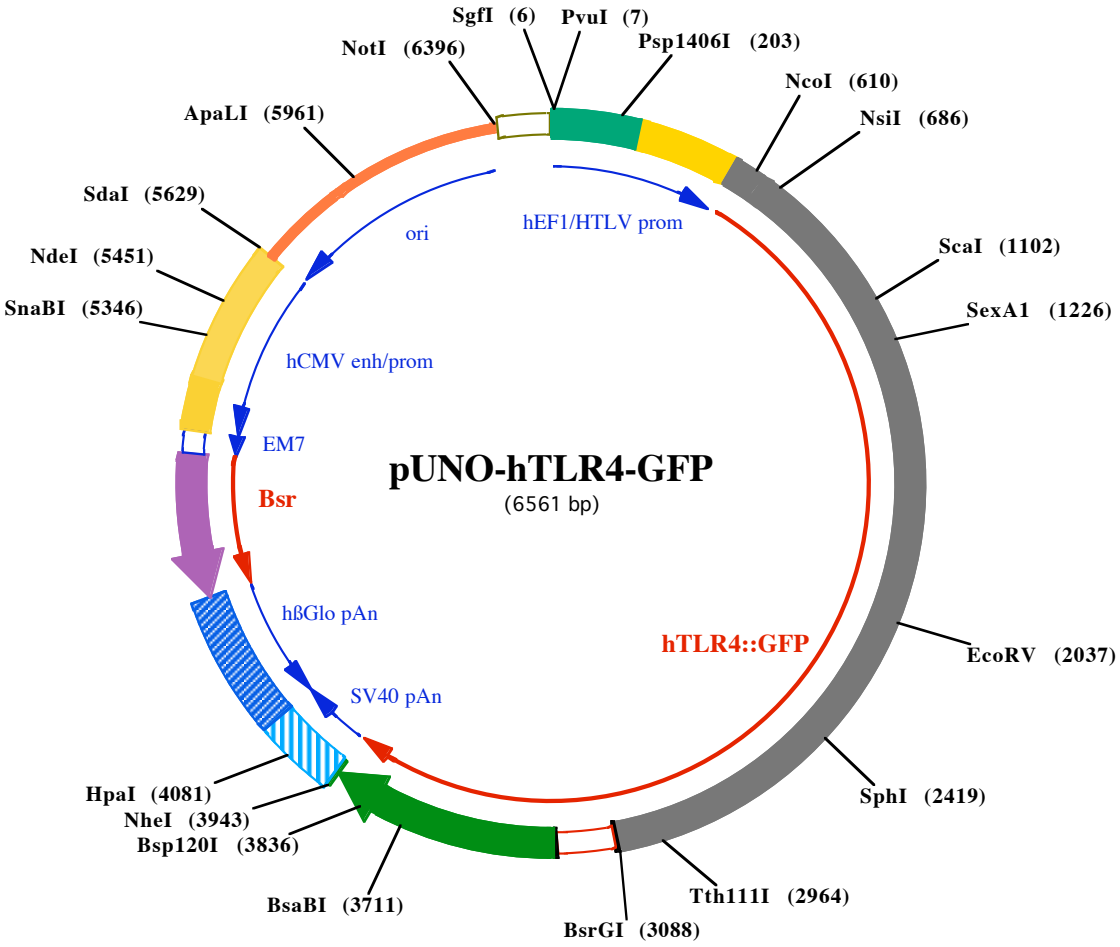
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PvuI (7)
Psp1406I (203)
NcoI (610)
NsiI (686)
ScaI (1102)
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SphI (2419)
Tth111I (2964)
BsrGI (3088)
BsaBI (3711)
Bsp120I (3836)
NheI (3943)
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hCMV enh/prom
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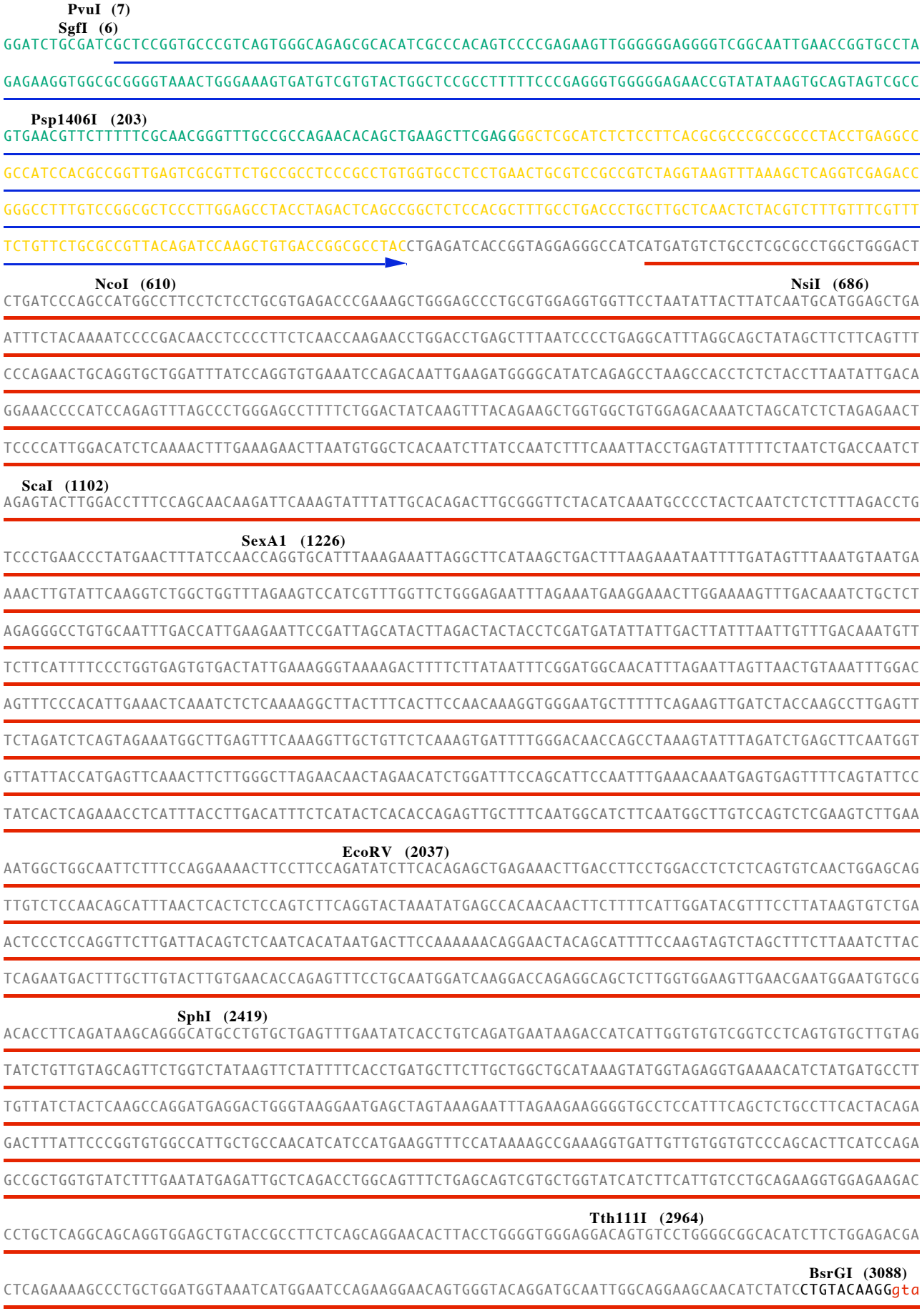
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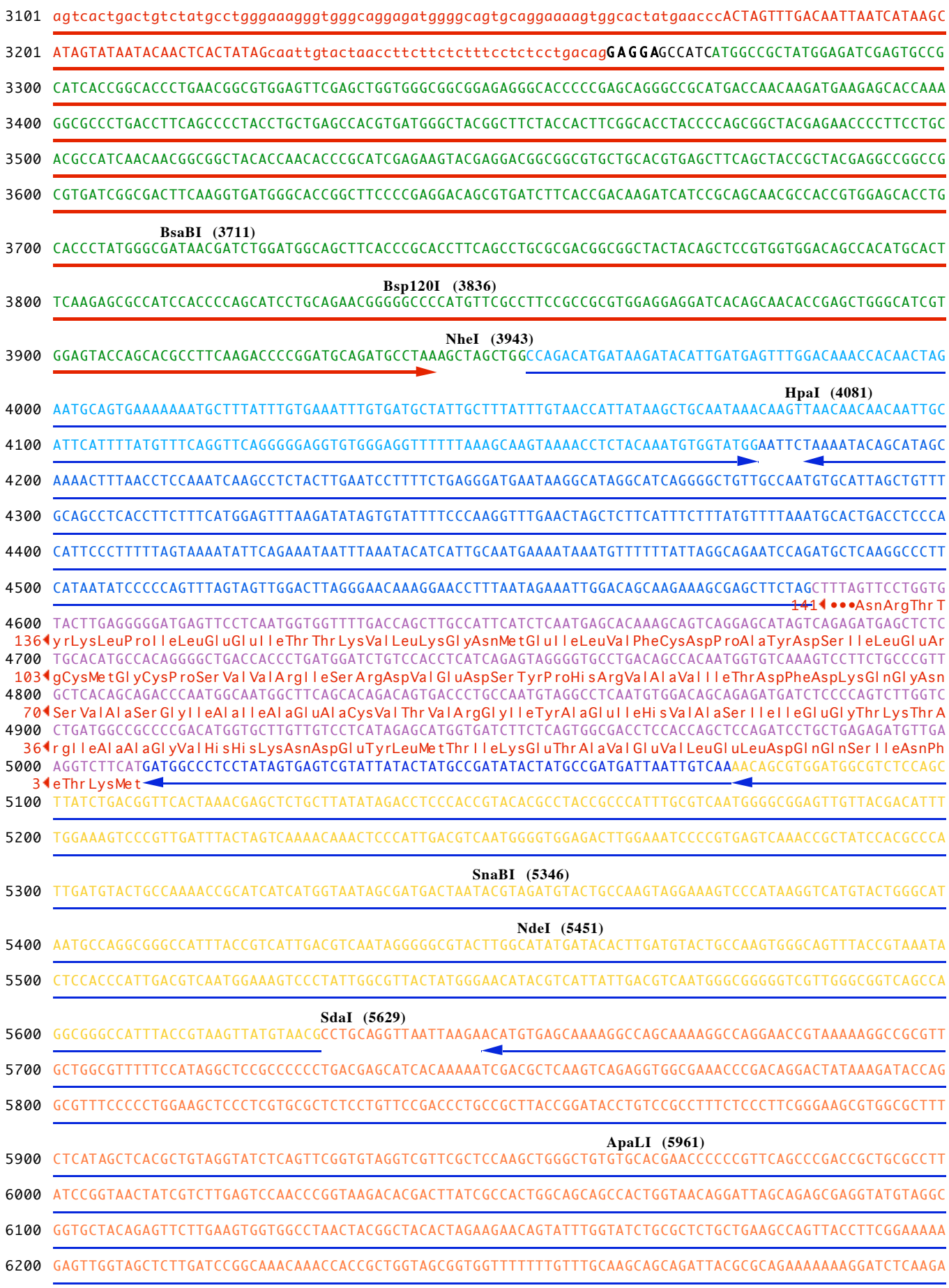
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