

# pUNO-hTLR5-GFP

A plasmid expressing the human TLR5 gene fused to a GFP gene

Catalog # phtr5-gfp

For research use only

Version # 10K03-MM

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized *E. coli* transformed with pUNO-hTLR5-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:

- hTLR5::GFP fusion gene has been fully sequenced, its fluorescence confirmed and its function tested in HEK293 cells coexpressing 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 composite 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 TLR5::GFP fusion gene (3429 bp)

TLR5 is the Toll-like molecule that recognizes flagellin from both Gram-positive and Gram-negative bacteria. TLR5 was identified by the presence of the TIR domain and is expressed in spleen, peripheral blood leukocytes and epithelial cells. Activation of the receptor stimulates the production of pro-inflammatory cytokines, such as TNF-α, through signaling via the adaptor protein MyD88 and the serine kinase IRAK<sup>1,2</sup>. TLR5 can generate a pro-inflammatory signal as a homodimer suggesting that it might be the only TLR required for flagellin recognition<sup>2</sup>.

The hTLR5::GFP fusion gene was generated by fusing at the C terminus of the human TLR5 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 hTLR5::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<sup>3</sup> 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<sup>4</sup>. 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. Gewirtz AT. et al. (2001). J Immunol, 167(4):1882-5.
2. Hayashi F. et al. (2001). Nature, 410(6832):1099-103.
3. Kim et al. (1990). Gene 2: 217-223.
4. Takebe et al. (1988). 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.

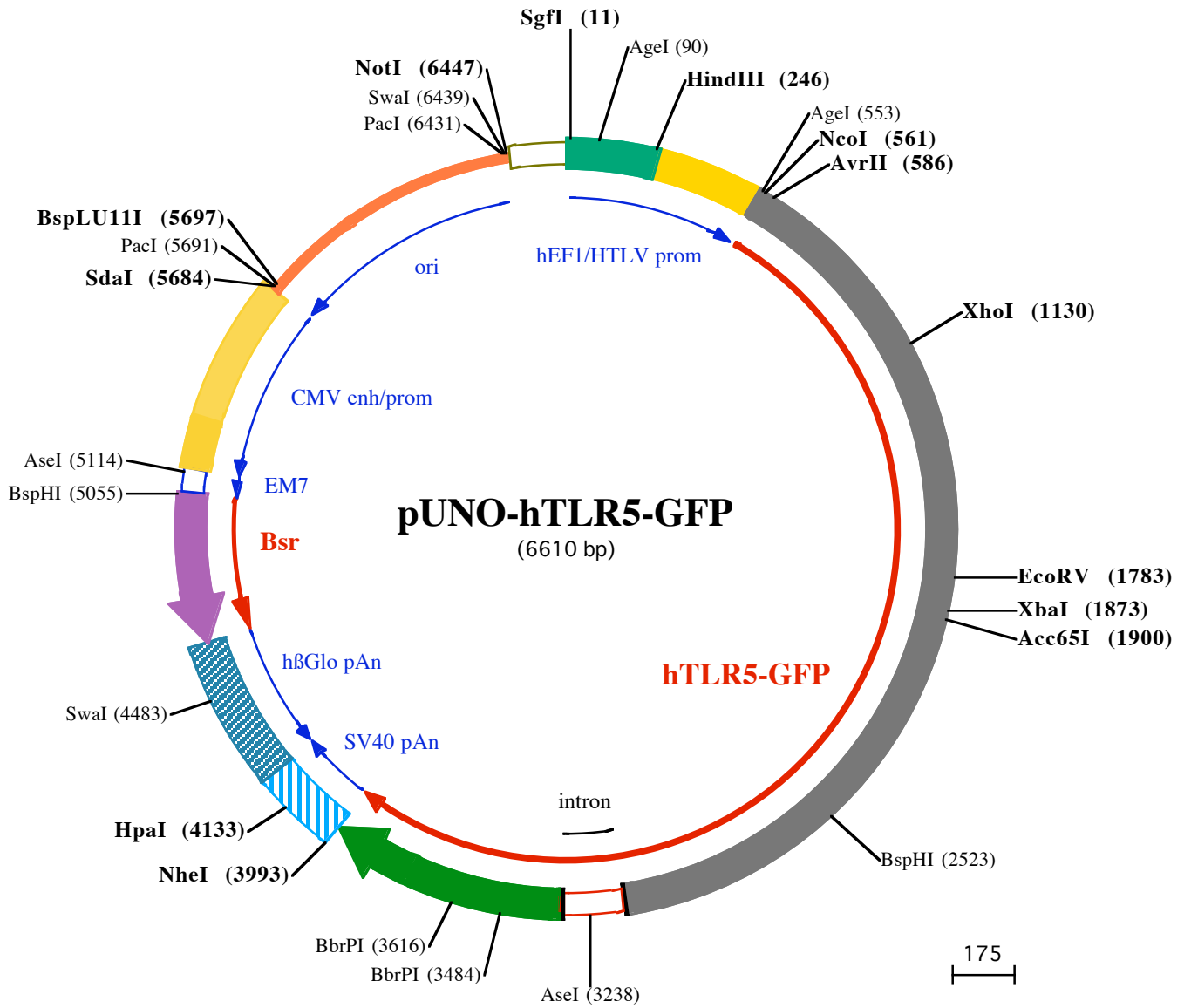
- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask.
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave 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.

### TECHNICAL SUPPORT

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BbrPI (3616)  
3601 CGGCGCGTGTGCACGTGAGCTTACGCTACCGCTACGAGCGCGCGCTGATCGGCGACTTCAAGGTGATGGGCACCGGCTTCCCGAGGACACGGTG  
1013▶pGI yGI yVal LeuHi sVal Ser PheSer TyrArgTyrGI uAl aGI yA rgVal I I eGI yAspPheLysVal MetGI yThr GI yPheP roGI uAspSer Val  
3701 ATCTTACCGACAAGATCATCCGACGCAACGCCACCGTGGAGCAGCTGCACCCTATGGGCGATAACGATCTGGATGGCAGCTTACCCCGACCTTCAGCC  
1047▶I I ePheThrAspLysI I eI I eArgSerAsnAl aThr Val GI uHi sLeuHi sP RoMeTGI yAspAsnAspLeuAspGI ySer PheThr A rgThr PheSer L  
3801 TGC GCGACGGCGCTACACAGCTCCGTTGGGACAGCCACATGCACCTCAAGAGCGCCATCCACCCAGCATCTGCAGAACGGGGGCCCATGTTCGC  
1080▶euArgAspGI yGI yTyrTyrSer Ser Val I Val AspSer Hi sMeThi sPheLysSer Al a I eHi sP roSer I I eLeuGI nAsnGI yGI yP roMeT PheAl  
NheI (3993)  
3901 CTTCCGCGCTGGAGGAGGATCACAGCAACCCAGCTGGGCATCGTGGAGTACCAGCAGCCTTCAAGACCCGGATGCAGATGCCTAAAGTAGCTAGCTG  
1113▶aPheArgArgVal GI uGI uAspHi sSerAsnThr GI uLeuGI yI I eVal GI uTyrGI nHi sAl aPheLysThr P roAspAl aAspAl a●●●  
4001 GCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTA  
HpaI (4133)  
4101 TTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGTTTTTAAAGCA  
4201 AGTAAACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTGAACTCTTTTCTGAGGGATG  
4301 AATAAGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGT  
SwaI (4483)  
4401 TTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCAGCTGACCTCCACATTCCTTTTTAGTAAAATATTAGAAATAATTTAAATACATCATTGCAAT  
4501 GAAAAATAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAA  
4601 TAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCA  
141▶●●●AsnArgThr TyrLysLeuP roI I eLeuGI uGI uI I eThr Thr LysVal LeuLysGI yAsnMe  
4701 TCTCAATGAGCACAAAGCAGTCCAGGAGCATAGTCAGAGATGAGCTCTGCACATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATAGA  
119▶tGI uI I eLeuVal I PheCysAspP roAl aTyrAspSer I I eLeuGI uArgCysMetGI yCysP roSer Val I Val A rGI I eSerArgAspVal GI uAspSer  
4801 GTAGGGGTGCTGACAGCCACAATGGTGTCAAAGTCTTCTGCCGTTGCTCACAGCAGCCCAATGGCAATGGCTTCAGCAGACAGTGAACCTGCCA  
86▶TyrP roHi sArgVal Al aVal I I eThrAspPheAspLysGI nGI yAsnSerVal Al aSer GI yI I eAl aI I eAl aGI uAl aCysVal I ThrVal A rGI yI  
4901 ATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCACTCTGGTCTGATGGCCGCCGACATGGTGTCTGTTGTCCTCATAGAGCATGGTGTCT  
52▶I eTyrAl aGI uI I eHi sVal Al aSer I I eI I eGI uGI yThr LysThr ArgI I eAl aAl aGI yVal I Hi sHi sLysAsnAspGI uTyrLeuMeT Thr I I eLy  
BspHI (5055)  
5001 TCTCAGTGGCGACCTCCACCAGCTCCAGATCTGTGAGAGATGTTGAAGTCTTTCATGATGGCCCTCTATAGTGAAGTATTATACTATGCCGATAT  
19▶sGI uThr Al aVal GI uVal LeuGI uLeuAspGI nGI nSer I I eAsnPheThr LysMeT  
AseI (5114)  
5101 ACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGACCTCCACCGTAC  
5201 ACGCCTACCGCCATTTGCGTCAATGGGCGGAGTTGTTACGACATTTTGAAAGTCCCCTTGATTACTAGTCAAAACAACTCCCATGACGTCATG  
5301 GGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAACCGCATCATATGGTAATAGCGATGACTAATACGT  
5401 AGATGTACTCCAAGTAGGAAAGTCCCATAAAGTCATGTACTGGGCATAATGCCAGCGGGCCATTACCCTCATTGACGTCAATAGGGGGCGTACTTGG  
5501 CATATGATACACTTGATGTACTGCCAAGTGGGCGTTCACCGTAAACTCCACCCATTGACGTCATGGAAAGTCCCTATTGGCGTTACTATGGGAACA  
PacI (5691) SdaI (5684) BspLU11I (5697)  
5601 TACGTCATTATTGACGTCATGGGCGGGGCTGTTGGCGGTCAGCCAGCGGGCCATTTACCCTAAGTTATGTAACGCTGCAGGTTAATTAAGAACAT  
5701 GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAGGCCCGCTGCTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGA  
5801 CGCTCAAGTCAGAGGTGGCAAAACCGACAGGACTATAAGATACCAGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCTGCCGC  
5901 TTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAA  
6001 GCTGGGCTGTGTGCAGAACCCCGCTTCCAGCCGACCGCTGCGCTTATCCGTAATCTCGTCTTGGTCCAAACCGGTAAGACACGACTTATCGCCA  
6101 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA  
6201 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTGGTAGCGGTGTTTT  
6301 TTTTGTGGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGGAAACAAAAACTCA  
PacI (6431) SwaI (6439) NotI (6447)  
6401 CGTTAAGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGTTTTTGTG  
6501 TGAATCGTAACTAACATACGCTCTCCATCAAAACAAACGAAACAAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACATTT  
6601 CTCTATCGAA