pUNO-mTRIAD3A

An expression vector containing the mouse TRIAD3A open reading frame

Catalog # puno-mtriad3a

For research use only

Version # 05F16-JC07

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized *E. coli* bacteria, strain GT116 transformed by pUNO-mTRIAD3A
- Strain genotype is: *F-, mcrA , Δ(mrr-hsdRMS-mcrBC) , Ø80lacZΔM15 , ΔlacX74, recA1 , endA1, Δdcm, ΔsbcC-sbcD .*
- 4 pouches of *E. coli* Fast-Media® Blas.

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:

Plasmid construct has been confirmed by restriction analysis and ORF sequencing. Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pUNO are ready-made expression vectors containing a Toll-Like Receptor (TLR) gene, a TLR-associated gene (co-receptor, adaptor, involved in TLR signaling, etc...), or gene otherwise implicated in the innate immune response.

TLRs play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs) that include various bacterial cell wall components, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade involving several proteins, and leads to the activation of the transcription factor NF-kB which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

pUNO may be used for:

Obtaining a TLR-related gene to subclone into another vector. Two restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Stable gene expression in mammalian cells. pUNO plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO plasmids contain the blasticidin resistance gene (*bsr*) driven by the SV40 promoter in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli* AND the selection of stable clones in mammalian cells. pUNO allows a high level of expression and secretion of the gene product.

mTRIAD3A gene may be cut out by using NcoI and NheI enzymes.
AgeI, XmaI, BspEI, NgoMIV and SgrAI are compatible. NcoI, BspHI and BspLU11I are compatible. NheI, XbaI, SpeI, and AvrII are compatible.

PLASMID FEATURES

• **EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter[1] and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a "house keeping" gene. It is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[2] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **mTRIAD3 gene, isoform A:**
Intronless ORF from the ATG to the stop codon.
ORF Size (bp): 2562
Cloning fragment size (bp): 2579

• **SV40 polyA:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

• **SpAn:** A synthetic polyadenylation site and strong pause site, placed downstream of the pMB1 Ori, to limit transcriptional interference between both transcription units. The synthetic polyA site is based on the highly efficient polyA signal from the rabbit ß-globin gene[4].

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **SV40 promoter:** The Simian Virus 40 promoter allows the expression of the blasticidin resistance gene in mammalian cells.

• **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. The *bsr* gene is driven by the SV40 promoter in tandem with the bacterial EM7 promoter. Therefore each pBLAST plasmid can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **bGh polyA:** The bovine growth hormone (bGh) polyadenylation (pAn) signal and a transcriptional pause are placed 3' of the blasticidin gene. The bGh pAn has been shown to be as efficient as SV40 and HSV1tk polyadenylation signals in many different cell types[5]. The use of bGH pAn minimizes interference and possible recombination events with the SV40 polyadenylation signal. The pause site prevents transcriptional interference and read-through.

References

1- Kim et al (1990). Gene 2: 217-223.
2- Takebe et al (1988). Mol. Cell Biol. 1: 466-472.
3- Carswell et al(1989). Mol. Cell Biol. 10: 4248-4258.
4- Levitt *et al.* (1989). Genes Dev. 7: 1019-1025
5- Goodwin *et al.* (1992). J. Biol. Chem. 23: 16330-16334

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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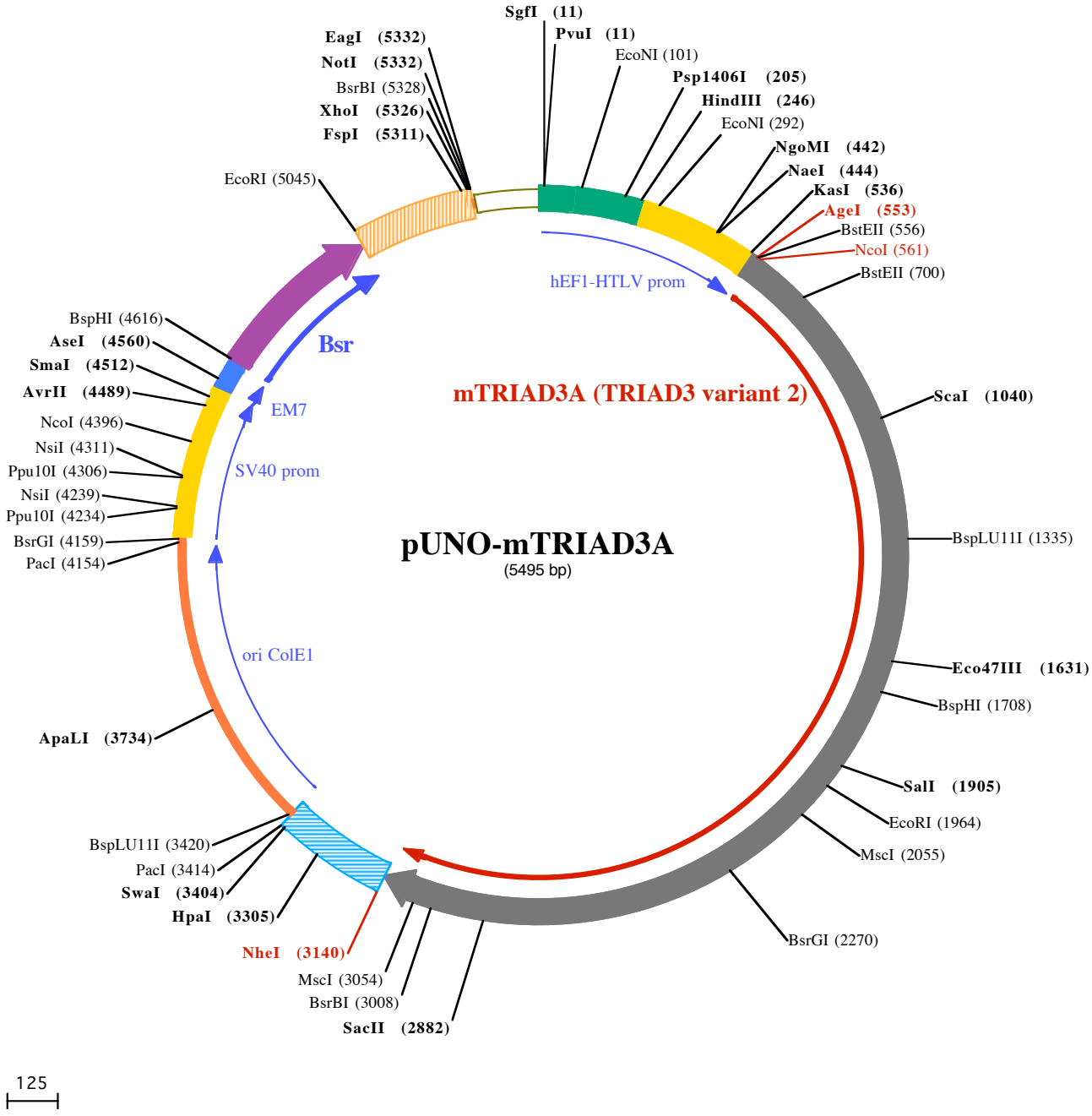
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(5495 bp)
mTRIAD3A (TRIAD3 variant 2)
hEF1-HTLV prom
Bsr
EM7
SV40 prom
ori ColE1
SgfI (11)
PvuI (11)
EcoNI (101)
Psp1406I (205)
HindIII (246)
EcoNI (292)
NgoMI (442)
NaeI (444)
KasI (536)
AgeI (553)
BstEII (556)
NcoI (561)
BstEII (700)
ScaI (1040)
BspLU11I (1335)
Eco47III (1631)
BspHI (1708)
SalI (1905)
EcoRI (1964)
MscI (2055)
BsrGI (2270)
SacII (2882)
BsrBI (3008)
MscI (3054)
NheI (3140)
HpaI (3305)
SwaI (3404)
PacI (3414)
BspLU11I (3420)
ApaLI (3734)
PacI (4154)
BsrGI (4159)
Ppu10I (4234)
NsiI (4239)
Ppu10I (4306)
NsiI (4311)
NcoI (4396)
AvrII (4489)
SmaI (4512)
AseI (4560)
BspHI (4616)
EcoRI (5045)
EagI (5332)
NotI (5332)
BsrBI (5328)
XhoI (5326)
FspI (5311)
125


PvuI (11)
SgfI (11)
EcoNI (101)
Psp1406I (205)
HindIII (246)
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NgoMI (442)
NaeI (444)
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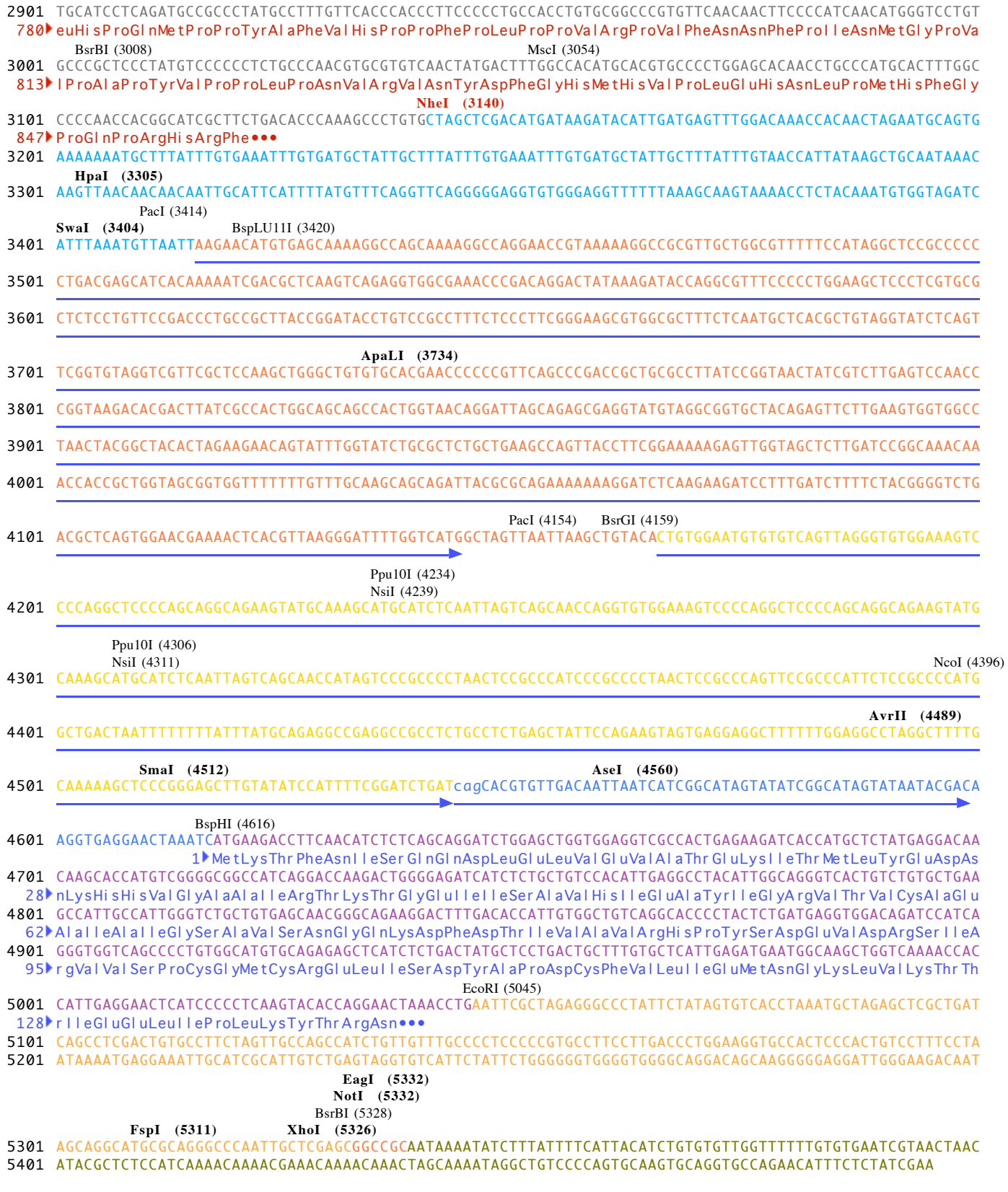
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BsrBI (3008)
MscI (3054)
3001 GCCCGCTCCCTATGTCCCCCCTCTGCCCAACGTGCGTGTCAACTATGACTTTGGCCACATGCACGTGCCCCTGGAGCACAACCTGCCCATGCACTTTGGC
813 lProAlaProTyrValProProLeuProAsnValArgValAsnTyrAspPheGlyHisMetHisValProLeuGluHisAsnLeuProMetHisPheGly
NheI (3140)
3101 CCCCAACCACGGCATCGCTTCTGACACCCAAAGCCCTGTGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTG
847 ProGlnProArgHisArgPhe•••
3201 AAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAC
HpaI (3305)
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PacI (3414)
SwaI (3404)
BspLU11I (3420)
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ApaLI (3734)
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BsrGI (4159)
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NsiI (4239)
4201 CCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATG
Ppu10I (4306)
NsiI (4311)
NcoI (4396)
4301 CAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATG
AvrII (4489)
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SmaI (4512)
AseI (4560)
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BspHI (4616)
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95 rgValValSerProCysGlyMetCysArgGluLeuIleSerAspTyrAlaProAspCysPheValLeuIleGluMetAsnGlyLysLeuValLysThrTh
EcoRI (5045)
5001 CATTGAGGAACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAATTCGCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGAT
128 rIleGluGluLeuIleProLeuLysTyrThrArgAsn•••
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EagI (5332)
NotI (5332)
BsrBI (5328)
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XhoI (5326)
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