

pDUO-hMD2/TLR4A

A plasmid coexpressing the human MD2 and TLR4A genes

Catalog code: pduo-hmd2tlr4a

<https://www.invivogen.com/pduo-md2-tlr4>

For research use only

Version 20H26-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-hMD2/TLR4A provided as DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors, homologues of the *Drosophila* Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharides (LPS), peptidoglycans and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade that involves a number of proteins, such as MyD88 and IRAK. This signaling cascade leads to the activation of the transcription factor NF-κB which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

To date ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. In many instances, TLRs require the presence of a co-receptor to initiate the signaling cascade. One example is TLR4 which interacts with MD2 and CD14 to induce NF-κB in response to LPS stimulation.

pDUO is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other.

The genes cloned into pDUO comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

• **Human MD2 (480 bp) / Human TLR4A (2517 bp)** TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS^{1,2}. TLR4 physically associates with MD2, and together with a third protein called CD14, this complex is responsible for LPS recognition and signaling³.

• **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 protein⁴. 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 plasmids⁵.

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, 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 enhancer⁶.

• **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.*⁷

• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression⁸.

TECHNICAL SUPPORT

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- EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.
- **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.

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 H₂O. 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.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

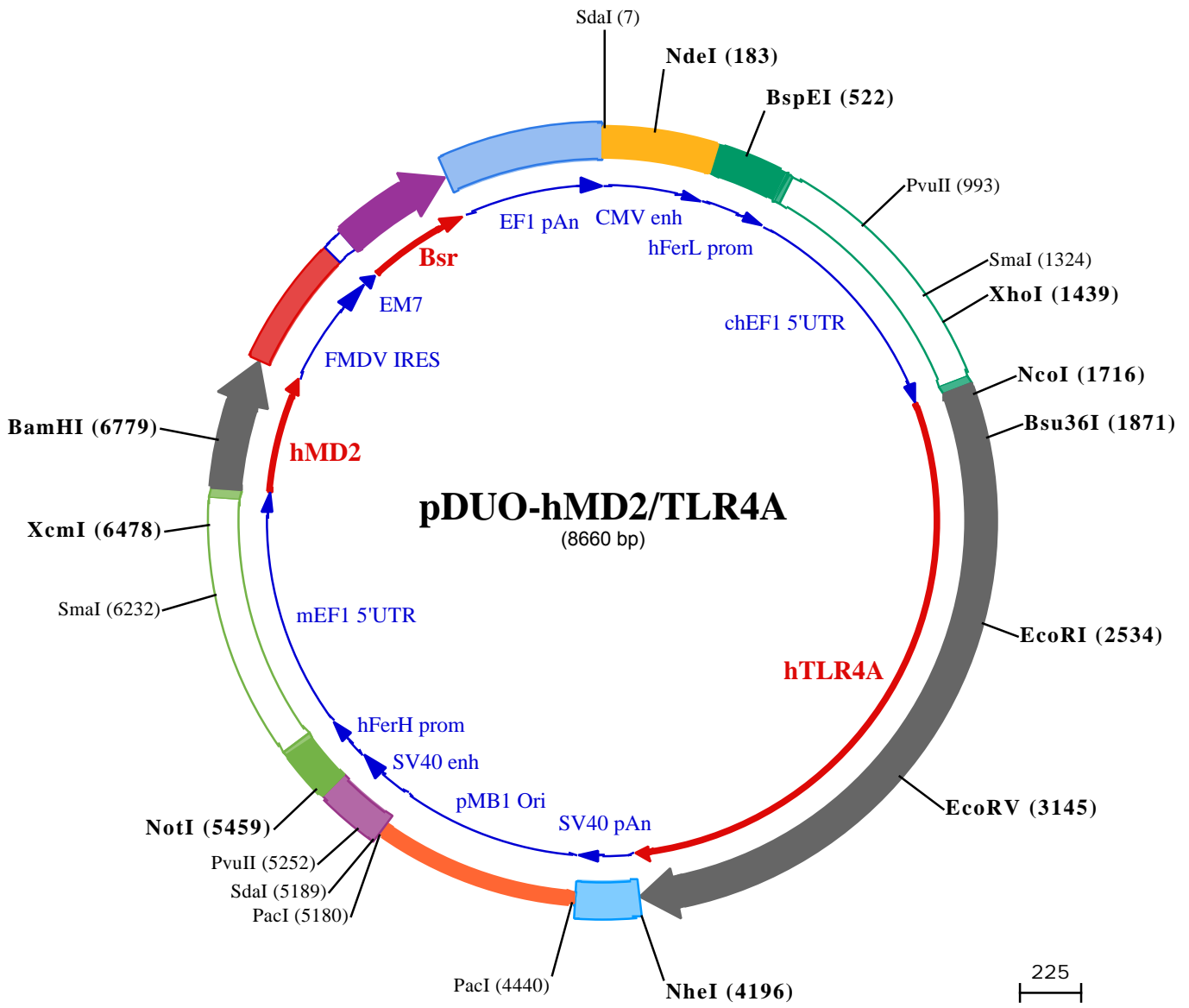
References

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2. Nagai Y. *et al.*, 2002. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol*. 3(7):667-72.
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8. Ramesh N *et al.*, 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.

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SdaI (7)

1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (183)

101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGCTGGATAGCGGTTTACTCAGGGGATTTCGAAGTCTCCACCCCAATTGACGTCAATG

401 GGAGTTTGTTTGACTAGTCAGGGCCCAACCCCCCAAGCCCAATTTACAACACGCTGGCGCTACAGGCGCTGACTTCCCCTTGCTTTGGGGCGG

BspEI (522)

501 GGGCTGAGACTCCTATGTCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCGAGCGCCCTGCC

601 TCCGAGGGCCGGCGACCATAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTCGGCGGTCCCGGGTCTGTCTCAAGCTTGGCCGAGAACACAGG

701 taagtgccgtgtgtggttccccggggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc

801 ttgatcccagccttcgggttgggaagtggtgggagagttcgaggccttgcgcttaaggagcccttcgcctcgtgcttgagttgaggcctggcctggcg

PvuII (993)

901 ctggggcccgcgctgctaatctggtggcaccttcgcgctgtctcgctgctttcgtaagtctctagccatttaaattttgat aaccagctgcgacg

1001 cttttttctggcgagatagctctgtaaatcgggccaagatctgcacactggtatttcggtttttggggccggggcgacggggcccgctgcgtccc

1101 agcgcacatgctcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggggtagtctcaactggccgctgctctggtgctggcctcgc

1201 gcccgctgtatcggccgctggggcgcaagctggccggctcgaccagttcgctgagcggaaagatggccgcttcccgccctgctgcagggagc

SmaI (1324)

1301 tcaaatggaggacggcgccccgggagagcggggcgggtgagtcaaccaacaaggaagggccttctctcctcatcctgcttcatgtgactcca

XhoI (1439)

1401 cggagtaccgggcccctccaggcacctcgattagttctcgagctttggagtagctgctcttaggttgggggagggtttatgcgtaggatttc

1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaaatttgcctttttgagtttgatcttgcctcatc

1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAACACTACCCTAAAAGCCACCATGATGCTGCCTCGCGCTGGCTGG

MetMetSerAlaSerArgLeuAlaGI

NcoI (1716)

1701 GACTCTGATCCCAGCCATGGCCTTCCCTCCTGCGTGAGACCCGAAAGCTGGGAGCCCTGCGTGAGGTTGGTTCCTAATATTACTTATCAATGCATGGAG

yThrLeul leProAlaMetAlaPheLeuSerCysValArgProGluSerTrpGluProCysValGluValValProAsnl leThrTyrGlnCysMetGlu

Bsu36I (1871)

1801 CTGAATTTCTACAAAATCCCCGACAACCTCCCTTCTCAACCAAGAACCTGGACCTGAGCTTTAATCCCCTGAGGCATTTAGGCAGCTATAGCTTCTTCA

LeuAsnPheTyrLysl leProAspAsnLeuProPheSerThrLysAsnLeuAspLeuSerPheAsnProLeuArgHisLeuGlySerTyrSerPheS

43 GTTCCCAGAACTGCAGGTGCTGGATTATCCAGGTGTAATCCAGACAATGAAGATGGGGCATATCAGAGCCTAAGCCACTCTACCTTAATATT

76 erPheProGluLeuGlnValLeuAspLeuSerArgCysGluI leGlnThrI leGluAspGlyAlaTyrGlnSerLeuSerHisLeuSerThrLeuI leLe

2001 GACAGAAAACCCATCCAGAGTTTAGCCCTGGGAGCCTTTCTGGACTATCAAGTTTACAGAAGCTGGTGGCTGTGGAGACAAAATAGCATCTCTAGAG

109 uThrGlyAsnProI leGlnSerLeuAlaLeuGlyAlaPheSerGlyLeuSerSerLeuGlnLysLeuValAlaValGluThrAsnLeuAlaSerLeuGlu

2101 AACTCCCATTGGACATCTCAAACCTTGAAGAATTAAGTGGCTCAAACTTATCCAACTTTCAAATCTTCAAATACCTGAGTATTTTCTAATCTGACCA

143 AsnPheProI leGlyHisLeuLysThrLeuLysGluLeuAsnValAlaHisAsnLeul leGlnSerPheLysLeuProGluTyrPheSerAsnLeuThrA

2201 ATCTAGACTACTGGACCTTTCCAGCAACAAGATCAAAGTATTTATTGGACAGACTTGGGGTTCTACATCAAATGCCCTACTCAATCTCTTTAGA

176 snLeuGluTyrLeuAspLeuSerSerAsnLysl leGlnSerI leTyrCysThrAspLeuArgValLeuHisGlnMetProLeuLeuAsnLeuSerLeuAs

2301 CCTGTCCTGAACCTATGAACCTTATCAACACAGGTGCATTTAAAGAAATTAGGCTTCATAAGCTGACTTTAAGAAATAATTTGATAGTTTAAATGTA

209 pLeuSerLeuAsnProMetAsnPheI leGlnProGlyAlaPheLysGluI leArgLeuHisLysLeuThrLeuArgAsnAsnPheAspSerLeuAsnVal

2401 ATGAAAACCTGTATTCAAGGTCTGGCTGGTTTGAAGTCCATCGTTGGTTCTGGGAGAATTTAGAATGAAGGAAACTTGGAAAAGTTTGACAAATCTG

243 MetLysThrCysl leGlnGlyLeuAlaGlyLeuGluValHisArgLeuValLeuGlyGluPheArgAsnGluGlyAsnLeuGluLysPheAspLysSerA

EcoRI (2534)

2501 CTCTAGAGGGCTGTGCAATTTGACCATTGAAGAATTCGATTAGCATACTTAGACTACTACCTCGATGATATTATTGACTTATTTAATTTGTTGACAAA

276 laLeuGluGlyLeuCysAsnLeuThrI leGluGluPheArgLeuAlaTyrLeuAspTyrTyrLeuAspAspI leI leAspLeuPheAsnCysLeuThrAs

2601 TGTTTCTTCAATTTCCCTGGTGGTGTGACTATTGAAAGGTTAAAAGACTTTTCTTATAATTTCCGATGGCAACATTTAGAATTAGTTAACTGTAATTT

309 nValSerSerPheSerLeuValSerValThrI leGluArgValLysAspPheSerTyrAsnPheGlyTrpGlnHisLeuGluLeuValAsnCysLysPhe

2701 GGACAGTTTCCCACATGAAACTCAAATCTCTCAAAGGCTTACTTCACTTCAACAAAGGTGGGAATGCTTTTTCAGAAGTTGATCTACCAAGCCTTG

343 pGlyGlnPheProThrLeuLysSerLeuLysArgLeuThrPheThrGluHisLeuAspPheGlyGlyAsnAlaPheSerGluValAspLeuProSerLeuG

2801 AGTTTCTAGATCTCAGTAGAAATGGCTTGGTTTCAAAGGTTGCTGTTCTCAAAGTATTTTGGGACAACCGCCTAAAGTATTTAGATCTGAGCTTCAA

376 luPheLeuAspLeuSerArgAsnGlyLeuSerPheLysGlyCysCysSerGlnSerAspPheGlyThrThrSerLeuLysTyrLeuAspLeuSerPheAs

2901 TGTTGTTATTACCATGAGTTCAAACCTTCTGGGCTTAGAACAAC TAGAACATCTGGATTCCAGCATTCCAATTTGAAACAAATGAGTGAGTTTTCAGTA

409 nGlyVal I leThrMetSerSerAsnPheLeuGlyLeuGluHisLeuAspPheGlnHisLeuSerAsnLysGlyHisAsnAlaPheSerGluValAspLeuProSerLeuG

3001 TTCCTACTCAGAAACCTCATTTACCTTGACATTTCTCATACTCACACCAGAGTTGCTTTCATGGCATCTTCAATGGCTTGTCCAGTCTCGAAGTCT

443 PheLeuSerLeuArgAsnLeul leTyrLeuAspI leSerHisThrHisThrArgValAlaPheAsnGlyI lePheAsnGlyLeuSerSerLeuGluValI

EcoRV (3145)

3101 TGAAAATGGCTGGCAATTTCTTCCAGAAAACCTTCCAGATATCTCACAGAGCTGAGAAAACCTTGACCTTCTGACCTCTCTCAGTGTCAACTGGA

476 euLysMetAlaGlyAsnSerPheGlnGluAsnPheLeuProAspI lePheThrGluLeuArgAsnLeuThrPheLeuAspLeuSerGlnCysGlnLeuGI

3201 GCAGTTGCTCCAACAGCATTAACTCACTCTCCAGTCTCAGGTAATAATAGGCCACAACAACCTTCTTTTCATTGGATACGTTTCTTATAAGTGT

509 uGlnLeuSerProThrAlaPheAsnSerLeuSerSerLeuGlnValLeuAsnMetSerHisAsnAsnPhePheSerLeuAspThrPheProTyrLysCys

3301 CTGAACCTCCCTCCAGGTTCTTGATTACAGTCTCAATCACATAATGACTTCCAAAAACAGGAACTACAGCATTTCCTCAAGTAGTCTAGCTTTCTTAAATC
543 ▶ LeuAsnSerLeuGlnValLeuAspTyrSerLeuAsnHisI leMetThrSerLysLysGlnGluLeuGlnHisPheProSerSerLeuAlaPheLeuAsnL
3401 TTACTCAGAATGACTTTGCTTGTACTTGTGAACACCAGAGTTTCTGCAATGGATCAAGACCAGAGCAGCTCTTGGTGAAGTTGAACGAATGGAATG
576 ▶ euThrGlnAsnAspPheAlaCysThrCysGluHisGlnSerPheLeuGlnTrpI leLysAspGlnArgGlnLeuValGluValGluArgMetGluCy
3501 TGCACACCTTCAGATAAGCAGGGCATGCCTGTGCTGAGTTTGAATATCACCTGTGAGTGAATAAGACCATCATTGGTGTGTCGGTCCCTCAGTGTGCTT
609 ▶ sAlaThrProSerAspLysGlnGlyMetProValLeuSerLeuAsnI leThrCysGlnMetAsnLysThrI leI leGlyValSerValLeuSerValLeu
3601 GTAGTATCTGTGTAGCAGTTCTGGTCTATAAGTTCTATTTTCCACCTGATGCTTCTTGTGCTGCATAAAGTATGGTAGAGGTGAAAACATCTATGATG
643 ▶ ValValSerValAlaValLeuValTyrLysPheTyrPheHisLeuMetLeuLeuAlaGlyCysI leLysTyrGlyArgGlyGluAsnI leTyrAspA
3701 CCTTTGTTATCTACTCAAGCCAGGATGAGGACTGGGTAAGGAATGAGCTAGTAAAGAATTTAGAAGAAGGGTGCCTCCATTTACAGTCTGCCTTCACTA
676 ▶ laPheVal I leTyrSerSerGlnAspGluAspTrpValArgAsnGluLeuValLysAsnLeuGluGluGlyValProProPheGlnLeuCysLeuHisTy
3801 CAGAGACTTTATTTCCCGGTGTGGCCATTGCTGCCAACATCATCCATGAAGTTTCCATAAAGCCGAAAGGTGATTGTTGTGGTGTCCAGCACTTCATC
709 ▶ rArgAspPheI leProGlyValAlaI leAlaAlaAsnI leI leHisGluGlyPheHisLysSerArgLysVal I leValValValSerGlnHisPheI le
3901 CAGACCCGCTGGTGTATCTTTGAATATGAGATTGCTCAGACCTGGCAGTTTCTGAGCAGTCTGTGGTATCATCTTCATTGCTCAGAAAGGTGGAGA
743 ▶ GlnSerArgTrpCysI lePheGluTyrGluI leAlaGlnThrTrpGlnPheLeuSerSerArgAlaGlyI leI lePheI leValLeuGlnLysValGluL
4001 AGACCTGCTCAGGCAGCAGGTGGAGCTGTACCCCTTCTCAGCAGGAACACTTACCTGGGGTGGGAGGACAGTGTCTGGGGCCGACATCTCTGGAG
776 ▶ ysThrLeuLeuArgGlnGlnValGluLeuTyrArgLeuLeuSerArgAsnThrTyrLeuGlyTrpGluAspSerValLeuGlyArgHisI lePheTrpAr

NheI (4196)

4101 ACGACTCAGAAAAACCCTGCTGGATGGTAAATCATGGAATCCAGAAGGAACAGTGGGTACAGGATGCAATTGGCAGGAAGCAACATCTATCTGAGCTAGC
809 ▶ gArgLeuArgLysAlaLeuLeuAspGlyLysSerTrpAsnProGluGlyThrValGlyThrGlyCysAsnTrpGlnGluAlaThrSerI le•••
4201 TGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAACACTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTT

4301 TATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAACAACAATTGCATTTCATTTATGTTTTCAGGTTACAGGGGAGGTGTGGGAGGTTTTTAAAG

PacI (4440)

4401 CAAGTAAAACCTCTACAAATGTGGTATGGAATGTTAATTAAC TAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCG

4501 TAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTGCAAAACAAAAAACCCGCTACCAGCGGTGTTTGTGTTGCC

4601 GGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGACAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCAC

4701 TTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGTGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACT

4801 CAAGACGATAGTTACCGGATAAAGCGCAGCGGTCGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGAGCGCAACGACCTACACCGAACTGAGATA

4901 CCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGGGCGGACAGGTATCCGGTAAGCGCGAGGGTCGGAACAGGAGAGCGCACGAGG

5001 GAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTCTGCTCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGGA

PacI (5180) SdaI (5189)

5101 GCCTATGGAAAAACGCCAGCAACCGGCCTTTTTACGGTTCCTGGCCTTTTGTGTCACATGTTCTTAATTAACCTGCAGGGCCTGAAATA

PvuII (5252)

5201 ACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCTGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAG

5301 CAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCT

NotI (5459)

5401 CAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGAGGGCCTCCAGCGGCGCCCTCCCCACAGCAGGGCGGGGTCGCCGCCAC

5501 CGGAAGGAGCGGGCTCGGGCGGGCGCGCTGATTGGCCGGGGCGGCTGACGCCAGCGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGAGCT

5601 TCTTCGCCAAGCTTCCGCTCAGAACGAGgtgagggcggggtgtggcttccgcgggcgccgagctggaggtcctgctccgagcgggcgggccccgct

5701 gtcgctcggggggattagctgagcattcccgcttcgagttgcgggcgggcgggagggagagctgagggcctagggcaaccctgagcctcgctcg

5801 tgtccgcttgaggcctagcgtggtgtccgcccggcgccgctgctactccggcgcaactcgtgctttttttttttgtgtgtgtgcccctgctgct

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6001 agtggcggtgagggcccggccttcggagcacatgtccgagcccactggatggggcgaggcctggggtttttcccgaagcaaccaggctggggttag

6101 cgtgcccaggccatgtggcccagcaccggcagcatctggcttggcgggcgccgcttgccctgectccctaacctagggtgagggcctcccgtccggcac

SmaI (6232)

6201 cagttgctgctgctggaagatggccgctcccgggcccgtgtgcaaggagctcaaatggaggacgcccagcccggaggcgggcggggtgagtcacca

6301 cacaaggaagagggcctggtccctcaccggctgctgcttctgtgacccgctggtcctatcgccgcaatagtcacctcgggcttttgagcagggctag

XcmI (6478)

6401 tcgcccggggggaggggatgtaatggcgttgaggtttgttcacattgggtgggtggagactagtcaggccagcctggcgctggaagtcattttggaa

6501 ttgtccccttgagtttgagcggagctaatctcgggcttcttagcgggtcaagagatcttttaaaccttttttagGTGTTGTGAAAAACCACCGCTAA

6601 TTCAAAGCAATCATGTTACCATTCTGTTTTTCCACCCTGTTTTCTTCCATATTTACTGAAGCTCAGAAGCAGTATTGGTCTGCAACTCATCCGATG

▶ MetLeuProPheLeuPhePheSerThrLeuPheSerSerI lePheThrGluAlaGlnLysGlnTyrTrpValCysAsnSerSerAspA

BamHI (6779)

6701 CAAGTATTTACATACCTACTGTGATAAAATGCAATACCAATTTCAATTAATGTTAACCCCTGTATAGAATTGAAAGGATCCAAAGGATTATTGCACAT

30 ▶ laSerI leSerTyrThrTyrCysAspLysMetGlnTyrProl leSerI leAsnValAsnProCysI leGluLeuLysGlySerLysGlyLeuLeuHisI

6801 TTTCTACATTCCAAGGAGAGATTTAAAGCAATTATATTTCAATCTCTATATAACTGTCAACACCATGAATCTTCCAAGCGCAAAGAAGTTATTTGCCGA
63 ▶ ePheTyrI leProArgArgAspLeuLysGlnLeuTyrPheAsnLeuTyrI leThrValAsnThrMetAsnLeuProLysArgLysGluVal I leCysArg
6901 GGATCTGATGACGATTACTCTTTTTGCAGAGCTCTGAAGGGAGAGACTGTGAATACAACAATATCATTCTCCTTCAAGGGAATAAAATTTCTAAGGGAA
97 ▶ GlySerAspAspAspTyrSerPheCysArgAlaLeuLysGlyGluThrValAsnThrThrI leSerPheSerPheLysGlyI leLysPheSerLysGlyL
7001 AATACAAATGTGTTGTTGAAGCTATTTCTGGGAGCCAGAAGAAATGCTCTTTTGCTTGGAGTTTGTCTCTACACCAACCTAATTCAAATTAGCTAGG
130 ▶ ysTyrLysCysValValGluAlaI leSerGlySerProGluGluMetLeuPheCysLeuGluPheVal I leLeuHisGlnProAsnSerAsn•••
7101 AGCAGGTTTCCCAATGACACAAAACGTGCAACTGAAACTCCCGCTGCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCT
CGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCAAAA
7201
7301 GCCACGCCACACGGGCCGTCATGTGTGAACCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCCACACACTGG
TGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGGGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTAAAAA
7401
7501 GCTTCTATGCCTGAATAGGTGACCGGAGTGGCGACCTTTCCTTTGCAATTACTGACCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAG
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7601
1 ▶ MetLysThrPheAsnI leSerGlnGlnAspLeuGluLeuValGluValAlaThrGlu
7701 AAGATCACCATGCTCTATGAGGACAACAAGCACCATGTGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGTGTCCACATTGAGGCCTACA
20 ▶ LysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI leSerAlaValHisI leGluAlaTyrI
7801 TTGGCAGGGTCACTGTCTGTCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTGAGGCCCCCTA
53 ▶ leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrI leValAlaValArgHisProTy
7901 CTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAG
86 ▶ rSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyrAlaProAspCysPheValLeuI leGlu
8001 ATGAATGGCAAGCTGGTCAAAACACCATTGAGGAACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAATTAATTCGCTAGGATTATCCCTAATAC
120 ▶ MetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgAsn•••
8101 CTGCCACCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAAC
AATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTGTGGACCACCTTTGGTTTTCTTTTTTTCGCTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAA
8201
8301 CAGTACTTTTTAATGGAAACAACTTGACCAAAAATTTGTACAGAATTTTGAGACCCATTAATAAGTTAAATGAGAAACCTGTGTGTTCTTTGGTCAA
CACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAACTTCTTGAAAATGTAATTTCTGAGTTAACACTTCTGGGTGGAGAATA
8401
8501 GGGTTGTTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTT
8601 GCTGATTGCCTGTCACTAAAAACAGGCCAAAAAAGTGAATCCTTGGGTTGCATAGAAAGCTG