

# pDUO-hCD14/TLR2

A plasmid coexpressing the human CD14 and TLR2 genes

Catalog code: pduo-hcd14tlr2

<https://www.invivogen.com/pduo-cd14-tlr2>

For research use only

Version 20H25-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pDUO-hCD14/TLR2 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 CD14 (1125 bp) / Human TLR2 (2352 bp)**

TLR2 is involved in the recognition of multiple products of Gram-positive bacteria, mycobacteria and yeast. The first studies reported that TLR2 mediated LPS response but TLR2 has since been shown to confer responsiveness to the lipopeptides present in LPS preparations. However, it seems that some types of LPS can activate TLR2<sup>1</sup>. TLR2 is known to heterodimerize with other TLRs, a property believed to extend the range of PAMPs that TLR2 can recognize. TLR2 cooperates with TLR6 in the response to peptidoglycan<sup>2</sup> and diacylated mycoplasmal lipopeptide, and associates with TLR1 to recognize triacylated lipopeptides. Furthermore, pathogen recognition by TLR2 is strongly enhanced by CD14.

- **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<sup>3</sup>. 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<sup>4</sup>.

- **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 enhancer<sup>5</sup>.

- **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.*<sup>6</sup>

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

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

- **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 expression<sup>7</sup>.
- **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 H<sub>2</sub>O. 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

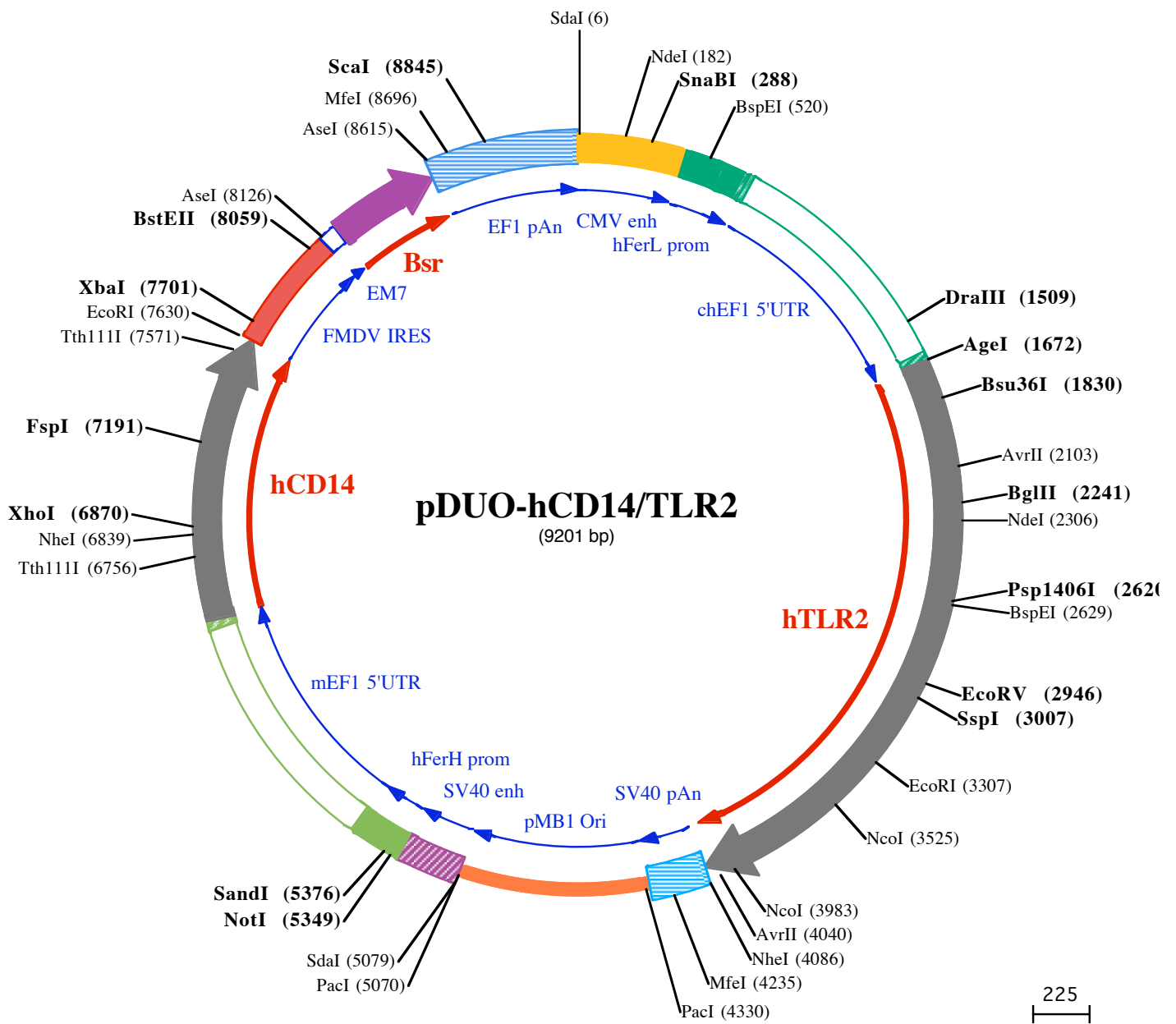
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4. **Dean DA. et al., 1999.** Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22.
5. **Boshart M. et al., 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141(2):521-30.
6. **Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258.
7. **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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 InvivoGen Hong Kong: +852 3622-3480  
 E-mail: [info@invivogen.com](mailto:info@invivogen.com)





SdaI (6)  
1 CCTGCAGGCGTTACATAAATTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (182)  
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCC

SnaBI (288)  
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATG

401 GGAGTTTGTGTTGACTAGTCAGGGCCCCAACCCCCAAGCCCCATTTACAACACGCTGGCGTACAGGCGGTGACTTCCCTTGTCTTGGGGCGG

BspEI (520)  
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCC

601 TCCGAGGGCGGGCCACCATAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTGCGCGGTCCCGGGTCTGTCTCAAGCTTCCGCCAGAACACAGg

701 taagtgcgctgtgtggttccgcgggctggcctctttacgggttatggccttgcgtgcttgaattacttccatgcccctggctgcagtacgtgattc

801 ttgatcccagcctcgggttgaagtgggtgggagagttcaggccttgcgcttaaggagcccttcgctcgtgcttgagtgaggcctggcttggcg

901 ctggggccgctgctaatctggtggcaccttcgcgctgtctcgtgcttctcgttaagtctctagcatttaaaatTTTTgataaccagctgcgacg

1001 cTTTTTctggcgagatagcttgtaaatgccccagatctgcacactggatatttcggTTTTTggggccgccccggcgaggggccccgtgctccc

1101 agcgcacatgttcggcgagggggctgcgagcgcggccaccgagaatcggacggggtagtctcaactggccggcctgctctggtgctggcctcgc

1201 gccgctgtatcgcggccttggcggaaggtggccggtcggcaccagttgctgagcggaaagatggcggcttccggcctgctgcaggggagc

1301 tcaaatggaggacgccccgggagagcggcggtgagtcaccacacaaggaagggcctttcctcctcatcctgctcgtcctcatgtgactcca

1401 cggagtaccgggccccgctccaggcactcgtattagttgctgagctttggagtagctgctcttaggttggggggggggttttatgcatggagttcc

DraIII (1509)  
1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggatttgcctttttagtggatcttgcctcattc

AgeI (1672)  
1601 tcaagcctcagacagtggttcaagattttttctccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGTAGGAGGGCCAGCATGCCACATAc

1701 TTTGTGGATGGTGTGGGCTTGGGGTCTCATCAGCCTCTCAAGGAAGAATCCTCCAATCAGGCTTCTCTGTCTTGTGACCGCAATGGTATCTGCAAG  
4rLeuTrpMetValTrpValLeuGlyValIleIleSerLeuSerLysGluGluSerSerAsnGlnAlaSerLeuSerCysAspArgAsnGlyIleCysLys  
1MetProHisThr

Bsu36I (1830)  
1801 GGCAGCTCAGGATCTTTAACTCCATTCCCTCAGGGCTCAGAGAAGCTGTAAGGCTTACCTGTCCAACAACAGGATACCTACATTAGCAACAGTG  
38GlySerSerGlySerLeuAsnSerIleProSerGlyLeuThrGluAlaValLysSerLeuAspLeuSerAsnAsnArgIleThrTyrIleSerAsnSerA  
1901 ACCTACAGAGGTGTGTGAACCTCCAGGCTCTGGTGTGACATCCAATGGAATTAACACAATAGAGGAAGATTCTTTTCTCCCTGGGCAGTCTTGAACA  
71spLeuGlnArgCysValAsnLeuGlnAlaLeuValLeuThrSerAsnGlyIleAsnThrIleGluGluAspSerPheSerSerLeuGlySerLeuGluHi  
2001 TTTAGACTTATCCTATAATTACTTATCTAATTTATCGTCTTCTGGTTCAAGCCCTTCTTTTAACTTCTTAACTTACTGGGAAATCCTTACAAA  
104sLeuAspLeuSerTyrAsnTyrLeuSerAsnLeuSerSerSerTrpPheLysProLeuSerSerLeuThrPheLeuAsnLeuLeuGlyAsnPProTyrLys  
AvrII (2103)  
2101 ACCCTAGGGGAAACATCTCTTTTTCTCATCTCACAAAATTGCAAACTCTGAGAGTGGGAAATATGGACACCTTCACTAAGATTCAAAGAAAAGATTTG  
138ThrLeuGlyGluThrSerLeuPheSerHisLeuThrLysLeuGlnIleLeuArgValGlyAsnMetAspThrPheThrLysIleGlnArgLysAspPheA

BglIII (2241)  
2201 CTGGACTTACCTTCTTGGAACTTGGATTGATGCTTACAGTCTACAGACTATGAGCCAAAAAGTTTGAAGTCAATTGAAATGTAAGTCACTGTAT  
171IleGlyLeuThrPheLeuGluGluLeuGluIleAspAlaSerAspLeuGlnSerTyrGluProLysSerLeuLysSerIleGlnAsnValSerHisLeuI

NdeI (2306)  
2301 CCTTCATATGAAGCAGCATATTTACTGCTGGAGATTTTGTAGATGTTACAAGTTCCGTGGAATGTTTGAAGTGGCAGATACTGATTTGGACACTTTC  
204eLeuHisMetLysGlnHisIleLeuLeuLeuGluIlePheValAspValThrSerSerValGluCysLeuGluLeuArgAspThrAspLeuAspThrPhe  
2401 CATTTTTCAGAACTATCCACTGGTGAACAATTCATTGATTAAGGTTTACATTTAGAAATGTGAAATCACCGATGAAAGTTTGTTCAGGTTATGA  
238HisPheSerGluLeuSerThrGlyGluThrAsnSerLeuIleLysLysPheThrPheArgAsnValLysIleThrAspGluSerLeuPheGlnValMetL  
2501 AACTTTGAATCAGATTCTGGATTGTAGAATTAGAGTTTGTGACTGTACCTTAATGGAGTTGGTAATTTAGAGCATCTGATAATGACAGAGTTAT  
271ysLeuLeuAsnGlnIleSerGlyLeuLeuGluLeuGluIlePheAspAspCysThrLeuAsnGlyValGlyAsnPheArgAlaSerAspAsnAspArgValIle

BspEI (2629)  
2601 AGATCCAGGTAAGTGGAAACGTTAAACATCCGGAGGCTGCATATTCGAAGTTTACTTATTTTATGATCTGAGCACTTATATTCACCTACAGAAAAG  
304eAspProGlyLysValGluThrLeuThrIleArgArgLeuHisIleProArgPheTyrLeuPheTyrAspLeuSerThrLeuTyrSerLeuThrGluArg  
2701 GTTAAAAGAATCACAGTAGAAAACAGTAAAGTTTTCTGGTCTCTGTTACTTTTACAACATTTAAAATCATTAGAATACTGGATCTCAGTGAAAAT  
338ValLysArgIleThrValGluAsnSerLysValPheLeuValProCysLeuLeuSerGlnHisLeuLysSerLeuGluIleThrAspGluSerLeuPheGlnValMetL  
2801 TGATGGTTGAAGAATCTTGAATACTTCAAGGATGCTGGCCCTCTACAACTTAAATTTAAGGCAAAATCATTGGCATCTTGGAAAA  
371euMetValGluGluTyrLeuLysAsnSerAlaCysGluAspAlaTrpProSerLeuGlnThrLeuIleLeuArgGlnAsnHisLeuAlaSerLeuGluLys  
EcoRV (2946)  
2901 AACCGGAGAGACTTGTCTCACTCTGAAAACTTACTAACATGATATCAGTAAGAATAGTTTCTATTCTATGCCTGAAACTTGTGAGTGGCCAGAAAAG  
404sThrGlyGluThrLeuLeuThrLeuLysAsnLeuThrAsnIleAspIleSerLysAsnSerPheHisSerMetProGluThrCysGlnTrpProGluLys  
SspI (3007)  
3001 ATGAAATATTTGAATTCACAGCACAGCAATACACAGTGTAAACAGGCTGCATTCCTCAAGACTGGAAATTTAGATGTTAGCAACAACAATCTCAATT  
438MetLysTyrLeuAsnLeuSerSerThrArgIleHisSerValThrGlyCysIleProLysThrLeuGluIleLeuAspValSerAsnAsnAsnLeuAsnL  
3101 TATTTTCTTGAATTTGCCGCAACTCAAAGAACTTTATATTTCCAGAAATAAGTTGATGACTTACAGATGCCTCCCTCTTACCCATGTTACTAGTATT  
471euPheSerLeuAsnLeuProGlnLeuLysGluLeuTyrIleSerArgAsnLysLeuMetThrLeuProAspAlaSerLeuLeuProMetLeuLeuValLe  
3201 GAAAATCAGTAGGAATGCAATAACTACTTTTTCTAAGGAGCAACTTACTCATTTCACACACTGAAGACTTTGGAAGCTGGTGGCAAACTTCAATTTGC  
504uLysIleSerArgAsnAlaIleThrThrPheSerLysGluGlnLeuAspSerPheHisThrLeuLysThrLeuGluAlaGlyGlyAsnAsnPheIleCys

EcoRI (3307)  
3301 TCCTGTGAATTCCTCTCCTTCACTCAGGAGCAGCAAGCACTGGCCAAAGTCTTGATTGATTGGCCAGCAAATTACCTGTGTGACTCTCCATCCCATGTGC  
538▶ Ser CysGluPheLeuSerPheThrGlnGluGlnAlaLeuAlaLysValLeuIleAspTrpProAlaAsnTyrLeuCysAspSerProSerHisValAla  
3401 GTGGCCAGCAGGTTCCAGGATGTCCGCCTCTCGGTGTCGGAATGTACAGGACAGCACTGGTGTCTGGCATGTGCTGTCTGTTCTCTGATCCTGTGCT  
571▶ rGlyGluGlnValGluAspValArgLeuSerValSerGluCysHisArgThrAlaLeuValSerGlyMetCysCysAlaLeuPheLeuLeuIleLeuLeu  
NcoI (3525)  
3501 CACGGGGTCTGTGCCACCGTTTCCATGGCCTGTGGTATATGAAAATGATGTGGCCTGGCTCCAGGCCAAAAGGAAGCCAGGAAAGCTCCAGCAGG  
604▶ uThrGlyValLeuCysHisArgPheHisGlyLeuTrpTyrMetLysMetMetTrpAlaTrpLeuGluAlaLysArgLysProArgLysAlaProSerArg  
3601 AACATCTGCTATGATGCATTTGTTTCTTACAGTGAGCAGGATGCCTACTGGGTGGAGAACCCTTATGGTCCAGGAGCTGGAGAACCCTCAATCCCCCTTCA  
638▶ AsnIleCysTyrAspAlaPheValSerTyrSerGluGluAspAlaTyrTrpValGluAsnLeuMetValGluGluLeuGluAsnPheAsnProProPheL  
3701 AGTTGTGCTTCATAAGCGGGACTTCATTCTGGCAAGTGGATCATTGACAATATCATTGACTCCATTGAAAAGAGCCACAAAACGTCTTTGTGCTTTC  
671▶ ysLeuCysLeuHisLysArgAspPheIleProGluLysTrpIleIleAspAsnIleIleAspSerIleGluLysSerHisLysThrValPheValLeuSe  
3801 TGAAAACCTTTGTGAAGAGTGAGTGGTGAAGTATGAACCTGGACTTCTCCCATTTCCGCTTTTTGATGAGAACAATGATGCTGCCATTCTATTCTTCTG  
704▶ rGluAsnPheValLysSerGluTrpCysLysTyrGluLeuAspPheSerHisPheArgLeuPheAspGluAsnAsnAspAlaAlaIleLeuIleLeuLeu  
NcoI (3983)  
3901 GAGCCCATTTGAAAAAAGCCATTTCCAGCGCTTCTGCAAGCTGCGGAAGATAATGAACACCAAGACCTACCTGGAGTGGCCATGGACGAGGCTCAGC  
738▶ GluProIleGluLysLysAlaIleProGluArgPheCysLysLeuArgLysIleMetAsnThrLysThrTyrLeuGluTrpProMetAspGluAlaGluAla  
AvrII (4040) NheI (4086)  
4001 GGGAAGGATTTGGTAAATCTGAGAGCTGCGATAAAGTCCTAGGTTCCATATTTAAGACCAGTCTTTGTCTAGTTGGGATCTTCTAGCTGGCCAGC  
771▶ rGluGluGlyPheTrpValAsnLeuArgAlaAlaIleLysSer●●●  
4101 ATGATAAGATACATTGATGAGTTGGACAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA  
MfeI (4235)  
4201 CCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAA  
PaeI (4330)  
4301 CCTCTACAAATGTGGTATGGAATGTTAATTAAGTACAGCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAA  
4401 TCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCTAATCTGCTGCTTGAACAACAAAAACCCAGCTACCAGCGGTGGTTTGTGGCCGATCAAGA  
4501 GCTACCAACTCTTTTCCGAAGTAAGTGGCTTCCAGCAGAGCGCAGATACCAAACTACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAC  
4601 TCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTCAAGACGAT  
4701 AGTTACCAGATAAGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTGGAGCGAACGACCTACACGAACTGAGATACCTACAGCG  
4801 TGAGCTATGAGAAAGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGAGGGTCGGAACAGGAGAGCGCAGGGGACTTCCA  
4901 GGGGAAACGCTGATCTTTATAGTCTGCTGGGTTTCCGACCTCTGACTTGAGCGTCGATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGA  
PaeI (5070) SdaI (5079)  
5001 AAAACGCCAGCAACCGGCTTTTTACGGTCTCGGCTTTTGTGCGCTTTTGTCCACATGTTCTTAATTAACCTGACGGGCTGAAATAACCTCTGAA  
5101 AGAGGAACCTGGTTAGGTACCTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
5201 GTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAAAGCATGCATCTCAATTAGTC  
NotI (5349) SmaI (5376)  
5301 AGCAACCATAGTCCCACTAGTTCGCCAGAGCGCGGAGGGCTCCAGCGGCCGCCCTCCCCACAGCAGGGGCGGGTCCCGCGCCACCAGGAAAGGAG  
5401 CGGGCTCGGGCGGGCGGCGCTGATTGGCCGGGCGGGCTGACGCCGACGCGCTATAAGAGACCACAAGCGACCCGAGGGCCAGACGTTCTTCGCCG  
5501 AAGCTTGGCGTCAGAACGCAAGtgaggggagggtgtggcttcgagggcgccgagctggaggctctgctccgagggggccggcccgctgctcggc  
5601 ggggattagctgagcattccgcttcgagttgagggcgccgagggcagagtgagggcctagcggcaacccttagcctcgctcgtgctcggc  
5701 tgagggcctagcgtggtgctcggcggcgccgctgctacccggcgcactctggtctttttttttttgtgtgtgtgctcctgctcctcgattgcc  
5801 gttcagcaataggggctaacaaggagggtgagggcttctcggcggagccggagaggtcatggtggggaggaatggagggacaggagtgaggc  
5901 tggggccgccccttcggagcactgctcggcggcaccctggatggggcagggcctggggtttttccgaagcaaccaggctgggggttagctgctcggag  
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6201 agagggcctggtccctcaccggctgctgcttctgtgacccggtgctctatcggcggcaatagtcacctcgggctttttagcacggctagtcgaggc  
6301 ggggaggggatgtaatggcgttggagttgttcaatgttggggggagagtagtcaggccagcctggcgtggaagtcatttttggaaatgtccct  
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6501 ATCATGGAGCGCGCTCTGCTGTTGCTGCTGCTGCTGCCGTGGTGACAGTCTCTGCGACCACGCCAGAACCCTTGTGAGCTGGACGATGAAGATTTC  
▶MetGluArgAlaSerCysLeuLeuLeuLeuLeuProLeuValHisValSerAlaThrThrProGluCysGluLeuAspAspGluAspPheA  
6601 GCTGCTGCAACTTCTCCGAACCTCAGCCGACTGGTCCGAAGCCTTCCAGTGTGTGCTGCAAGTGGAGATCCATGCCGGCGGTCTCAACCT  
33▶ rGlyValCysAsnPheSerGluProGluProAspTrpSerGluAlaPheGluCysValSerAlaValGluValGluIleHisAlaGlyGlyLeuAsnLe  
Th1111 (6756)  
6701 AGAGCCGTTTCTAAAGCGCTGATGCGGACGCGACCCGCGGAGTATGCTGACACGGTCAAGGCTCTCCGCTGCGGCGGCTCACAGTGGGAGCCGCA  
66▶ uGluProPheLeuLysArgValAspAlaAspAlaAspProArgGluN-TyrAlaAspThrValLysAlaLeuArgValArgArgLeuThrValGlyAlaAla

NheI (6839) XhoI (6870)

6801 CAGGTTCTGCTCAGTACTGGTAGGCGCCCTGCGTGTGCTAGCGTACTCCCGCCTCAAGGAAGTACGCTCGAGGACCTAAAGATAACCGCACCATGC  
100▶ Gl nVal P roAl aGl nLeuLeuVal l Gl yAl aLeuArgVal l LeuAl aTyrSer ArgLeuLysGl uLeuThr LeuGl uAspLeuLys l l eThr Gl yThr Met P  
6901 CTCCGCTGCCTCTGGAAGCCACAGGACTTGACATTTCCAGCTTGCGCCTACGCAACGTGTCTGGGCGACAGGGCGTTCTTGGCTCGCCGAGCTGCAGCA  
133▶ r oP roLeuGl uAl aThr Gl yLeuAl aLeuSer Ser LeuArgLeuArgAsnVal l Ser TrpAl aThr Gl yArgSer TrpLeuAl aGl uLeuGl nGl  
7001 GTGGCTCAAGCCAGGCCTCAAGGTACTGAGCATTGCCAAGCACACTCGCTGCTTTCTCTGCGAACAGGTTCGCGCTTCCCGCCCTTACCAGCCTA  
166▶ nTrpLeuLysP roGl yLeuLysVal l LeuSer l l eAl aGl nAl aHi sSer P roAl aPheSer CysGl uGl nVal l ArgAl aPheP roAl aLeuThr Ser Leu

FspI (7191)

7101 GACCTGTCTGACAATCCTGGACTGGGCGAACGCGGACTGATGGCGGCTCTGTGTCCCAAGTTCGCGCATCCAGAATCTAGCGCTGCGCAACACAG  
200▶ AspLeuSer AspAsnP roGl yLeuGl yGl uArgGl yLeuMe tAl aAl aLeuCysP roHi sLysPheP roAl a l l eGl nAsnLeuAl aLeuArgAsnThr G  
7201 GAATGGAGACGCCCCACAGGCGTGTGCGCCGACTGGCGGCGGAGGTGTGCAGCCCCACAGCCTAGACCTCAGCCACAACCTCGCTGCGCGCCACCGTAAA  
233▶ l yMe tGl uThr P roThr Gl yVal l CysAl aAl aLeuAl aAl aGl yVal l Gl nP roHi sSer LeuAspLeuSer Hi sAsnSer LeuArgAl aThr Val l As  
7301 CCCTAGCGCTCCGAGATGCATGTGGTCCAGCGCCTGAACCTCCCAATCTGTCTGCTGGCTGGAACAGGTGCCTAAAGGACTGCCAGCCAAGCTC  
266▶ nP roSer Al aP roArgCysMe tTrpSer Ser Al aLeuAsnSer LeuAsnLeuSer PheAl aGl yLeuGl uGl nVal l P roLysGl yLeuP roAl aLysLeu  
7401 AGAGTGTCTGATCTCAGCTGCAACAGACTGAACAGGGCGCGAGCCTGACGAGCTGCCGAGGTGGATAACCTGACACTGGACGGGAATCCCTTCTCTGG  
300▶ ArgVal l LeuAspLeuSer CysAsnArgLeuAsnArgAl aP roGl nP roAspGl uLeuP roGl uVal l AspAsnLeuThr LeuAspGl yAsnP roPheLeuV

Th111I (7571)

7501 TCCCTGGAAGTCCCTCCCCACGAGGGCTCAATGAACTCCGGCGTGGTCCAGCCTGTGCACGTTTCGACCTGTCTGGTGGGGTGTCTGGAAACCTTGGT  
333▶ a l P roGl yThr Al aLeuP roHi sGl uGl ySer Me tAsnSer Gl yVal l Val l P roAl aCysAl aArgSer Thr LeuSer Val l Gl yVal l Ser Gl yThr LeuVa

EcoRI (7630)

7601 GCTGCTCAAGGGGCCGGGCTTTGCTGAATTCGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTCCAGG  
366▶ l LeuLeuGl nGl yAl aArgGl yPheAl a●●●

XbaI (7701)

7701 TCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCG  
7801 TGGAACTCCTCCTTGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCGTCATGTGTGCAACCCACAGCAGCCGACTTTACTGCGAAA  
7901 CCCACTTAAAGTGACATTGAACTGGTACCCACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCGAGGTAACACGCGACACTCGGGATCTGA

BstEII (8059)

8001 GAAGGGGACTGGGCTTCTATAAAGCGCTCGGTTTAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCCGCACCTTTCCTTGGCAATTACTGACCC

AseI (8126)

8101 TATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACA  
1▶ Me tLysThr PheAsn l  
8201 TCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAA  
6▶ l eSer Gl nGl nAspLeuGl uLeuVal l Gl uVal l Al aThr Gl uLys l l eThr Me tLeuTyrGl uAspAsnLysHi sHi sVal l Gl yAl aAl l l eArgThr Ly  
8301 GACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCATATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGG  
39▶ sThr Gl yGl u l l e l l eSer Al aVal l Hi s l l eGl uAl aTyr l l eGl yArgVal l Thr Val l CysAl aGl uAl a l l eAl a l l eGl ySer Al aVal l SerAsnGl y  
8401 CAGAAGGACTTTGACACCATTTGGCTGTGAGGACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCCAGCCCTGTGGCATGTGAGAGAGC  
73▶ Gl nLysAspPheAspThr l l eVal l Al aVal l ArgHi sP roTyrSerAspGl uVal l AspArgSer l l eArgVal l Val l Ser P roCysGl yMe tCysArgGl uL  
8501 TCATCTCTGACTATGCTCCTGACTGCTTTGTCTCATTGAGATGAATGGCAAGCTGGTCAAAAACACCATTGAGGAACTCATCCCCCTCAAGTACACCCAG  
106▶ eul l eSerAspTyrAl aP roAspCysPheVal l Leu l l eGl uMe tAsnGl yLysLeuVal l LysThr Thr l l eGl uGl uLeu l l eP roLeuLysTyrThr Ar

AseI (8615) MfeI (8696)

8601 GAACTAAACCTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCAGTGGTGAAGAAGGCTCTCAGAAGTGTGTTTCAATT  
139▶ gAsn●●●  
8701 GGCCATTAAGTTTAGTAGTAAAGACTGGTAAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTTC

ScaI (8845)

8801 TTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGGAACAACCTTGACCAAAAATTTGTACAGAAATTTTGAGCCCAT  
8901 TAAAAAGTTAAATGAGAACTGTGTCTTTGGTCAACCCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGAAACTTCTT  
9001 GAAATGTAATCTTGAGTAAACACTTCTGGGTGAGAATAGGGTGTGTTTTCCCCACATAATTGGAAGGGAAGGAATATCATTTAAAGCTATGGGAG  
9101 GGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACCTGAGTCTTTGGGTTGCATAGAAAGCT  
9201 G