

pDUO-hTLR1/TLR2

A plasmid coexpressing the human TLR1 and TLR2 genes

Catalog code: pduo-hTLR1TLR2

<https://www.invivogen.com/pduo-tlr1-tlr2>

For research use only

Version 20H25-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-hTLR1/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 TLR1** (2358 bp) / **Human TLR2** (2352 bp)

TLR1, the first member of the Toll-Like Receptor family was identified by the presence of a domain homology found in both *Drosophila* Toll and human IL-1 receptors. So far, no direct ligands for TLR1 have been identified however it seems to act as a coreceptor. TLR1 has been shown to associate with TLR2 in response to triacylated lipopeptides¹, but not diacylated lipopeptides². These observations suggest that TLR1 is able to discriminate among lipoproteins by recognizing the lipid configuration.

- **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 protein³. 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 plasmids⁴.

- **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 enhancer⁵.

- **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.*⁶

- **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 expression⁷.

TECHNICAL SUPPORT

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

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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.

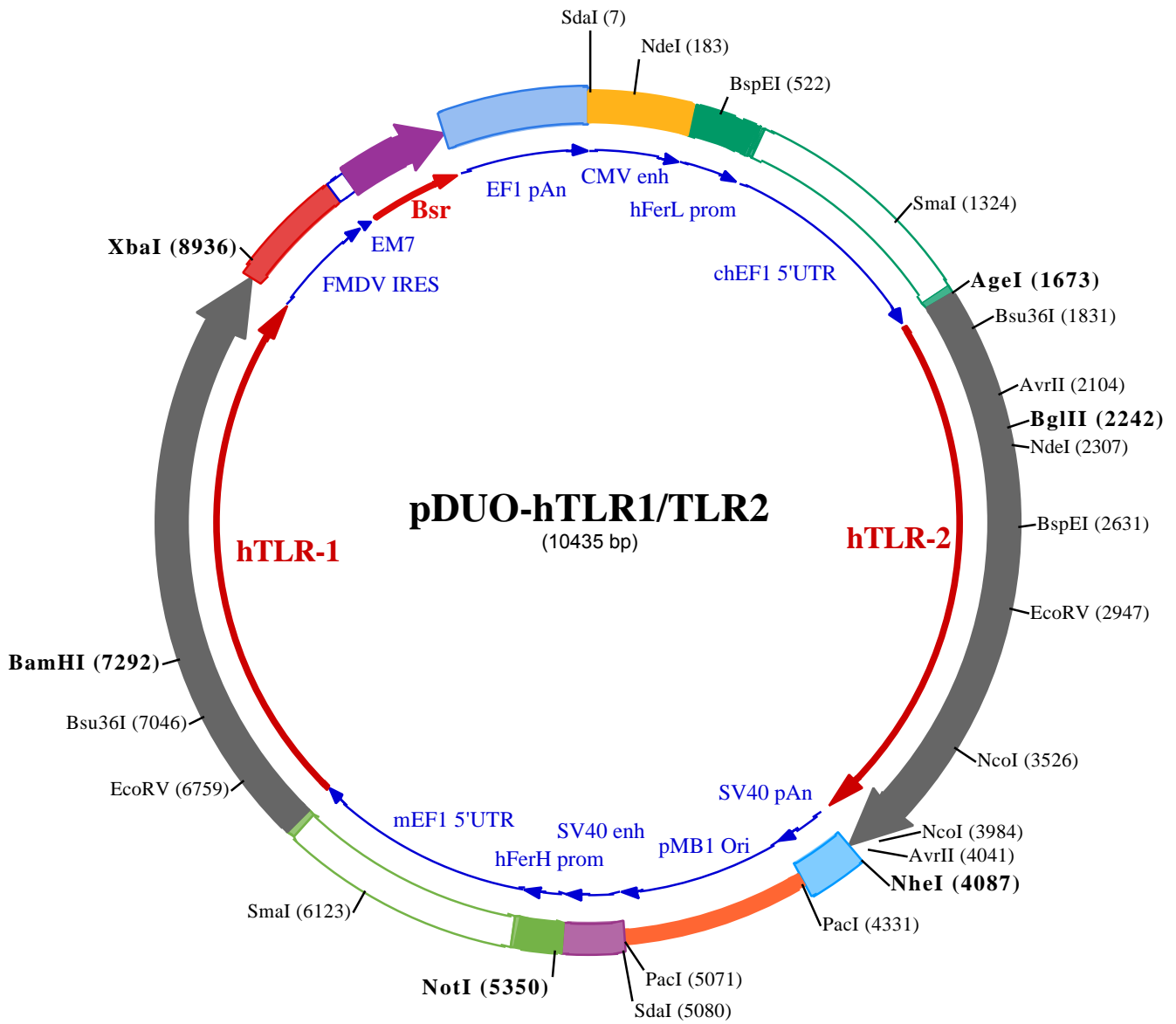
References

1. **Takeuchi O. et al., 2002.** Cutting edge: Role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol*, 169(1):10-14.
2. **Takeuchi O. et al., 2001.** Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol*, 13(7): 933-40.
3. **Eisenstein RS. & Munro HN. 1990.** Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
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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250

SdaI (7)

1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGGTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (183)

101 CGCCAAATAGGACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATG

401 GGAGTTTGTTTGACTAGTCAGGGCCCAACCCCCCAAGCCCAATTCACAACAGCTGGCGCTACAGGCGGTGACTTCCCTTGTCTTGGGGCGG

BspEI (522)

501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCGCTCCTGCCACCGCAGATTGGCCGTAGGCCTCCCGAGCGCCCTGCC

601 TCCGAGGGCCGGCGCACCATAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTCGGCGGTCCCGCGGTCTGTCTCAAGCTTGGCCGAGAACACAG

701 taagtgccgtgtgtggtccccgcccctggcctctttacgggtatggccttgcgtgccttgaattacttccatgccctggctgcagtacgtgattc

801 ttgatcccagacttcgggttgggaagtgggtgggagagttcgaggccttgcgttaaggagcccttcgctcgtgcttgagttgaggcctggcttgggccc

901 ctggggcccccgcgtgctaatctggtggcaccttcgcccctgtctcgtgctttcgtctaaagctcttagccatttaaaatttttgataaccagctgcgacg

1001 cttttttctggcgagatagctctgtaaatgcccggcaggatctgcacactggtatctcggttttggggccgcccggcgacggggcccctgctccc

1101 agcgcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcgagcgggggtagtctcaactggccgctgctctggtgcctggcctcgc

1201 gccgcccgtgtatcggcccccctgggcccgaagctggcccgtcgccaccagttgcgtgagcggaaagatggccgcttcccggcctgctgcagggagc

SmaI (1324)

1301 tcaaatggaggacgcccggggagagcgggcccgggtgagtcacccacacaaagaaaaggccttcttccatccctcgtcgttcatgtgactcca

1401 cggagtagcgggcccgtccaggcacctcgattagttgctgagccttggagtagcgtctttaggtggggggaggggtttatcgatggagtttcc

1501 ccacactgagtggggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaaatttgcctttttgagttggatcttgcctcattc

AgeI (1673)

1601 tcaagcctcagacagtgggttcaagttttttctccatttcagGTGTCGTGAAAACCTACCCTAAAAGCCACCGGTAGGAGGGCCAGCATGCCACATAC

1701 TTTGTGGATGGTGTGGTCTTGGGGTTCATCATCAGCCTCTCCAAGGAAGAATCCTCCAATCAGGCTTCTCTGTCTGTGACCGCAATGGTATCTGCAAG
4 rLeuTrpMetValTrpValLeuGlyVal I IeI leSerLeuSerLysGluGluSerSerAsnGlnAlaSerLeuSerCysAspArgAsnGlyI leCysLys

Bsu36I (1831)

1801 GGCAGCTCAGGATCTTTAACTCCATTCCTCAGGGCTCACAGAAGCTGTA AAAAGCCTTGACCTGTCCAACAACAGGATCACCTACATTAGCAACAGTG

1901 ACCTACAGAGGTGTGTGAACCTCAGGCTCTGGTGTGACATCCAATGGAATTAACACAATAGAGGAAGATTCTTTTCTCCCTGGGCAGTCTTGAACA

2001 TTTAGACTTATCCTATAATTACTTATCTAATTTATGCTTCTCTGGTTCAGCCCTTTCTTCTTAAACATTCTAAACTACTGGAAATCCTTACAAA

2101 ACCCTAGGGGAAACATCTCTTTTTTCTCATCTCACAAAATTGCAAAATCCTGAGAGTGGGAAATATGGACACCTTACTAAGATTCAAAGAAAAGATTTTG

138 ThrLeuGlyGluThrSerLeuPheSerHisLeuThrLysLeuGln I leLeuArgValGlyAsnMetAspThrPheThrLysI leGlnArgLysAspPheA

BglII (2242)

2201 CTGGACTTACCTTCTTGGAACTTGAGATTGATGCTTACAGTCTACAGAGCTATGAGCCAAAAGTTTGAAGTCAATTCAGAATGTAAGTCTATCTGAT

171 laGlyLeuThrPheLeuGluGluLeuGlu I leAspAlaSerAspLeuGlnSerTyrGluProLysSerLeuLysSerI leGlnAsnValSerHisLeuI I

NdeI (2307)

2301 CCTTCATATGAAGCAGCATATTTTACTGCTGGAGATTTTTGTAGATGTTACAAGTTCCTGGAAATGTTTGAAGTGGCAGATACTGATTTGGACACTTTC

2401 CATTTTTTCAGAACTCCACTCGGTGAAACAAATTCATTGATTA AAAAGTTTACTTTTACATTTAGAAATGTGAAAATCACCAGTAAAGTTTTCAGGTTATGA

2501 AACTTTTGAATCAGATTTCTGGATTGTAGAAATTAGAGTTTGTAGTACTGTACCCTTAATGGAGTTGGTAATTTAGAGCATCTGATAATGACAGAGTTAT

271 ysLeuLeuAsnGlnI leSerGlyLeuLeuGluLeuPheAspAspCysThrLeuAsnGlyValGlyAsnPheArgAlaSerAspAsnAspArgVal I I

BspEI (2631)

2601 AGATCCAGGTAAGTGGAAACGTTAAACAATCCGAGGCTGCATATTTCCAAGTTTTACTTATTTTATGATCTGAGCACTTTATATTCACCTACAGAAAAGA

2701 GTTAAAAGAAATCAGAGTAAAGAAACAGTAAAGTTTTCTGGTTCCTGTTTACTTTCACAACATTTAAAATCATTAGAAATCTGGATCTCAGTAAAAT

2801 TGATGGTTGAAGAATACTGAAAATTCAGCCTGTGAGGATGCCTGGCCCTCTCACAACTTAAATTTAAGGCAAAATCATTGGCATCATTGGAAAA

301 ATGAAATATTTGAACTTATCCAGCACACGAATACACAGTGAACAGGCTGCATTTCCCAAGCACTGGAAATTTAGATGTTAGCAACAACAACTCAATT

3101 TATTTTCTTTGAAATTTGGCCAACTCAAAGAATTTATATTTCCAGAAATAAGTTGACTACTACCAGATGCCTCCCTCTTACCCATGTTACTAGTATT

3201 GAAAATCAGTAGGAATGCAATAACTACGTTTTCTAAGGAGCAACTGACTATTTCCACACACTGAAGACTTTGGAAGCTGGTGGCAATAACTTCAATTTGC

504 uLysI leSerArgAsnAlaI leThrThrPheSerLysGluGlnLeuAspSerPheHisThrLeuLysThrLeuGluAlaGlyGlyAsnAsnPhel leCys

3301 TCCTGTGAATTCCTCTCCTTCACTCAGGAGCAGCAAGCACTGGCCAAAGTCTTGATTGATTGGCCAGCAAATTACCTGTGTGACTCTCCATCCCATGTGC
538▶ SerCysGluPheLeuSerPheThrGlnGlnGlnAlaLeuAlaLysValLeu leAspTrpProAlaAsnTyrLeuCysAspSerProSerHisValA
3401 GTGGCCAGCAGGTTCCAGGATGTCCGCTCTCGGTGTCGGAATGTCACAGGACAGCACTGGTGTCTGGCATGTGCTGTCTGTCTGTCTGATCCTGCT
571▶ rgGlyGlnGlnValGlnAspValArgLeuSerValSerGluCysHisArgThrAlaLeuValSerGlyMetCysCysAlaLeuPheLeuLeu leLeuLe
NcoI (3526)
3501 CACGGGGTCTGTGCCACCGTTTCCATGGCCTGTGGTATATGAAAATGATGTGGCCCTGGCTCCAGGCCAAAAGGCCAGGAAAGCTCCCAGCAGG
604▶ uThrGlyValLeuCysHisArgPheHisGlyLeuTrpTyrMetLysMetMetTrpAlaTrpLeuGlnAlaLysArgLysProArgLysAlaProSerArg
3601 AACATCTGCTATGATGCATTGTTTCTTACAGTGAGCAGGATGCCTACTGGTGGAGAACCTTATGGTCCAGGAGCTGGAGAACTCAATCCCCCTTCA
638▶ AsnI leCysTyrAspAlaPheValSerTyrSerGluGlnAspAlaTyrTrpValGluAsnLeuMetValGlnGluLeuGluAsnPheAsnProProPheL
3701 AGTTGTGTCTTCATAAGCGGGACTTCATTCTGGCAAGTGGATCATTGACAATATCATTGACTCCATTGAAAAGAGCCACAAAAGCTGTTTGTGCTTTC
671▶ ysLeuCysLeuHisLysArgAspPheI leProGlyLysTrpI leI leAspAsnI leI leAspSerI leGluLysSerHisLysThrValPheValLeuSe
3801 TGAAAACCTTTGTGAAGAGTGAGTGGTCAAGTATGAACTGGACTTCTCCATTCCGCTCTTTTGTGATGAGAACATGATGCTGCCATTCTCATTCTTCTG
704▶ rGluAsnPheValLysSerGluTrpCysLysTyrGluLeuAspPheSerHisPheArgLeuPheAspGluAsnAsnAspAlaAlaI leLeuI leLeuLeu
NcoI (3984)
3901 GAGCCATTGAGAAAAAGCCATTCCCAGCGTCTTGCAGCTGCGGAAGATAATGAACCAAGACCTACCTGGAGTGGCCATGGACGAGGCTCAGC
738▶ GluProl leGluLysLysAlaI leProGlnArgPheCysLysLeuArgLysI leMetAsnThrLysThrTyrLeuGluTrpProMetAspGluAlaGlnA
AvrII (4041) NheI (4087)
4001 GGAAGGATTTTGGGTAAATCTGAGAGCTGCGATAAAGTCCCTAGGTTCCCATATTTAAGACCAGTCTTTGTCTAGTTGGGATCTTGCTAGCTGGCCAGC
771▶ rgGluGlyPheTrpValAsnLeuArgAlaAlaI leLysSer•••
4101 ATGATAAGATACATTGATGAGTTTGGACAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA
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PacI (4331)
4301 CCTCTACAAATGTGGTATGGAATGTTAATTAACCTAGCCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAA
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4501 TCTGTAGACCCGCTACATACCTCGCTCTGTAATCCTGTTACCAGTGGCTGCTGCCAGTGGGATAAGTGTGCTTACCGGTTGGACTCAAGACGAT
4601 AGTTACCGGATAAGGCGCAGCGTCCGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCG
4701 TGAGCTATGAGAAAGCCACGCTTCCGAAGGGAGAAAGGGGACAGGTATCCGTAAGCGGCAGGGTCCGAACAGGAGAGCGCAGAGGGAGCTTCCA
4801 GGGGAAACGCTGCTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGA
4901 AAAACGCCAGCAACGGCGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCCTTTTGTCTACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAA
PacI (5071) SdaI (5080)
5001 AGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGAATGTGTGTCACTTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA
5101 GTATGCAAAGCATGCATCTCAATTAGTCAGAACAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTC
5201 AGCAACCATAGTCCCACTAGTTCCGCGAGAGCGCGAGGGCCTCCAGCGCCGCCCTCCCCACAGCAGGGGCGGGGTCCCGCGCCACCGGAAGGAG
NotI (5350)
5301 CGGGCTCGGGCGGGCGGCTGATTGGCCGGGCGGGCTGACGCCAGCGGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGACGTTCTTCGCGG
5401 AAGCTTCCCGTCAGAACGCAGgtgaggggagggtgtggcttccgcgggcccgagctggaggtcctgctccgagcgggccccgctgtcgtcggc
5501 ggggattagctcgcgagcattcccgcttcgagttgcgggcgggcgggagcagagtcgagggcctagcggcaacccttagcctcgcctcgtgtccggt
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6501 ▶MetThrSerI lePheHisPheAlaI leI lePheMetLeuI leLeuGlnI leArgI leGlnLeuSerGluGluSerGluPheLeuValAspArgSerL
6601 AAAACGGTCTCATCCAGTTTCCATAAAGACCTATCCAGAAAACAACAATCTTAATATATCGCAAAATATATATCTGAGCTTTGGACTTCTGACATCTT
33▶ ysAsnGlyLeuI leHisValProLysAspLeuSerGlnLysThrThrl leLeuAsnI leSerGlnAsnTyrI leSerGluLeuTrpThrSerAspI leLe
EcoRV (6759)
6701 ATCACTGTCAAACTGAGGATTTTATAATTTCTCATAATACAATCCAGTATCTTGATATCAGTGTTTTCAAATTCACCAGGAATTGGAATACTTGGAT
66▶ uSerLeuSerLysLeuArgI leLeuI leI leSerHisAsnThrl leGlnTyrLeuAspI leSerValPheLysPheAsnGlnGluLeuGluTyrLeuAsp

6801 TTGTCCACAACAAGTTGGTGAAGATTTCTTCCACCCTACTGTGAACCTCAAGCACTTGGACCTGTCATTTAATGCATTTGATGCCCTGCCTATATGCA
100 ▶ LeuSerHisAsnLysLeuValLysI leSerCysHisProThrValAsnLeuLysHisLeuAspLeuSerPheAsnAlaPheAspAlaLeuProI leCysL
6901 AAGAGTTTGGCAATATGTCTCAACTAAAATTTCTGGGGTTGAGCACCACACACTTAGAAAAATCTAGTGTGTGCGCAATTGCTCATTGAAATATCAGCAA
133 ▶ ysGluPheGlyAsnMetSerGlnLeuLysPheLeuGlyLeuSerThrThrHisLeuGluLysSerSerValLeuProI leAlaHisLeuAsnI leSerLy
Bsu36I (7046)

7001 GGTCTTGCTGGTCTTAGGAGAGACTTATGGGAAAAAGAAGACCCTGAGGGCCTCAAGACTTTAACACTGAGAGTCTGCACATTGTGTTCCCCACAAC
166 ▶ sValLeuLeuValLeuGlyGluThrTyrGlyGluLysGluAspProGluGlyLeuGlnAspPheAsnThrGluSerLeuHisI leValPheProThrAsn

7101 AAAGAATTCATTTTATTTGGATGTCTCAGTCAAGACTGTAGCAAATCTGGAATATCTAATATCAAATGTGTGCTAGAAGATAACAAATGTTCTTACT
200 ▶ LysGluPheHisPheI leLeuAspValSerValLysThrValAlaAsnLeuGluLeuSerAsnI leLysCysValLeuGluAspAsnLysCysSerTyrP

BamHI (7292)

7201 TCCTAAGTATTCTGGCGAACTTCAAACAAATCCAAAGTTATCAAATCTTACCTTAAACAACATTGAAACAACCTGGAATTCCTTATTAGGATCCCTCCA
233 ▶ leuLeuSerI leLeuAlaLysLeuGlnThrAsnProLysLeuSerAsnLeuThrLeuAsnAsnI leGluThrThrTrpAsnSerPheI leArgI leLeuGI
7301 GCTGGTTTGGCATAACAACCTGTATGGTATTCTCAATTTCAAACGTGAAGCTACAGGGTACAGTGGACTTCAGAGATTTGATTATTCTGGCACTTCCTTG
266 ▶ nLeuValTrpHisThrThrValTrpTyrPheSerI leSerAsnValLysLeuGlnGlyLeuAspPheArgAspPheAspTyrSerGlyThrSerLeu
7401 AAGGCCTTGTCTATACCAAGTTGTCAGCGATGTGTTCCGTTTCCGCAAAGTTATATCTATGAAATCTTTTTCGAATATGAACATCAAAAATTTTCACAG
300 ▶ LysAlaLeuSerI leHisGlnValValSerAspValPheGlyPheProGlnSerTyrI leTyrGluI lePheSerAsnMetAsnI leLysAsnPheThrV
7501 TGTCTGGTACACGCATGGTCCACATGCTTTCGCCATCCAAAATTAGCCCGTTCCTGCATTTGGATTTTTCGAATAATCTCTTAACAGACACGGTTTTTGA
333 ▶ alSerGlyThrArgMetValHisMetLeuCysProSerLysI leSerProPheLeuHisLeuAspPheSerAsnAsnLeuLeuThrAspThrValPheGI
7601 AAATGTGGGCACCTTACTGAGTGGAGACACTATTTTACAAATGAATCAATTAAGAAGACTTCAAAAATAGCTGAAATGACTACACAGATGAAGTCT
366 ▶ uAsnCysGlyHisLeuThrGluLeuGluThrLeuI leLeuGlnMetAsnGlnLeuLysGluLeuSerLysI leAlaGluMetThrThrGlnMetLysSer
7701 CTGCAACAATTGGATATTAGCCAGAATTCTGTAAGCTATGATGAAAAGAAAGGAGACTGTTCTTGGACTAAAAGTTTATTAAGTTTAAATATGCTTCAA
400 ▶ leuGlnGlnLeuAspI leSerGlnAsnSerValSerTyrAspGluLysLysGlyAspCysSerTrpThrLysSerLeuLeuSerLeuAsnMetSerA
7801 ATATACTTACTGACACTATTTTCAGATGTTTACCTCCAGGATCAAGGTACTGTATCTTCACAGCAATAAAAATAAGAGCATTCTCAAAACAGTCGTAAA
433 ▶ snI leLeuThrAspThrI lePheArgCysLeuProProArgI leLysValLeuAspLeuHisSerAsnLysI leLysSerI leProLysGlnValValLy
7901 ACTGGAAGCTTTCGAAGAAGCTCAATGTTGCTTTCATTTCTTAACTGACCTTCCTGGATGTGGCAGCTTTAGCAGCCTTCTGTATTGATCATTGATCAC
466 ▶ sLeuGluAlaLeuGlnGluLeuAsnValAlaPheAsnSerLeuThrAspLeuProGlyCysGlySerPheSerSerLeuSerValLeuI leI leAspHis
8001 AATTCAGTTTCCCACCCATCGGCTGATTCTTCCAGAGCTGCCAGAGATGAGGTCAATAAAAAGCAGGGGACAATCCATTCCAATGTACCTGTGAGCTAG
500 ▶ AsnSerValSerHisProSerAlaAspPhePheGlnSerCysGlnLysMetArgSerI leLysAlaGlyAspAsnProPheGlnCysThrCysGluLeuG
8101 GAGAAATTTGCAAAAATATAGACCAAGTATCAAGTGAAGTGTAGAGGGCTGGCCGTGATTCTTATAAGTGTGACTACCCGGAAGTTATAGAGGAACCT
533 ▶ lyGluPheValLysAsnI leAspGlnValSerSerGluValLeuGluGlyTrpProAspSerTyrLysCysAspTyrProGluSerTyrArgGlyThrLe
8201 ACTAAAGGACTTTCACATGTCTGAATTATCTGCAACATAACTCTGCTGATCGTACCCTCGTTGCCACCATGCTGGTGTGGCTGTGACTGTGACCTCC
566 ▶ uLeuLysAspPheHisMetSerGluLeuSerCysAsnI leThrLeuLeuI leValThrI leValAlaThrMetLeuValLeuAlaValThrValThrSer
8301 CTCTGCAGCTACTTGGATCTGCCCTGGTATCTCAGGATGGTGTGCCAGTGGACCCAGCCGCGCAGGGCCAGGAACATACCCTTAGAAGAACTCCAAA
600 ▶ LeuCysSerTyrLeuAspLeuProTrpTyrLeuArgMetValCysGlnTrpThrGlnThrArgArgAlaArgAsnI leProLeuGluGluLeuGlnA
8401 GAAATCTCCAGTTTCATGCATTTATTTCATATAGTGGGCACGATTTCTTCTGGTGAAGAATGAATTTGCCAAACCTAGAGAAGAAGGTATGCAGAT
633 ▶ rgAsnLeuGlnPheHisAlaPheI leSerTyrSerGlyHisAspSerPheTrpValLysAsnGluLeuLeuProAsnLeuGluLysGluGlyMetGlnI I
8501 TTGCCTTCATGAGAGAACTTTGTTCTGGCAAAGAGCATTGTGAAAATATCATCACCTGCATTGAGAAGAGTTACAAGTCCATCTTTGTTTGTCTCCC
666 ▶ eCysLeuHisGluArgAsnPheValProGlyLysSerI leValGluAsnI leI leThrCysI leGluLysSerTyrLysSerI lePheValLeuSerPro
8601 AACTTTGTCAGAGTGAATGGTCCATTATGAACTCTACTTTGCCATCACAATCTCTTTCATGAAGGATCTAATAGCTTAATCCTGATCTTGTCTGGAAC
700 ▶ AsnPheValGlnSerGluTrpCysHisTyrGluLeuTyrPheAlaHisHisAsnLeuPheHisGluGlySerAsnSerLeuI leLeuI leLeuLeuGluP
8701 CCATTCGCGAGTACTCCTTCTAGCAGTTATCACAAGCTCAAAAGTCTCATGGCCAGGAGACTTATTGGAATGGCCCAAGGAAAAGCAAAACGTTGG
733 ▶ roI leProGlnTyrSerI leProSerSerTyrHisLysLeuLysSerLeuMetAlaArgArgThrTyrLeuGluTrpProLysGluLysSerLysArgGI
8801 CCTTTTTGGGCTAACTTAAAGGCAGCCATTAAATTAAGCTGACAGAGCAAGCAAGAAAGAAATAGTCTAGGCTAGGAGCAGGTTTCCCAATGACACAAAA
766 ▶ yLeuPheTrpAlaAsnLeuArgAlaAlaI leAsnI leLysLeuThrGluGlnAlaLysLys • • •

XbaI (8936)

8901 CGTGAACCTTGAACCTCCGCTGGTCTTTCCAGGCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCAGCTCGATCCACTGGCGAGTGTAGTAAC
9001 AGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACCTCCTCTGGTAAACAAGGACCCACGGGGCCAAAAGCCACGCCACAGGGCCCGTCA
9101 TGTGAACCCAGCAGCGGACTTTACTGCGAAACCACTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTCAGG
9201 TACCCGAGGTAACAGCGCACCTCGGGATCTGAGAAGGGGACTGGGGCTCTATAAAAAGCGCTCGGTTAAAAAGCTTCTATGCCTGAATAGGTACCC
9301 GAGGTGGGACCTTTCCTTTCGAATTAAGTACCTATGAATACACTGACTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACT
9401 CACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGTGCCTACTGAGAAGATCACCATGCTCTATGAGGACA
1 ▶ MetLysThrPheAsnI leSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysI leThrMetLeuTyrGluAspA
9501 ACAAGCACCATGTCCGGGCGCCATCAGGACCAAGACTGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGCTGTGTGA
28 ▶ snLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI leSerAlaValHisI leGluAlaTyrI leGlyArgValThrValCysAlaGI
9601 AGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGAGGACTTTGACACCATTTGGCTGTGAGGACCCCTACTCTGATGAGGTGGACAGATCCATC
61 ▶ uAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrI leValAlaValArgHisProTyrSerAspGluValAspArgSerI le
9701 AGGTTGGTACGCCCTGTGGCATGTGCAGAGACTCATCTGACTATGCTCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAAACCA
95 ▶ ArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyrAlaProAspCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrT
9801 CCATTGAGGAACCTATCCCTCAAGTACACCGAAGTAAACCTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCCCTCTTAATACAGTG
128 ▶ hrI leGluGluLeuI leProLeuLysTyrThrArgAsn • • •

9901 TGAAGAACGGTCTCAGAAGCTGTTGTTCAATGGCCATTAAGTTTAGTAGTAAAAGACTGGTAAATGATAACAATGCATCGTAAAACCTTCAGAAGG
10001 AAAGGAGAATGTTTTGTGGACCCTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGAAAACAACCT
10101 GACCAAAAATTTGTCACAGAATTTTGGACCCATTAAGAAAGTAAATGAGAAACCTGTGTCTCTTTGGTCAACCCGAGACATTTAGGTGAAAGACA
10201 TCTAATCTGGTTTTACGAATCTGGAACCTTCTGAAAATGTAATCTTTGAGTTAACACTTCTGGGTGGAGAATAGGTTGTTTTCCCCACATAATTG
10301 GAAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTAAAACAGG
10401 CCAAAAACCTGAGTCTTGGGTTGCATAGAAAGCTG