

pDUO2-mCD14/TLR2

A plasmid coexpressing the murine CD14 and TLR2 genes

Catalog code: pduo2-mcd14tlr2

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

For research use only

Version 19123-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO2-mCD14/TLR2 provided as lyophilized DNA
- 1 ml of Hygromycin B Gold at 100 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 Hygromycin B Gold 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 toll-like receptors have been reported in humans (TLR1 to TLR10) and only nine in mice (TLR1 to TLR9). 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.

pDUO2 is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other. The genes cloned into pDUO2 comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

- **Murine CD14 (1098 bp)/Murine 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 TLR2¹. 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 peptidoglycan² 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 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*.
- **Hph (hygromycin resistance gene):** confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* 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.

Hygromycin B usage:

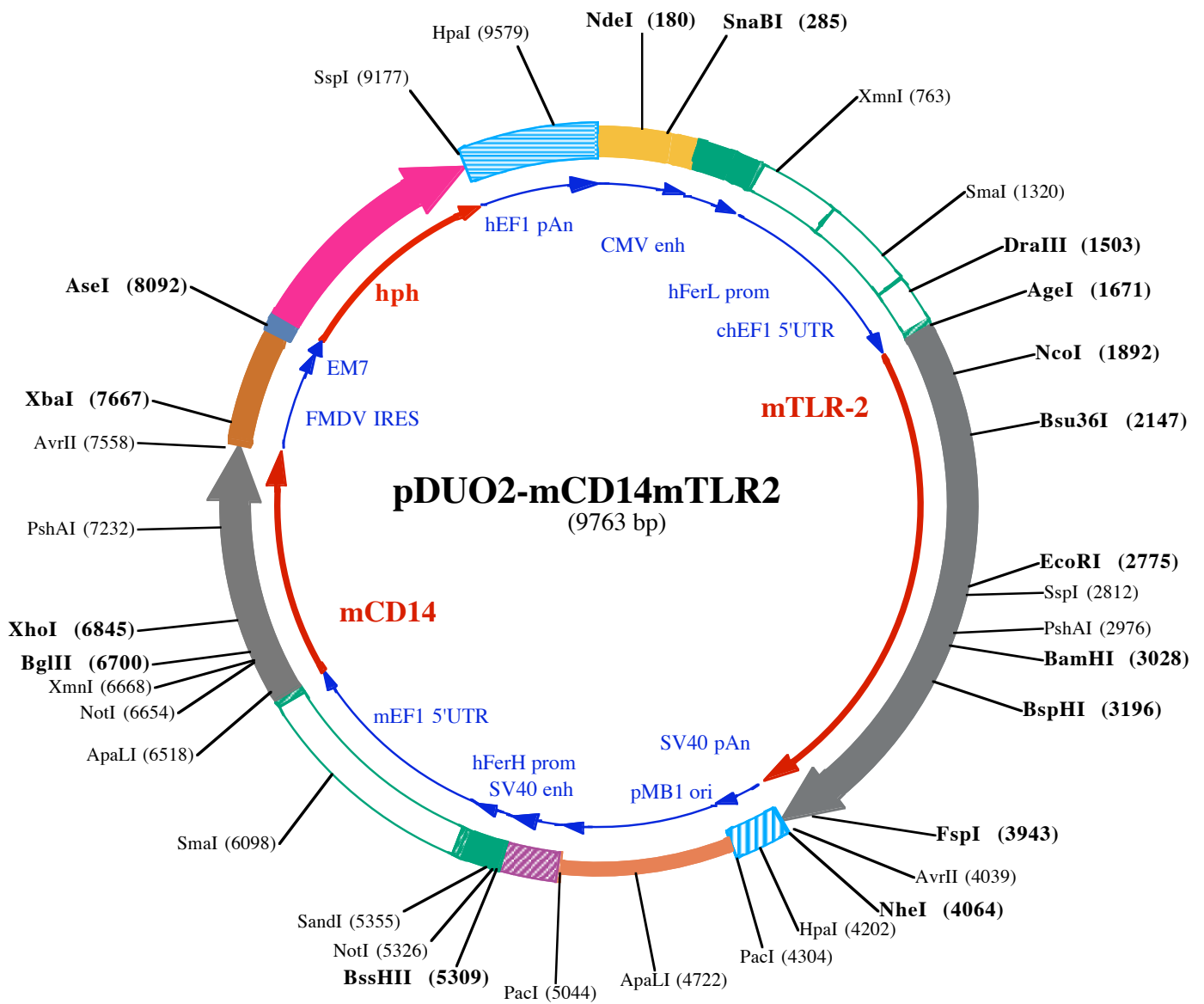
This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

References

1. Netea MG, et al. 2002. Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? Trends Immunol, 23(3):135-9.
2. Ozinsky A, et al. (2000). The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. PNAS 97(25):13766-71.
3. Eisenstein RS, and 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., and 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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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
NdeI (180)
101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
SnaBI (285)
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCAATTGACGTCAATG
401 GGAGTTTGTTTTGACTAGTCAGGGCCCAACCCCAAGCCCCATTTCACAACACGCTGGCGTACAGGCGGTGACTTCCCCTTGCTTTGGGGCGG
501 GGGCTGAGACTCCTATGTGCTCCGATTGGTCAGGCACGGCCTTCGGCCCCGCTCCTGCCACCGCAGATTGGCCGCTAGGCCCTCCCGAGCGCCCTGCC
601 TCCGAGGGCCGGCGCACCATAAAGAAGCCGCTTAGCCAGTCCCTCGAGTTCCGGCGTCCCGGGTCTGTCTCAAGCTTCCCGCAGAACACAGg
XmnI (763)
701 taagtgcctgtgtggttcccgcggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc
801 ttgatcccagacttcgggttgaagtgggtgggagagttcaggccttgcgcttaaggagccccttcgctcgtgcttgagttgaggcctggcttggcg
901 ctggggccgctgctaactcgtgtggcacttcgctcgtctcgtgctttcgtaagtctctagccatttaaatttttgataaccagctgcgacg
1001 cttttttctggcgagatagcttgaatgcccagcagctgcacactggatatttcggttttggggccgccccggcgacggggccctgctgctccc
1101 agcgacatgttcggcgaggcgggctgcgagcggccaccgagaatcggaggggtagtctcaactggccgctgctctggtgctggcctcgc
1201 gccgctgtatcggccctggcggcaaggctggccggctggcaccagttgcgtgagcggaaagatggccgcttccggccctgctgcagggagc
SmaI (1320)
1301 tcaaatggaggacgcccgggagagcggcggggtgagtcaccacacaaaggaaaaggcctttccttctcatccgctgcttcatgtgactcca
1401 cggagtaccggcgccgtccaggcacctcgatttagttgtcagacttttggagtacgtcgtctttaggttgggggaggggttttatgcatggagtttc
DraIII (1503)
1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaaatttgcctttttgagtttgatcttgcctcattc
AgeI (1671)
1601 tcaagcctcagacagtggttcaagttttttcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGGTAGGAGGGCCAGCATGCTACGAGC
1701 TCTTTGGCTCTTCTGGATCTTGGTGGCCATAACAGTCTCTTTCAGCAAACGCTGTTCTGCTCAGGAGTCTCTGTCATGTGATGCTTCTGGGGTGTGTGAT
4 aLeuTrpLeuPheTrpIleLeuValAlaIleThrValLeuPheSerLysArgCysSerAlaGlnGluSerLeuSerCysAspAlaSerGlyValCysAsp
NcoI (1892)
1801 GGCCGCTCCAGTCTTTACCTCTATTCCCTCCGACTCACAGCAGCCATGAAAAGCCTTGACCTGTCTTTCAACAAGATCACCTACATTGGCCATGGTG
38 GlyArgSerArgSerPheThrSerIleProSerGlyLeuThrAlaAlaMetLysSerLeuAspLeuSerPheAsnLysIleThrTyrIleGlyHisGlyA
1901 ACCTCCGAGCGTGTGCGAACCTCCAGGTTCTGATTTTGAAGTCCAGCAGAATCAATAACAATAGAGGGAGACGCCTTTATCTCTGGGCAGTCTTGAACA
71 spLeuArgAlaCysAlaAsnLeuGlnValLeuIleLeuLysSerSerArgIleAsnThrIleGluGlyAspAlaPheTyrSerLeuGlySerLeuGluHi
2001 TTTGGATTTGCTGATAATCACCTATCTAGTTTATCTTCTCCTGGTTCCGGCCCCCTTCTCTTTGAAATACTTAACTTAATGGAAATCCTTACCAG
104 sLeuAspLeuSerAspAsnHisLeuSerSerLeuSerSerSerTrpPheGlyProLeuSerSerLeuLysTyrLeuAsnLeuMetGlyAsnPProTyrGln
Bsu36I (2147)
2101 AACTGGGGTAACATCGCTTTTTCCCAATCTCACAAATTTACAAACCTCAGGATAGGAAATGTAGAGACTTTCAGTGAGATAAGGAGAATAGATTTTG
138 ThrLeuGlyValThrSerLeuPheProAsnLeuThrAsnLeuGlnThrLeuArgIleGlyAsnValGluThrPheSerGluIleArgArgIleAspPheA
2201 CTGGGCTGACTTCTCTCAATGAACTGAAATTAAGGCATTAAGTCTCCGGAATTATCAGTCCCAAAGTCTAAAGTCGATCCGCGACATCCATCACCTGAC
171 laGlyLeuThrSerLeuAsnGluLeuGluIleLysAlaLeuSerLeuArgAsnTyrGlnSerGlnSerLeuLysSerIleArgAspIleHisHisLeuTh
2301 TCTTCACTTAAGCGAGTCTGCTTTCCTGCTGGAGATTTTTGCAGATATTTCTGAGTTCTGTGAGATATTTAGAACTAAGAGATACTAACTTGGCCAGGTTT
204 rLeuHisLeuSerGluSerAlaPheLeuLeuGluIlePheAlaAspIleLeuSerSerValArgTyrLeuGluLeuArgAspThrAsnLeuAlaArgPhe
2401 CAGTTTTCCACTGCCCCGATAGATGAAGTACAGTCCCGATGAAGAAGCTGGCATTCCGAGGCTCGGTTCTCACTGATGAAAGCTTTAACGAGCTCCTGA
238 GlnPheSerProLeuProValAspGluValSerSerProMetLysLysLeuAlaPheArgGlySerValLeuThrAspGluSerPheAsnGluLeuLeuL
2501 AGCTGTTGCTTACATCTTGAACCTGTCGGAGGTAGAGTTTCGACGACTGTACCCTCAATGGGCTCGGCGATTCAACCCCTCGGAGTCAGAGTGTGAG
271 ysLeuLeuArgTyrIleLeuGluLeuSerGluValGluPheAspAspCysThrLeuAsnGlyLeuGlyAspPheAsnPProSerGluSerAspValValSe
2601 CGAGCTGGGTAAGTAGAAACAGTCACTATCCGGAGTTGCATATCCCCAGTCTATTGTTTTATGACCTGAGTACTGTCTATCCCTCCTGGAGAAG
304 rGluLeuGlyLysValGluThrValThrIleArgArgLeuHisIleProGlnPheTyrLeuPheTyrAspLeuSerThrValTyrSerLeuLeuGluLys
EcoRI (2775)
2701 GTGAAGCGAATCACAGTAGAGAACAGCAAGTCTTCTCGTTCCCTGCTGTTCTCCAGCATTAAAAATCATTAGAATTTTAGACCTCAGCGAAAATC
338 ValLysArgIleThrValGluAsnSerLysValPheLeuValProCysSerPheSerGlnHisLeuLysSerLeuGluPheLeuAspLeuSerGluAsnL
SspI (2812)
2801 TGATGGTTGAAGAATTTTGAAGAAGTCTGAGGAGCCTGGCCTTCTTACAAACCTTAGTTTTGAGCCAGAATCATTGAGATCAATGAAAA
371 euMetValGluGlyTyrLeuLysAsnSerAlaCysLysGlyAlaTrpProSerLeuGlnThrLeuValLeuSerGlnAsnHisLeuArgSerMetGlnLys
PshAI (2976)
2901 AACAGGAGAGATTTTGTGACTCTGAAAAACCTGACCTCCCTTGACATCAGCAGGAACACTTTTCATCCGATGCCCGACAGCTGTGAGTGGCCAGAAAAG
404 sThrGlyGluIleLeuLeuThrLeuLysAsnLeuThrSerLeuAspIleSerArgAsnThrPheHisProMetProAspSerCysGlnTrpProGluLys
BamHI (3028)
3001 ATGCGCTTCTGAATTTGTCCAGTACAGGATCCGGGTGGTAAAAACGTCATTCTCAGACGCTGGAGGTGTGGATGTTAGTAACAACAATCTTGACT
438 MetArgPheLeuAsnLeuSerSerThrGlyIleArgValValLysThrCysIleProGlnThrLeuGluValLeuAspValSerAsnAsnAsnLeuAspS

3101 CATTTCCTTTGTTCTTGCCTCGGCTGCAAGAGCTCTATATTTCCAGAAATAAGCTGAAAACACTCCCAGATGCTTCGTTGTTCCCTGTGTTGCTGGTCAT
471 er PheSer LeuPheLeuProArgLeuGlnGluLeuTyrIleSerArgAsnLysLeuLysThrLeuProAspAlaSerLeuPheProValLeuLeuValMe
3201 GAAAATCAGAGAGAATGCAGTAAGTACTTTCTCTAAAGACCAACTTGGTTCTTTCCAAAACCTGGAGACTCTGGAAGCAGGCAGCAACCACTTTGTTGC
504 tLysIleArgGluAsnAlaValSerThrPheSerLysAspGlnLeuGlySerPheProLysLeuGluThrLeuGluAlaGlyAspAsnHisPheValCys
3301 TCCTGCGAACTCCTATCCTTTACTATGAGAGACGCCAGCTCTGGCTCAAATCTGGTTGACTGGCCAGACAGCTACCTGTGTACTCTCCGCTCGCCTGC
538 SerCysGluLeuLeuSerPheThrMetGluThrProAlaLeuAlaGlnIleLeuValAspTrpProAspSerTyrLeuCysAspSerProProArgLeuH
3401 ACGGCCACAGGCTTCAGGATGCCGGCCCTCCGCTTGGAAATGTCACCAGGCTGCACTGGTGTCTGGAGTCTGCTGTGCCCTTCTCTGTGATCTTGTCT
571 isGlyHisArgLeuGlnAspAlaArgProSerValLeuGluCysHisGlnAlaAlaLeuValSerGlyValCysCysAlaLeuLeuLeuLeuLeuLeuLe
3501 CGTAGGTGCCCTGTGCCACCATTTCCACGGACTGTGGTACCTGAGAATGATGTGGCGTGGCTCCAGGCCAAGAGGAAGCCCAAGAAAGCTCCCTGCAGG
604 uValGlyAlaLeuCysHisHisPheHisGlyLeuTrpTyrLeuArgMetMetTrpAlaTrpLeuGlnAlaLysArgLysProLysLysAlaProCysArg
3601 GACGTTTGCTATGATGCCTTTGTTTCTACAGTGAGCAGGATTCCTATTGGTGGAGAACCCTCATGGTCCAGCAGCTGGAGAAGCTGACCCGCCCTTTA
638 AspValCysTyrAspAlaPheValSerTyrSerGluGlnAspSerHisTrpValGluAsnLeuMetValGlnGlnLeuGluAsnSerAspProProPheL
3701 AGCTGTGTCTCCACAAGCGGGACTTCGTTCCGGGCAAATGGATCATTGACAACATCATCGATTCCATCGAAAAGAGCCACAAAACCTGTGTTCTGTGCTTTC
671 ysLeuCysLeuHisLysArgAspPheValProGlyLysTrpIleIleAspAsnIleIleAspSerIleGluLysSerHisLysThrValPheValLeuSe
3801 TGAGAACTTCGTACGGAGCGAGTGGTCAAGTACGAACTGGACTTCTCCACTTCAGGCTCTTTGACGAGAACACGACGCGGCCATCCTGTTTTGCTG
704 rGluAsnPheValArgSerGluTrpCysLysTyrGluLeuAspPheSerHisPheArgLeuPheAspGluAsnAsnAspAlaAlaIleLeuValLeuLeu

FspI (3943)

3901 GAGCCCATTGAGAGGAAAGCCATTCCTCCAGCGCTTCTGCAAACCTGCGCAAGATAATGAACACCAAGACCTACCTGGAGTGGCCCTTGGATGAAGGCCAGC
738 GluProIleGluArgLysAlaIleProGlnArgPheCysLysLeuArgLysIleMetAsnThrLysThrTyrLeuGluTrpProLeuAspGluGlyGlnG

AvrII (4039)

4001 AGGAAGTGTTTTGGGTAATCTGAGAACTGCAATAAAGTCCTAGGTTCTCCACCCAGTTCCTGAGCTAGCTAGCCAGACATGATAAGATACATTGATGAG
771 InGluValPheTrpValAsnLeuArgThrAlaIleLysSer

NheI (4064)

4101 TTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAC

HpaI (4202)

4201 AAGTTAAACAACAACAATTGCATTCAATTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGA

PaeI (4304)

4301 AATGTTAATTAAGTACCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC

4401 CTTTTTTTCTGCGCGTAATCTGCTGCTTGCACAAAAAACCACCGCTACCAGCGGTGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA

4501 GGTAAGTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTAGTGAGCCGTAGTTAGGCCACCACTTCAAGAAGCTGTAGCACCCTACATAC

4601 CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCAGGATAAGGCGCAGC

ApaLI (4722)

4701 GGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCAC

4801 GCTTCCCGAAGGGGAGAAAGCGGACAGGTATCCGTAAGCGGCAGGGTCCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTT

4901 TATAGTCTGTGCGGTTTTGCGCACCTCTGACTTGAAGCTGATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCT

PaeI (5044)

5001 TTTTACGGTTCCTGCCTTTTGTGCTGCTTTTGTCTCACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAAGCTTGGTTAGGTACC

5101 TTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCGAGGCTCCCGCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA

5201 ATTAGTCAGCAACCAGGTGTGGAAGTCCCGAGGCTCCCGCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGT

BssHIII (5309)

NotI (5326)

SmaI (5355)

5301 TCCGCCAGAGCGCGGAGGGCTCCAGCGGCGCCCTCCCCACAGCAGGGGCGGGTCCCGGCCACCAGGAGGAGCGGGCTCGGGCGGGCGGCGC

5401 TGATTGGCCGGGGCGGCTGACGCCGACGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGACGTTCTTCGCCGAAGCTTCCGTCAGAACGCAG

5501 GTGAGGGGCGGTGTGGCTTCCGCGGCGCCGAGCTGGAGTCTGCTCCGAGCGGGCGGGCCCCGCTGTCGTGCGGGGATTAGCTGCGAGCATTCC

5601 CCGCTTCAGTTGCGGGCGGCGGGAGGAGGAGTGCAGGCTAGCGGCAACCCGTAGCCTCGCCTCGTGTCCGGCTTGAAGCCTAGCGTGGTGTCCG

5701 CGCCCGCCGCGTCTACTCCGGCCGACTCTGGTCTTTTTTTTTTTTGTGTTGTTGTCCTGCTGCCTTCGATTGCCGTTACGCAATAGGGGCTAACA

5801 AAGGGAGGTTGCGGGCTTGTCTGCCCGAGCCGGAGAGGTCATGGTTGGGAGGAATGGAGGACAGGAGTGGCGGCTGGGGCCCGCCGCTTCGGA

5901 GCACATGTCCGACGCCACTGGATGGGGGAGGCTGGGTTTTTCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCG

SmaI (6098)

6001 GCACGATCTGGCTTGGCGGCGCGGTTGCCCTGCCCTCCCTAACTAGGGTGAAGCCATCCCGTCCGGCACCAGTTGCGTGCCTGGAAGATGGCCGCTCC

6101 CGGGCCCTGTTGCAAGGAGCTCAAATGGAGGACGCGGCAGCCCGTGGAGCGGGCGGGTGAAGTACCCACACAAAGGAAGGGCTGGTCCCTCACCG

6201 GCTGCTGCTTCTGTGACCCGTGGTCTATCGGCCAATAGTCACCTCGGGCTTTTGTAGCACGGCTAGTCGCGCGGGGGGAGGGATGTAATGGCGT

6301 TGGAGTTTGTTCACATTTGGTGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGGAAGTCATTTTTGGAATTTGTCCCTTGAAGTTTGTAGCGGAGCTAAT

6401 TCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTAAACCCCTTTTATAGTGTGTGAAAACCACCGCTAATTCAAAGCAATCATGGAGCGTGTGCTTGGC
1 Met Gl u Arg Val Leu Gl y

ApaI (6518)

6501 TTGTTGCTGTTGCTTCTGGTGCACGCCTCTCCGCCACCAGAGCCCTGCGAGCTAGACGAGGAAAGTTGTTCTGCAACTTCTCAGATCCGAAGCCAG
7 Leu Leu Leu Leu Leu Val Hi s Al a Ser Pro Al a P ro P ro Gl u P ro Cys Gl u Leu Asp Gl u Gl u Ser Cys Ser Cys Asn Phe Ser Asp P ro Lys P ro A

NotI (6654) XmnI (6668)

6601 ATTGTCACGCGCTTCAATTGTTGGGGCGGCAGATGTGGAATTGTACGGCGGCGCCGAGCCTGGAATACCTTCTAAAGCGTGTGGACACGGAAGC
40 sp Trp Ser Ser Al a Phe Asn Cys Leu Gl y Al a Al a Asp Val Gl u Leu Tyr Gl y Gl y Gl y A rg Ser Leu Gl u Tyr Leu Leu Lys Arg Val Asp Thr Gl u Al
BglIII (6700)

6701 AGATCTGGGGCAGTTCACTGATATTATCAAGTCTCTGTCTTAAAGCGGCTTACGGTGGGGCCGCGCGGATTCTAGTCGGATTCTATTGGAGCCCTG
73 a Asp Leu Gl y Gl n Phe Thr Asp I l e I l e Lys Ser Leu Ser Leu Lys Arg Leu Thr Val A rg Al a Al a Arg I l e P ro Ser Arg I l e Leu Phe Gl y Al a Leu

XhoI (6845)

6801 CGTGTGCTCGGATTCCGGCCTCCAGGAAGTACTCTTGAAGTCTCGAGGTAACCGGCACCGCCGCCACCGCTTCTGGAAGCCACCGGACCCGATC
107 Arg Val Leu Gl y I l e Ser Gl y Leu Gl n Gl u Leu Thr Leu Gl u Asn Leu Gl u Val Thr Gl y Thr Al a P ro P ro Leu Leu Gl u Al a Thr Gl y Pro Asp L

6901 TCAACATCTTGAACCTCCGCAACGTGCTGGGCAACAAGGGATGCCTGGCTCGCAGAACTGCAGCAGTGGCTAAAGCCTGGACTCAAGTACTGAGTAT
140 eu Asn I l e Leu Asn Leu Arg Asn Val Ser Trp Al a Thr Arg Asp Al a Trp Leu Al a Gl u Leu Gl n Gl n Trp Leu Lys P ro Gl y Leu Lys Val Leu Ser I l

7001 TGCCCAAGCACACTCACTCACTTTTCTGCAACAGGTCCGCGTCTTCCCTGCCCTCTCCACCTTAGACCTGTCTGACAATCCTGAATTGGGCGAGAGA
173 e Al a Gl n Al a Hi s Ser Leu Asn Phe Ser Cys Gl u Gl n Val A rg Val Phe P ro Al a Leu Ser Thr Leu Asp Leu Ser Asp Asn P ro Gl u Leu Gl y Gl u Arg

7101 GGACTGATCTCAGCCCTCTGTCCCTCAAGTTCGCCACCCTCAAGTTTTAGCGCTGCGTAACGCGGGGATGGAGACGCCACGCGCGTGTCTCTGCGC
207 Gl y Leu I l e Ser Al a Leu Cys P ro Leu Lys Phe P ro Thr Leu Gl n Val Leu Al a Leu Arg Asn Al a Gl y Met Gl u Thr P ro Ser Gl y Val Cys Ser Al a L

PshAI (7232)

7201 TGGCCGAGCAAGGTCAGCTGCAAGGACTAGACCTTAGTACAATCACTGCGGGATGCTGCAGGCGCTCCGAGTTGTACTGGCCAGTCAGCTAAA
240 eu Al a Al a Arg Val Gl n Leu Gl n Gl y Leu Asp Leu Ser Hi s Asn Ser Leu Arg Asp Al a Al a Gl y Al a P ro Ser Cys Asp Trp P ro Ser Gl n Leu As

7301 CTCGCTCAATCTGTCTTCACTGGGCTGAAGCAGGTACCTAAAGGGCTGCCAGCCAAGCTCAGCGTCTGGATCTCAGTTACAACAGGCTGGATAGGAAC
273 n Ser Leu Asn Leu Ser Phe Thr Gl y Leu Lys Gl n Val I P ro Lys Gl y Leu P ro Al a Lys Leu Ser Val Leu Asp Leu Ser Tyr Asn Arg Leu Asp Arg Asn

7401 CCTAGCCAGATGAGCTGCCCAAGTGGGAACTGTCACTTAAAGGAAATCCCTTTTGGACTCTGAATCCCACTCGGAGAAGTTTAACTCTGGCGTAG
307 P ro Ser P ro Asp Gl u Leu P ro Gl n Val Gl y Asn Leu Ser Leu Lys Gl y Asn P ro Phe Leu Asp Ser Gl u Ser Hi s Ser Gl u Lys Phe Asn Ser Gl y Val V

AvrII (7558)

7501 TCACCGCGGAGCTCCATCATCCCAAGCAGTGGCCTTGTGAGAACTCTGGCTTTGCTCCTAGGAGATCGCCTCTTTGTTTAAAGGAACATTTGCATCCTC
340 a l Thr Al a Gl y Al a P ro Ser Ser Gl n Al a Val Al a Leu Ser Gl y Thr Leu Al a Leu Leu Leu Gl y Asp Arg Leu Phe Val •••

XbaI (7667)

7601 CTGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAAGTCCGCTGGTCTTTCCAGGCTAGAGGGGTAACACTTTGTACTGCGTTTGGC
7701 TCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGTAACAAGGACCCACGG
7801 GGCCAAAAGCCACGCCACAGGGCCGCTCATGTGTGCAACCCAGCACGGCAGCTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCCA
7901 CACACTGGTGACAGGCTAAGGATGCCCTTCAAGTACCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAGCGCTCGG

AseI (8092)

8001 TTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCATGAATAACAACCTGACTGTTTGACAATTAATCA
8101 TCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAACTGAACTGACAGCAACTTCTGTTGAGAAGTTTCTCATT
1 Met Lys Lys P ro Gl u Leu Thr Al a Thr Ser Val Gl u Lys Phe Leu I l e

8201 GAAAAATTTGATTCTGTTTCTGATCTCATGCAGTGTCTGAAGTGAAGAAAGCAGAGCCTTTTCTTTGATGTTGGAGGAAGAGTTATGTTCTGAGGG
17 Gl u Lys Phe Asp Ser Val Ser Asp Leu Met Gl n Leu Ser Gl u Gl y Gl u Gl u Ser Arg Al a Phe Ser Phe Asp Val Gl y Gl y Arg Gl y Tyr Val Leu Arg V

8301 TCAATCTTGTGCTGATGGTTTTTACAAGACAGATATGTTTACAGACACTTTCCTCTGCTGCTCTGCCAATCCAGAAGTTCTGGACATTGGAGAATT
50 a l Asn Ser Cys Al a Asp Gl y Phe Tyr Lys Asp Arg Tyr Val Tyr Arg Hi s Phe Al a Ser Al a Al a Leu P ro I l e P ro Gl u Val Leu Asp I l e Gl y Gl u Ph

8401 TTCTGAATCTCTCACTACTGCATCAGCAGAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCT
83 e Ser Gl u Ser Leu Thr Tyr Cys I l e Ser Arg Arg Al a Gl n Gl y Val Thr Leu Gl n Asp Leu P ro Gl u Leu P ro Al a Val Leu Gl n P ro Val Al a

8501 GAAGCAATGGATGCCATTGACAGCAGTGTCTGAGCCAAACCTCTGGATTTGGTCTTTGGTCCCAAGGCAATGGTCACTGACACACTGGAGGGATT
117 Gl u Al a Met Asp Al a I l e Al a Al a Asp Leu Ser Gl n Thr Ser Gl y Phe Gl y P ro Phe Gl y P ro Gl n Gl y I l e Gl y Gl n Tyr Thr Thr Trp Arg Asp P

8601 TCATTTGTGCCATTGCTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAACTCATGCT
150 he I l e Cys Al a I l e Al a Asp P ro Hi s Val Tyr Hi s Trp Gl n Thr Val Met Asp Asp Thr Val Ser Al a Ser Val Al a Al a Gl n Al a Leu Asp Gl u Leu Met Le

8701 GTGGCAGAAGATTGCTGAAAGTCAGACACTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCATTGAC
183 u Trp Al a Gl u Asp Cys P ro Gl u Val A rg Hi s Leu Val Hi s Al a Asp Phe Gl y Ser Asn Asn Val Leu Thr Asp Asn Gl y A rg I l e Thr Al a Val I l e Asp

8801 TGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTGGCTGGCTGCATGGAACAACAACAAGATATTTTG
217 Trp Ser Gl u Al a Met Phe Gl y Asp Ser Gl n Tyr Gl u Val Al a Asn I l e Phe Phe Trp Arg P ro Trp Leu Al a Cys Met Gl u Gl n Gl n Thr Arg Tyr Phe G

8901 AAAGAAGACACCCAGAACTGGCTGTTCCCCAGACTGAGAGCTACATGCTCAGAAATGGCCTGGACCAACTGTATCAATCTCTGGTTGATGAAACT
250 I u Arg Arg Hi s P ro Gl u Leu Al a Gl y Ser P ro Arg Leu Arg Al a Tyr Met Leu Arg I l e Gl y Leu Asp Gl n Leu Tyr I l e Ser Leu Val Asp Gl y Asn Ph

9001 TGATGATGCTGCTGGGCACAAGGAGATGTGATGCCATTTGAGGCTGGTCTGGAAGTGTGGAAGAACTCAAATGGCAAGAGGCTGCTGCTGTT
283 e Asp Asp Al a Al a Trp Al a Gl n Gl y A rg Cys Asp Al a I l e Val A rg Ser Gl y Al a Gl y Thr Val Gl y A rg Thr Gl n I l e Al a A rg Arg Ser Al a Al a Val

9101 TGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAAACAGGAGACCCTCCACAAGACCCAGAGCCAAGGAATGAATATTAGCTAGATTATCCCTAA
317 TrpThrAspGlyCysVal GluVal LeuAl aAspSer GlyAsnArgArgProSer ThrArgProArgAl aLysGlu●●●

9201 TACCTGCCACCCCACTCTTAATCAGTGGTGGGAAGAACGGTCTCAGAAGCTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGAT

9301 AACAAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAA

9401 AATCAGTACTTTTTAATGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGT

9501 CAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGA
HpaI (9579)

9601 ATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCAT

9701 GTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAAGCTG

