

pDUO2-mMD2/CD14

A plasmid coexpressing the mouse MD2 and CD14 genes

Catalog code: pduo2-mmd2cd14

<https://www.invivogen.com/pduo-md2-cd14>

For research use only

Version 19I24-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO2-mMD2/CD14 provided as 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 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.

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

- **Mouse MD2 (483 bp) / Mouse CD14 (1101 bp)**

MD2 and CD14 are necessary for proper LPS-induced TLR4 signaling. TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). TLR4 alone is not sufficient to confer LPS responsiveness. MD-2 is a secreted molecule that functionally interacts with LPS^{1,2}. TLR4 physically associates with MD2 and CD14 to form the complex 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*.
- **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:

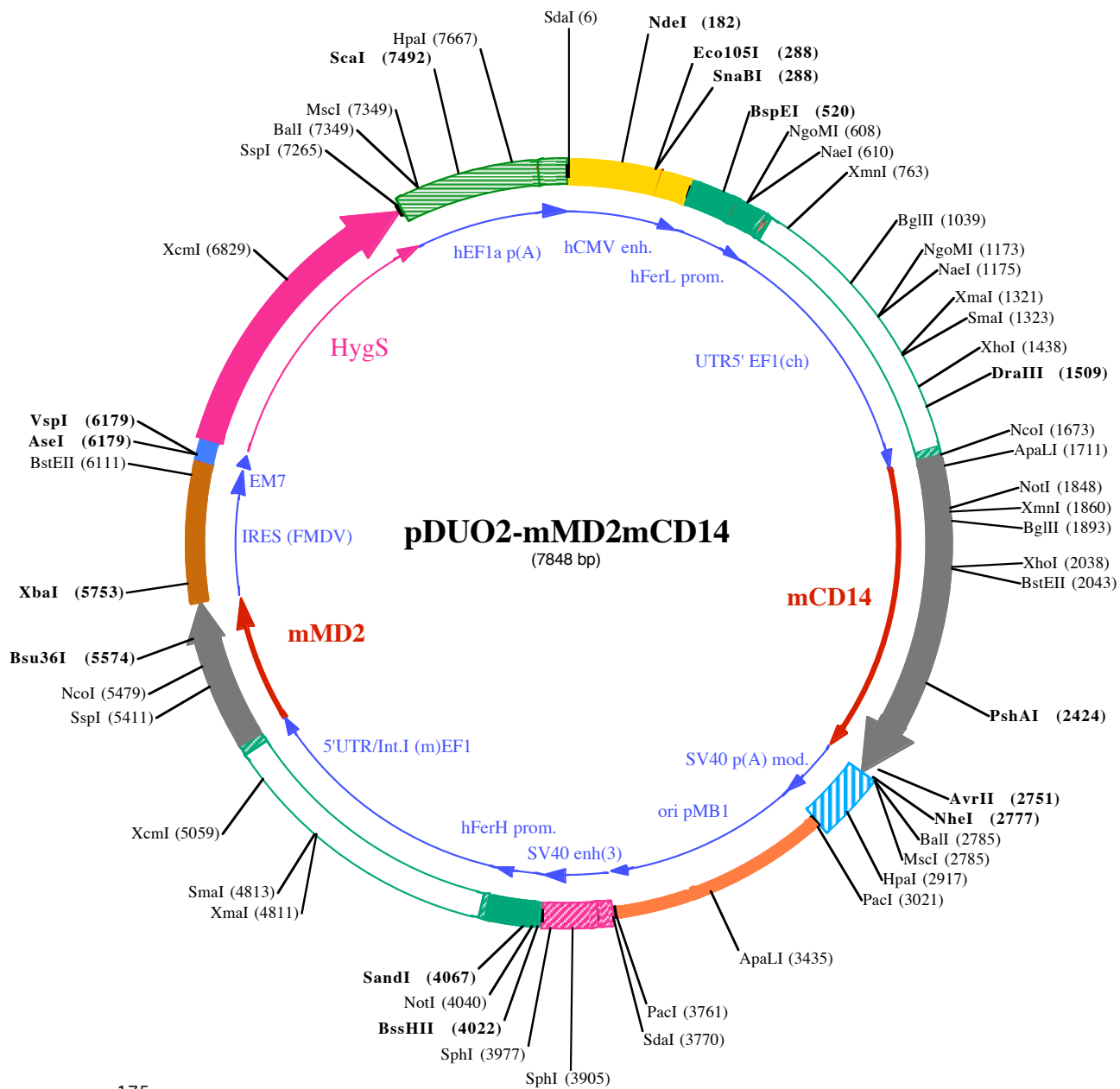
This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

References

1. Shimazu R. *et al.*, 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*, 189(11):1777-82.
2. Nagai Y. *et al.*, 2002. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol*. 3(7):667-72.
3. da Silva Correia J. *et al.*, 2001. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem*. 276(24):21129-35.
4. Eisenstein RS. and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
5. Dean DA. *et al.* 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell Res*. 253:713-22.
6. 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.
7. 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.
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 (6)
1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (182)
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

SnaBI (288)
Eco105I (288)
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAAGTCTCCACCCCATTTGACGTCAATG

401 GGAGTTTTGTTTTGACTAGTCAAGGCCCAACCCCCCAAGCCCATTTACACAACACGCTGGCGCTACAGCGCGTGACTTCCCTTGTCTTGGGGCGGG

BspEI (520)
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCGCCTCTGCCACCAGATTGGCCGCTAGCCTCCCCGAGCGCCCTGCC

NgoMI (608)
NaeI (610)
601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCGCCCTCGCAGTTCGGCGGTCCCGGGTCTGTCTCAAGCTTGCCGCCAGAACAAGg

XmnI (763)
701 taagtccgctgtgtggttcccgccggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgccctggctgcagtacgtgattc

801 ttgatcccagacttccgggttgaagtggtgggagagttcgaggccttgcgcttaaggagcccttcgctcgtgcttgagttgagccctggcttggcg

901 ctggggcccgcgctgctaactctggtggcaccttcgcgctgtctcgtgctttcgctaagtctctagccatttaaaattttgataaccagctgcgacg

BglII (1039)
1001 cttttttctggcgagatagtcttgtaaatgcccgaagatctgcacactggtatttcggtttttggggccgcccggcgagccgggcccgtgcgtccc

NgoMI (1173)
NaeI (1175)
1101 agcgcacatgttcggcgaggccggcctgcgagcgcggccaccgagaatcggacggggtagtctcaaaactggccggcctgctctggtgcctggcctcgc

1201 gccgccgtgtatcggcccccctggcggaagctggcccgtcggcaccagttgcgtgagcggaaagatggccgcttccggccctgctgcagggagc

XmaI (1321)
SmaI (1323)
1301 tcaaaatggaggacgcccggggagagcgggctgagtcacccacacaaggaaaaggccttccctctcatccgtcgtcctcatgtgactcca

XhoI (1438)
1401 cggagtagcgggcccgtccaggcacctcgattagttctcgagcttttggagtacgtcgtctttaggttggggggaggggtttatgcatggagtttcc

DraIII (1509)
1501 ccacactgagtggtggagactgaagagtaggcccagcttggcacttgatgtaattctccttggaaatttgcctttttgagtttgatcttgcctcattc

Neol (1673)
1601 tcaagcctcagacagtggttcaaagtttttctccattcagGTGTCGTGAAAACCTACCCCTAAAAGCCACCATGGAGCGTGTGCTTGGCTTGTGCT
1-MetGI uArgVal LeuGI yLeuLeuLe

ApaLI (1711)
1701 GTTGCTTCTGGTGCACGCCTCTCCCGCCACCAGAGCCCTGCGAGCTAGACGAGAAAGTTGTTCCTGCAACTTCTCAGATCCGAAGCCAGATTGGTCC
9uLeuLeuLeuVal Hi sAl aSer P roAl aP roP roGI uP roCysGI uLeuAspGI uGI uSer CysSer CysAsnPheSer AspP roLysP roAspT rpSer

NotI (1848) XmnI (1860) BglIII (1893)
1801 AGCGCTTTC AATTGTTTGGGGCGGCAGATGTGGAATTGTACGGCGGGCGCCGACGCTTGAATACCTTCTAAAGCGTGTGGACACGGAAAGCAGATCTGG
43Ser Al aPheAsn CysLeuGI yAl aAl aAspVal GI uLeuTyrGI yGI yGI yA rgSer LeuGI uTyrLeuLeuLysArgVal AspThr GI uAl aAspLeuG

1901 GGCAGTTCAC TGA TTTAAGTCTCTGTCTTAAAGCGCTTACGGTGGCGCCGCGCGGATTCTAGTCGGATTCTATTCCGAGCCCTGCGTGTGCT
76I yGI nPheThr AspI l eI l eLysSer LeuSer LeuLysArgLeuThr Val ArgAl aAl aArgI l eP roSer ArgI l eLeuPheGI yAl aLeuArgVal Le

BstEII (2043)
XhoI (2038)
2001 CGGGATTTCCGGCCTCCAGGAAGTACTCTTGAATACTCGAGGTAACCGGCACCGCCGCCACCCTTCTGGAAGCCACGGACCCGATCTCAACATC
109uGI yI l eSer GI yLeuGI nGI uLeuThr LeuGI uAsnLeuGI uVal I Thr GI yThr Al aP roP roP roLeuLeuGI uAl aThr GI yProAspLeuAsnI l e

2101 TTGAACCTCCGCAACGTGCTGGGCAACAAGGATGCCTGGCTCGCAGAAGTGCAGCAGTGGCTAAAGCCTGGACTCAAGGTAAGTATTGCCAAG
143LeuAsnLeuArgAsnVal Ser T rpAl aThr ArgAspAl aT rpLeuAl aGI uLeuGI nGI nT rpLeuLysP roGI yLeuLysVal LeuSer I l eAl aGI nA

2201 CACACTCACTCAACTTTTCTGCGAACAGGTCCGCGTCTCCCTGCCCTCTCCACCTTAGACCTGTCTGACAATCCTGAATTGGGCGAGAGAGGACTGAT
176I aHi sSer LeuAsnPheSer CysGI uGI nVal A rgVal I PheP roAl aLeuSer Thr LeuAspLeuSer AspAsnP roGI uLeuGI yGI uArgGI yLeuI l

2301 CTCAGCCCTCTGCTCCCTCAAGTTCGCCACCTCCAAGTTTTAGCGCTGCGTAACGCGGGGATGGAGACGCCACGCGCGTGTGCTCTGCGCTGGCCCA
209eSer Al aLeuCysP roLeuLysPheP roThr LeuGI nVal LeuAl aLeuArgAsnAl aGI yMetGI uThr P roSer GI yVal CysSer Al aLeuAl aAl a

PshAI (2424)
2401 GCAAGGGTACAGCTGCAAGGACTAGACCTTAGTCACAATTCACCTGCGGGATGTGTCAGGCGCTCCGAGTTGTGACTGGCCAGTCAGCTAAACTCGCTCA
243Al aArgVal GI nLeuGI nGI yLeuAspLeuSer Hi sAsnSer LeuArgAspAl aAl aGI yAl aP roSer CysAspT rpSer GI nLeuAsnSer LeuA

2501 ATCTGTCTTTCAC TGGCGTGAAGCAGGTACCTAAAGGCTGCCAGCCAAAGCTCAGCGTGTGATCTCAGTTACAACAGCTGGATAGGAACCCCTAGCCCA
276snLeuSer PheThr GI yLeuLysGI nVal P roLysGI yLeuP roAl aLysLeuSer Val LeuAspLeuSer TyrAsnArgLeuAspArgAsnP roSer P r

2601 AGATGAGTGC CCAAGTGGGAACTGTCACTTAAAGAAAATCCCTTTTTGACTCTGAATCCCACTCGGAGAAGTTAACTCTGGCGTAGTACCCGCC
309oAspGI uLeuP roGI nVal GI yAsnLeuSer LeuLysGI yAsnP roPheLeuAspSer GI uSer Hi sSer GI uLysPheAsnSer GI yVal Val Thr Al a

MscI (2785)
Ball (2785)
NheI (2777)

AvrII (2751)

2701 GGAGCTCCATCATCCAAGCAGTGGCCTTGTCCAGGAACCTGGCTTTGCTCCTAGGAGATCGCCTCTTTGTTTAAAGCTAGCTGGCAGACATGATAAGA
343▶ GI yAl aP roSer Ser GI nAl aVa lAl aLeuSer GI yThr LeuAl aLeuLeuLeuGI yAspArgLeuPheVal ●●●

2801 TACATTGATGAGTTTGGACAAACCACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAA

HpaI (2917)

2901 GCTGCAATAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAA

PaeI (3021)

3001 ATGTGGTATGAAATGTTAATTAAGTAGCCATGACCAAATCCCTTAACGTGAGTTTTCTTCCACTGAGCGTCAGACCCGTAGAAAAGATCAAAGGAT
▶

3101 CTTCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGCACAAACAAAAACCACCGCTACCAGCGGTGTTTGTTCGCGGATCAAGAGCTACCAAC

3201 TCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCA

3301 CCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGTTGGACTCAAGACGATAGTTACCGG

ApaLI (3435)

3401 ATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGCACACAGCCAGCTTGGAGCGAACGACCTACACGAACTGAGATACCTACAGCGTGAGCTATG

3501 AGAAAAGCGCCACGCTTCCGAAGGGAGAAAAGCGGACAGGTATCCGGTAAAGCGGCAGGGTGGAAACAGGAGCGCACGAGGGAGCTTCCAGGGGAAAC

3601 GCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCA

PaeI (3761) SdaI (3770)

3701 GCAACCGGGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCTTTTGTCCACATGTTCTTAATTAACCTGCAGGGCTGAAATAACCTCTGAAAGAGGAACT
▶

3801 TGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAA

SphI (3905) SphI (3977)

3901 GCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAT

BssHIII (4022) NotI (4040) SmaI (4067)

4001 AGTCCCAGTAGTCCGCCAGAGCGCGGAGGGCCTCCAGCGGCCGCCCTCCCCACAGCAGGGGCGGGTCCCAGCGCCACCGGAAGGAGCGGGCTCGG
▶

4101 GCGGGGCGGCGGTGATTGGCCGGGCGGGCCTGACGCCGACGGGCTATAAGAGACCACAAGCAGCCGAGGCCAGACGTTCTTCGCCAAGCTTGCC

4201 GTCAGAACGCAgTgagggggcgggtgtggttccgcgggcccgcgagctggaggtcctgctccgagcgggcccggcctgctgctgcccggggattag

4301 ctgagcagcattcccgcttcgagttgcgggcccgcgggagcagagtgagggcctagcggcaacccgtagcctgcctcgtgctcggcttgaggccta

4401 gctggtgtcctgcgcccgcggcgtgctactcggcccactctggtctttttttttttgttgttgttgcctgctgcttcgattgccgttcagcaa

4501 taggggctaacaaggagggtgcccgttgcctgcccggagcccggagaggtcatggttgggaggaatggaggacaggagtgccgctggggcccg

4601 cccgcttcggagcacatgtccgacgccacctggatggggcagggcctggggttttccgaagcaaccaggctggggttagcgtgccgagggcattgtgg

4701 cccagcaccggcagatctggcttggcggcggcgttgcctgcctcctaactaggggtgagggcattcccgtccggcaccagttcgtgctggtgaaa

XmaI (4811) SmaI (4813)

4801 gatggcgcctccgggcccctggtgcaaggagctcaaatggaggacgcccagcccgtggagcgggcccgggtgagtcaccacacaaggaagagggccct

4901 ggtccctcaccggctgctgcttctgtgaccccgtggtcctatcgccgcaatagtcacctgggcttttgagcacggctagtcgcccggggggagggg

XcmI (5059)

5001 atgtaatggcgttggagtttgcacatttgggtgggtggagactagtcaggccagcctggcgctggaagtcattttggatttgccttgagttttg

5101 agcggagctaattctcgggcttcttagcggttcaaggatcttttaaaccttttttagGTGTTGTGAAAACACCGTAATCAAAGCAATCATGTTG
▶ MetLeu

5201 CCATTTATCTCTTTTCGACGCTGCTTTCTCCATATTGACTGAATCTGAGAAGCAACAGTGGTTCTGCAACTCTCCGATGCAATTATTTCTACAGTT
▶ ProPheI l eLeuPheSer Thr LeuLeuSer P rol l eLeuThr GI uSer GI uLysGI nGI nTrpPheCysAsnSer SerAspAl a l e l l eSer TyrSer T

5301 ATGTGATCACTTGAATTCCTATTCAATTAGTTCTGAACCTGCATAAGACTGAGGGGAACCAATGGATTGTGCATGTTGAGTTCAATCCAAAGAGG
▶ yrCysAspHisLeuLysPheP rol l eSer l l eSer Ser GI uP roCys l l eArgLeuArgGI yThrAsnGI yPheVal Hi sVal GI uPhe l l eP roArgGI

SspI (5411) NcoI (5479)

5401 AAACCTAAAATATTTATATTTCAACCTATTTCATCAGTGTCAACTCCATAGAGTTGCCAAGCGTAAGGAAGTTCTGTGCCATGGACATGATGACTAT
69▶ yAsnLeuLysTyrLeuTyrPheAsnLeuPhe l l eSer ValAsnSer l l eGI uLeuProLysArgLysGI uVal LeuCysHi sGI yHi sAspAspAspTyr

Bsu36I (5574)

5501 TCTTTTTGCAGAGCTCTGAAAGGAGAGACTGTGAATACATCAATACCATTCTCTTTTCAGGGAATACTATTTCTAAGGGCATTACAGATGTGTTGCAG
103▶ Ser PheCysArgAl aLeuLysGI yGI uThr ValAsnThr Ser l l eP roPheSer PheGI uGI y l l eLeuPheP roLysGI yHi sTyrArgCysValAl aG

5601 AAGCTATTGCTGGGATACTGAAGAAAAGCTCTTCTGTTTGAATTTACCATCATTACCAGCGGTGATGCAATTAGAATATGCTGAGCTAGGAGCAGGT
136▶ l uAl a l eAl aGI yAspThr GI uGI uLysLeuPheCysLeuAsnPheThr l l e l l eHi sArgArgAspValAsn●●●

XbaI (5753)

5701 TTCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTCCAGGCTAGAGGGGTAACACTTTGACTGCGTTTGGCTCCACGCTCGATCCA
5801 CTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGC
5901 CCACACGGGCCCGTCATGTGTGCAACCCAGCACGGGCACTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCACACACTGGTGACAGG
6001 CTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTAAAAAGCTTCTA

BstEII (6111)

VspI (6179)
AseI (6179)

6101 TGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCTATGAATACAACCTGACTGTTTGACAATTAATCATCGGCATAGTATATC
6201 GGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAACTGACAGCAACTTCTGTTGAGAAGTTTCTCATTGAAAAATTTGATTCT
6301 GTTTCGTATCTCATGCACTGTCTGAAGGTGAAGAAAGCAGAGCCTTTCTTTTGGATGTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATTTCTGTGCTG
6401 ATGGTTTTTACAAAGACAGATATGTTTACAGACACTTTGCCTCTGCTGCTGCCAATTCAGAGTTCTGGACATTGGAGAATTTCTGAATCTCTCAC
6501 CTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCC
6601 ATTGACAGCAGCTGATCTGAGCCAAACCTCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCAGTACACCATTGGAGGGATTTCAATTTGTGCCATTG
6701 CTGATCCTCATGTCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAACTCATGCTGTGGCAGAAAGATTG
6801 TCCTGAAGTCAGACACCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATG

XcmI (6829)

6801 188 PheGlyAspSerGlnTyrGluValAlaAsnIlePhePheTrpArgProTrpLeuAlaCysMetGluGlnThrArgTyrPheGluArgArgHisProG
6901 222 PheGlyAspSerGlnTyrGluValAlaAsnIlePhePheTrpArgProTrpLeuAlaCysMetGluGlnThrArgTyrPheGluArgArgHisProG
7001 255 IuLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArgIleGlyLeuAspGlnLeuTyrGlnSerLeuValAspGlyAsnPheAspAlaAlaTr
7101 288 pAlaGlnGlyArgCysAspAlaIleValArgSerGlyAlaGlyThrValGlyArgThrGlnIleAlaArgArgSerAlaAlaValTrpThrAspGlyCys

SspI (7265)

7201 322 ValGluValLeuAlaAspSerGlyAsnArgArgProSerThrArgProArgAlaLysGlu...

MscI (7349)

BalI (7349)

7301 TCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAA

ScaI (7492)

7401 AACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTA
7501 ATGGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGGAGCCATTAAGTTAAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATT

HpaI (7667)

7601 TAGGTGAAAGACATCTAATCTGGTTTTACGAATCTGAAACTCTTGAAATGTAATCTTGAGTTAACACTCTGGGTGGAGAATAGGGTTGTTTTCC
7701 CCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTG
7801 TCACTAAAACAGGCCAAAACCTGAGTCCTGGGTTGCATAGAAAGCTG