

pDUO-mMD2/TLR4

A plasmid coexpressing the murine MD2 and TLR4 genes

Catalog code: pduo-mmd2tlr4

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

For research use only

Version 20H26-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-mMD2/TLR4 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

- **Murine MD2** (480 bp) / **Murine TLR4** (2505 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.

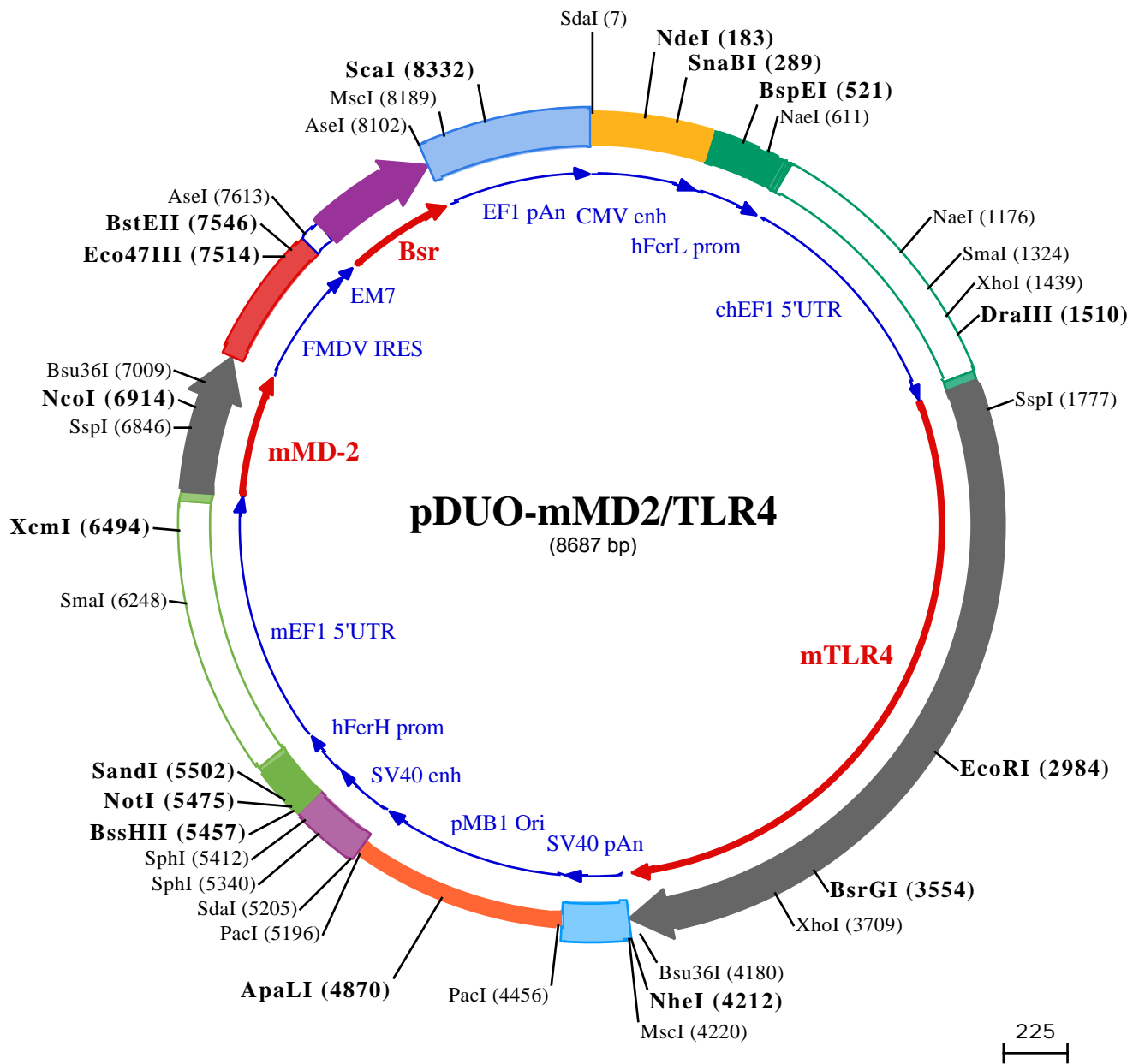
References

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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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SdaI (7)
1 CCTGCAGGCGTTACATAAATTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (183)
101 CGCCAATAGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

SnaBI (289)
201 TATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGGGTTTTGGCAGTACATCAATGGCGGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATG
401 GGAGTTTGTTTTGACTAGTCAGGGCCCCAACCCCAAGCCCCATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCTTGCTTTGGGGCGGG

BspEI (521)
501 GGGCTGAGACTCTATGTGCTCCGATTGGTCAGGCACGGCCTTCGGCCCCGCTCTGCCACCGCAGATTGGCCGTAGGCCTCCCGAGCGCCCTGCC

NaeI (611)
601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCTCCGAGTTCCGGCGGTCCCGGGTCTGTCTCAAGTTGCCCCAGAACACAGg
701 taagtgccgtgtgtggttcccggggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgccctggctgcagtacgtgatc
801 ttgatcccagactcgggttgggaagtgggtgggagagtgcaggccttgcgttaaggagcccttgcctcgtgcttgagtgaggcctggcttgggagc
901 ctggggccgccgctgctaactctggtggcaccttcgcgctgtctcgtgcttctcgttaagtctctagccatttaaaatTTTTGATAACCAGCTGCGAGC
1001 cttttttctggcgagatagcttgtataatggggccaagatctgcacactggtatcttgggttttggggccggggcgagggggcccgtgcgtccc

NaeI (1176)
1101 agcgcacatgttcggcgaggcggggcctgcgagcggccaccgagaatcggacggggtagtctcaaacggccgctgctcgtgctggcctggcctcgc
1201 gccgctgtatcggccgctggggcggaaggctggcccgtcggcaccagtgcgtgagcggaagatggccgcttcccggcctgctgcaggagc

SmaI (1324)
1301 tcaaaatggaggacgcgggcgggagagcggggcgggtgagt caccacacaaaggaaaaggcccttctctcctcatcctcgcttcatgtgactcca

XhoI (1439)
1401 cggagtaccgggcccgtccaggcacctcgattagttctcgagctttggagtagctcgtcttaggttggggggagggttttatcgatggagttccc

DraIII (1510)
1501 ccacactgagtgggtggagactgaagagttaggccacttggcacttgatgtaattccttggaaatggccttttgagttggatcttgcctcatctc
1601 tcaagcctcagacagtggttcaaagtTTTTTcttccatttcagGTGTCGTGAAACTACCCTAAAAGCCACCATGATGCCTCCTGGCTCCTGGCTAG

MetMetProTrpLeuLeuAlaAr
SspI (1777)
1701 GACTCTGATCATGGCACTGTTCTTCTCCTGCCTGACACCAGGAAGCTTGAATCCCTGCATAGAGGTAGTTCCTAATATTACCTACCAATGCATGGATCAG
9>gThrLeuI leMetAlaLeuPhePheSerCysLeuThrProGlySerLeuAsnProCysI leGluValValProAsnI leThrTyrGlnCysMetAspGln
1801 AAACTCAGCAAAGTCCCTGATGACATTCCTTCTCAACCAAGAACATAGATCTGAGCTTCAACCCCTGAAGATCTAAAAAGCTATAGCTTCTCAATT
43>LysLeuSerLysValProAspAspI leProSerThrLysAsnI leAspLeuSerPheAsnProLeuLysI leLeuLysSerTyrSerPheSerAsnP
1901 TTTCAGAACTTCAGTGGCTGATTTATCCAGGTGTAATTTGAAACAATTTAAGACAAGGCATGGCATGGCTTACACCACCTCTCAAACCTGATACTGAC
76>heSerGluLeuGlnTrpLeuAspLeuSerArgCysGluI leGluThrI leGluAspLysAlaTrpHisGlyLeuHisHisLeuSerAsnLeuI leLeuTh
2001 AGGAAACCTATCCAGAGTTTTTCCCGAGGAAGTTCTCTGACTAACAAGTTAGAGAATCTGGTGGCTGTGGAGACAAAATTGGCCTCTAGAAAAGC
109>rGlyAsnProI leGlnSerPheSerProGlySerPheSerGlyLeuThrSerLeuGluAsnLeuValAlaValGluThrLysLeuAlaSerLeuGluSer
2101 TTCCCTATTGGACAGCTTATAACCTTAAAGAAACTCAATGTGGCTCACAAATTTATACATTCCTGTAAGTTACCTGCATATTTTCCAATCTGACGAACC
143>PheProI leGlyGlnLeuI leThrLeuLysLysLeuAsnValAlaHisAsnPheI leHisSerCysLysLeuProAlaTyrPheSerAsnLeuThrAsnL
2201 TAGTACATGTGATCTTTCTTATACTATATTCAAACCTTACTGTCAACGACTTACAGTTTCTACGTGAAAATCCACAAGTCAATCTCTCTTTAGACAT
176>euValHisValAspLeuSerTyrAsnTyrI leGlnThrI leThrValAsnAspLeuGlnPheLeuArgGluAsnProGlnValAsnLeuSerLeuAspI I
2301 ATCTTTGAACCAATGACTTCATTAAGACCAAGCCTTTCAGGAAATTAAGCTCCATGAAGTACTCTAAGAGGTAATTTAATAGCTCAAATATAATG
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2501 TGGAAAGACTATGTGATGTGACCATTTGATGAGTTCAGGTTAACATATACAAATGATTTTTCAGATGATATTGTTAAGTTCATTTGCTTGGCGAATGTTTC
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2601 TGCAATGTCTCTGGCAGGTGTATCTATAAAATATCTAGAAGATGTTCCCTAAACATTTCAAATGGCAATCCTTATCAATCATTAGATGTCAACTAAGCAG
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2701 TTTCAACTCTGGATCTACCTTTCTTAAAAAGTTGACTTTAACTATGAACAAGGCTATCAGTTTTAAAAAGTGGCCCTACCAAGTCTCAGCTATC
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EcoRI (2984)
2901 CATCATTATGAGTGCCAATTTTCATGGGTCTAGAAGAGCTGCAGCACCTGGATTTTTCAGCACTCTACTTTAAAAAGGGTCCACAGAATTTCTCAGCGTTCTTA
409>al leI leMetSerAlaAsnPheMetGlyLeuGluGluLeuGlnHisLeuAspPheGlnHisSerThrLeuLysArgValThrLeuPheSerAlaPheLeu
3001 TCCCTTGAAGAGTACTTTACCTTGACATCTTATACTAACACCAAAATGACTTCGATGGTATATTTCTTGGCTTGACCAGTCTCAACACATTAATAAA
443>SerLeuGluLysLeuLeuTyrLeuAspI leSerTyrThrAsnThrLysI leAspPheAspGlyI lePheLeuGlyLeuThrSerLeuAsnThrLeuLysM
3101 TGCTGGCAATTTCTTCAAAGACAACACCCTTCAAATGCTTTGCAACACAAACAACTTGACATTCCTGGATCTTTCTAAATGTCAATTTGAACAATA
476>etAlaGlyAsnSerPheLysAspAsnThrLeuSerAsnValPheAlaAsnThrThrAsnLeuThrPheLeuAspLeuSerLysCysGlnLeuGluGlnI I
3201 ATCTTGGGGGTATTGTGACACCTCCATAGACTTCAATTAATAATATGAGTCAACAACATCTATTGTTTTGGATTCCCTCCATTAACCGCTGAT
509>eSerTrpGlyValPheAspThrLeuHisArgLeuGlnLeuLeuAsnMetSerHisAsnAsnLeuLeuPheLeuAspSerSerHisTyrAsnGlnLeuTyr

3301 TCCCTCAGCACTTTGATTGCAGTTTCAATCGCATAGAGACATCTAAAGGAATACTGCAACATTTTCCAAAGAGTCTAGCCTTCTCAATCTTACTAATA
543▶ SerLeuSerThrLeuAspCysSerPheAsnArgI leGluThrSerLysGlyI leLeuGlnHisPheProLysSerLeuAlaPhePheAsnLeuThrAsnA
3401 ATTCTGTTGCTGTATATGTGAACATCAGAAATTCCTGCAGTGGTCAAGGACCAGAAGCAGTTCTTGGTGAATGTTGAACAAATGACATGTGCAACACC
576▶ snSerValAlaCysI leCysGluHisGlnLysPheLeuGlnTrpValLysAspGlnLysGlnPheLeuValAsnValGluGlnMetThrCysAlaThrPr
BsrGI (3554)
3501 TGTAGAGATGAATACCTCCTTAGTGTGGATTTTAATAATTCTACCTGTTATATGTACAAGACAATCATCAGTGTGTGTCAGTGGTCAAGTGTGTTGGTA
609▶ oValGluMetAsnThrSerLeuValLeuAspPheAsnAsnSerThrCysTyrMetTyrLysThrI leI leSerValSerValValSerVal I leValVal
3601 TCCACTGTAGCATTTCTGATATACCCTTCTATTTTCACCTGATACTTATTGCTGGCTGTAAAAAGTACAGCAGAGGAGAAAAGCATCTATGATGCATTGG
643▶ SerThrValAlaPheLeu IeTyrHisPheTyrPheHisLeuI leLeuI leAlaGlyCysLysLysTyrSerArgGlyGluSerI leTyrAspAlaPheV
XhoI (3709)
3701 TGATCTACTCGAGTCAGAATGAGGACTGGGTGAGAAATGAGCTGGTAAAGAATTTAGAAGAAGGAGTGCCCCGCTTTCACCTCTGCCTTCACTACAGAGA
676▶ al I leTyrSerSerGlnAsnGluAspTrpValArgAsnGluLeuValLysAsnLeuGluGluGlyValProArgPheHisLeuCysLeuHisTyrArgAs
3801 CTTTATTCTGGTGTAGCCATTGCTGCCAATATCATCCAGGAAGGCTCCACAAGAGCCGGAAGGTTATTGTGGTAGTGTCTAGACACTTTATTTCAGAGC
709▶ pPheI leProGlyValAlaI leAlaAlaAsnI leI leGlnGluGlyPheHisLysSerArgLysVal I leValValSerArgHisPheI leGlnSer
3901 CGTTGGTGTATCTTTGAATATGAGATTGCTCAAACATGGCAGTTTCTGAGCAGCCACTCTGGCATCATCTTCATTGCTCTTGAGAAGGTTGAGAAGTCCC
743▶ ArgTrpCysI lePheGluTyrGluI leAlaGlnThrTrpGlnPheLeuSerSerHisSerGlyI leI lePheI leValLeuGluLysValGluLysSerL
4001 TGCTGAGGCAGCAGGTGGAATGTATCGCCTTCTTAGCAGAAACACCTACCTGGAATGGGAGGACAATCCTCTGGGAGGCACATCTTCTGGAGAAGACT
776▶ euLeuArgGlnGlnValGluLeuTyrArgLeuLeuSerArgAsnThrTyrLeuGluTrpGluAspAsnProLeuGlyArgHisI lePheTrpArgArgLe
Bsu36I (4180)
4101 TAAAAATGCCCTATTGGATGGAAAAGCCTCGAATCTGAGCAAACAGCAGAGGAAGAACAAGAAACGGCAACTTGGACCTGAGGAGAACAAAACCTCTGGG
809▶ uLysAsnAlaLeuLeuAspGlyLysAlaSerAsnProGluGlnThrAlaGluGluGluGlnGluThrAlaThrTrpThr•••
MscI (4220)
NheI (4212)
4201 GCCTAAACCCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAACCACAACCTAGAATGCAGTGAAAAAATGCCTTTATTTGTGAAATT
4301 TGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAACAACAATTGCATTCATTTTTATGTTTCAGGTTTCAGGGGAGGTGT
PacI (4456)
4401 GGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGAAATGTTAATTAAGTACCATGACAAAATCCTTAACGTGAGTTTTTCGTTCCAC
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4601 CCGTGGTTTTGTTTCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCC
4701 GTAGTTAGGCCACCCTTCAAGAAGCTGTAGCACCCTACATACCTCGCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTGT
ApaI (4870)
4801 CTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTGGAGCGAACGACCT
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5001 AGGAGAGCGCAGGAGGAGCTTCCAGGGGAAACCGCTGGTATCTTTATAGTCTGTGCGGTTTCCGACCTCTGACTTGAGCGTGGATTTTTGTGATGC
PacI (5196) SdaI (5205)
5101 TCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACCGGCCCTTTTTACGGTTCCTGGCCTTTTGTGCTCACATGTTCTTAATTAACC
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SphI (5340)
5301 TCCCCAGGCTCCCCAGCAGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGCAGAAGTA
SphI (5412) BssHIII (5457) NotI (5475)
5401 TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCGCCAGAGCGCGGAGGGCCTCCAGCGGCCCCCTCCCCACAGCAGGGGC
SandI (5502)
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SmaI (6248)
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6301 gcggtgagtcacccacaaaggaagggcctggtccctcaccggctgctgcttctgtgaccccggtgctcctatcgccgcaatagtcacctcgggc
XcmI (6494)
6401 tttttagcacggctagtcgcggggggggagggtgtaatggcgttggagttgttccatcttgggtgggtggagactagtcaggccagcctggcgctgg
6501 aagtcatttttggaaattgtccccttgagttttgagcggagctaatctcgggcttcttagcggttcaaaggtatcttttaacccttttttagCTGTTG

6601 **TGAAAACCACCGTAATTCAAAGCAAT**CATGTTGCCATTTATTCTCTTTTCGACGCTGCTTTCTCCCATATTGACTGAATCTGAGAAGCAACAGTGGTTC
MetLeuProPheI leLeuPheSerThrLeuLeuSerProl leLeuThrGluSerGluLysGlnGlnTrpPhe
6701 TGCAACTCCTCCGATGCAATTTTCTACAGTTATTGTGATCACTTGAAATTCCTATTTCAATTAGTTCTGAACCTGCATAAGACTGAGGGGAACCA
25 CysAsnSerSerAspAlaI leI leSerTyrSerTyrCysAspHisLeuLysPheProl leSerI leSerSerGluProCysI leArgLeuArgGlyThrA
SspI (6846)
6801 ATGGATTTGTGCATGTTGAGTTCATTCCAAGAGGAAACTTAAAAATTTTATATTTTCAACCTATTTCATCAGTGTCAACTCCATAGAGTTGCCGAAGCGTAA
58 snGlyPheValHisValGluPheI leProArgGlyAsnLeuLysTyrLeuTyrPheAsnLeuPheI leSerValAsnSerI leGluLeuProLysArgLy
NeoI (6914)
6901 GGAAGTTCTGTGCCATGGACATGATGACTATTCTTTTTGCAGAGCTGTGAAAGGAGAGACTGTGAATACATCAATACCATTCTTTTCGAGGGAATA
91 sGluValLeuCysHisGlyHisAspAspAspTyrSerPheCysArgAlaLeuLysGlyGluThrValAsnThrSerI leProPheSerPheGluGlyI le
Bsu36I (7009)
7001 CTATTTCCTAAGGGCCATTACAGATGTGTTGCAGAAGCTATTGCTGGGGATACTGAAGAAAAGCTCTTCTGTTTGAATTTACCACATATTCACCGCCGTC
125 LeuPheProLysGlyHisTyrArgCysValAlaGluAlaI leAlaGlyAspThrGluGluLysLeuPheCysLeuAsnPheThrI leI leHisArgArgA
7101 ATGTCAATTAGAATATGCTGAGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTTCCAGGTCTAGAGGGTAAC
158 spValAsn•••
7201 **ACTTTGACTCGCTTTGGCTCCACGCTCGATCCACTGGCGAGTGTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCTCT**
7301 **TGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCGTCATGTGTGCAACCCAGCAGCGGACTTTACTGCGAAACCCACTTTAAAGTG**
7401 **ACATTGAAACTGGTACCCACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGG**
Eco47III (7514) BstEII (7546)
7501 **CTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGGTCGGCACCTTTTCTTTGCAATTACTGACCCTATGAATACACTGA**
AseI (7613)
7601 **CTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGAT**
MetLysThrPheAsnI leSerGlnGlnAsp
7701 CTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCA
11 LeuGluLeuValGluValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI
7801 TCTCTGCTGTCCACATTGAGGCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGA
44 leSerAlaValHisI leGluAlaTyrI leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAs
7901 CACCATTGTGGCTGTGAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTAT
77 pThrI leValAlaValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyr
AseI (8102)
8001 GCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGGAACCTATCCCCCTCAAGTACACCAGGAACATAACCTGAA
111 AlaProAspCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgAsn•••
MscI (8189)
8101 **TTAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACTTAAATCAGTGGTGAAGAACGGTCTCAGAAGCTGTTTGTTCAAATGGCCATTTAAGTTT**
8201 **AGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGG**
ScaI (8332)
8301 **CAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAAACAACCTTGACCAAAAATTTGTACAGAATTTTGAGACCCATTAAAAAAGTTAAAT**
8401 **GAGAAACCTGTGTGTTTCTTTGGTCAACACCCGAGACATTTAGTGAAAGACATCTAATTTCTGGTTTTACGAATCTGGAAACTTCTTGAAATGTAATTCT**
8501 **TGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTTCTTTTGATTA**
8601 **CAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG**