

# pDUO-mMD1/RP105

A plasmid coexpressing the murine MD1 and RP105 genes

Catalog code: pduo-mmd1rp105

<https://www.invivogen.com/pduo-md1-rp105>

For research use only

Version 20H26-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pDUO-mMD1/RP105 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 MD1** (486 bp) / **Murine RP105** (1983 bp)

MD1 and RP105 (CD180) are physically associated with each other and are involved in the response to LPS. RP105 is a TLR-related protein, that acts as both an LPS sensor and a regulator of B cell proliferation<sup>1</sup>. Stable expression of MD-1 was shown to induce an increase in cell surface RP105 on a cell line that expresses RP105 alone, suggesting that MD1 is important for efficient cell surface expression of RP105<sup>2</sup>. When induced by LPS, the RP105/MD1 complex regulates B-cell proliferation, antibody production, and B7.2/CD86 up-regulation<sup>3</sup>.

- **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>4</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>5</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>6</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>7</sup>

- **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 expression<sup>8</sup>.

## 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 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

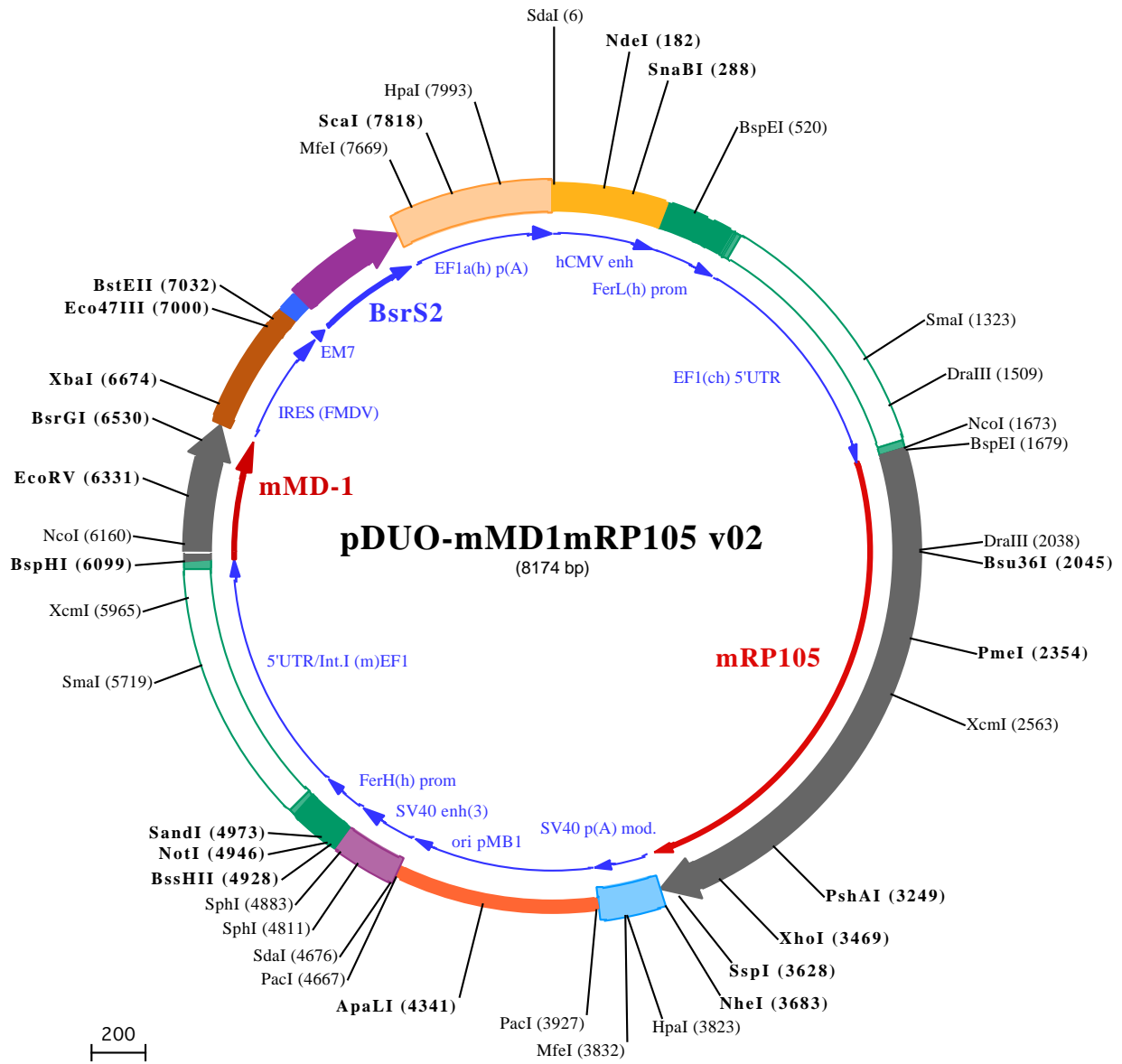
1. Miyake K. *et al.*, 2000. Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1. *J Endotoxin Res*, 6(5):389-91.
2. Miyake K. *et al.*, 1998. Mouse MD-1, a molecule that is physically associated with RP105 and positively regulates its expression. *J Immunol*, 161(3):1348-53.
3. Nagai Y. *et al.*, 2002. Requirement for MD-1 in cell surface expression of RP105/CD180 and B-cell responsiveness to lipopolysaccharide. *Blood*, 99(5):1699-705.
4. Eisenstein RS. & 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. & 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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SmaI (6)  
1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (182)  
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCC

SnaBI (288)  
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA CTACGCGGGATTTC AAGTCTCCACCCATTGACGTCAATG  
401 GGAGTTTGTTTGACTAGTCAGGGCCCAACCCCAAGCCCCATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCTTGTCTTGGGCGGG

BspEI (520)  
501 GGGCTGAGACTCCTATGTGCTCCGATTGGTCAGGCACGGCCTTCGGCCCGCTCCTGCCACGCAGATTGCCGCTAGGCCCTCCCAGCGCCCTGCC  
601 TCCGAGGGCCGGCGACCATAAAGAAGCGCCCTAGCCACGTCCCTCGAGTTCCGGCGTCCCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGg  
701 taagtgcggtgtgtggttccgcggcctggcctctttacgggttatggccttgctgctgaattacttccatgccccctggctgagtaacgtgattc  
801 ttgatcccagcttccgggttgaagtgggtgggagattcgaggccttgcgcttaaggagccccctgcctcgcttgagttgaggcctggctgggag  
901 ctggggccgcccgtgtaacttggtggcaccttgcgcctgtctgctgctttcgtaagtcttagccatttaaaatgataaccagctgagacg  
1001 cttttttctggcgagatagtcttgaatgcggccagatctgcacactggtatctcggttttggggccgcccggcgacggggcccgtgctccc  
1101 agcgacatgcttggcgaggggggctgagcgccgaccgagaatcggaggggtagtctcaaaactggcggcctgctctggtgctggcctgc  
1201 gccgctgtatcgccccctggcggaaggctggccggctggcaccagttgctgagcggaagatggcgccttccggcctgctgagggagc

SmaI (1323)  
1301 tcaaatggaggacggcgcccgggagagcgggctgagtcaccacacaaaggaaaggccttctctcatcctgcttcatgtgactcca  
1401 cggagtaacggcgccgtccaggcacctcgattagttgtcgagcttttgagtagctgctttaggttgggggaggggtttatgcatggagtttcc

DraIII (1509)  
1501 ccacactgagtggtggagactgaagagtaggcccagcttggcacttgatgtaattctccttggatttgcccttttgagttggatcttgcctcattc

BspEI (1679)  
1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAAC TACCCTAAAAGCCACCATGGCTCCGGACATCAGCTGCTTCTT

NcoI (1673)  
1701 TTTAGTGGCCCTCTTCTGGCCAGTTGCCAGCCACCACATCCTCAGATCAGAAGTGCATTGAGAAAGAGTCAACAAAACATACTGTGAAAATTTA  
9 eLeuValAlaLeuPheLeuAlaSerCysArgAlaThrThrSerSerAspGlnLysCysIeGluLysGluValAsnLysThrTyrAsnCysGluAsnLeu  
1801 GGTCTCAATGAAATTCCTGGCACTTTACCAAACCTCAACAGAATGTTTGGAGTTCAGCTTTAATGTCTTGCTACCATTCAAACACGACCTTCCAGCAGAC  
43 GlyLeuAsnGluIeProGlyThrLeuProAsnSerThrGluCysLeuGluPheSerPheAsnValLeuProThrIeGlnAsnThrThrPheSerArgL  
1901 TTATCAATCTCACCTTCTGGATTTAACAGGTGCCAGATTTACTGGATACATGAAGATACTTTCAAAGCCAACATCGGTTAGACACACTTGTACTAAC  
76 eulIeAsnLeuThrPheLeuAspLeuThrArgCysGlnIeTyrTrpIeHisGluAspThrPheGlnSerGlnHisArgLeuAspThrLeuValLeuTh

DraIII (2038) Bsu36I (2045)  
2001 CGCAATCCCCTGATATTTATGGCAGAGACAGCACTTAGTGGCCTAAGGCATTGAAGCATCTGTTCTTCATCCAAACAGGAATATCCAGTATTGATTT  
109 rAlaAsnProLeuIePheMetAlaGluThrAlaLeuSerGlyProLysAlaLeuLysHisLeuPhePheIeGlnThrGlyIeSerSerIeAspPhe  
2101 ATCCACTGCACAATCAAAAACTTGGAAAGTCTCTATCTCGGAAGCAACCATATTTCTCCATTAAGCTCCCAAGGTTTCCCAACAGAGAAGCTGA  
143 IeProLeuHisAsnGlnLysThrLeuGluSerLeuTyrLeuGlySerAsnHisIeSerSerIeLysLeuProLysGlyPheProThrGluLysLeuL  
2201 AGGTCCTGGATTTTCAATAATGCTATCATTACCTGCTAAAGAAGATATGAGCTCTCTCCAGCAAGCCACTAATCTGAGCCTTAACTTAAATGGAAA  
176 ysValLeuAspPheGlnAsnAsnAlaIeHisTyrLeuSerLysGluAspMetSerSerLeuGlnGlnAlaThrAsnLeuSerLeuAsnLeuAsnGlyAs

PmeI (2354)  
2301 TGACATTGCAGGAATAGAGCCGGGGCTTTCGACTCAGCTGTCTTCCAAAGTTAACTTTGGAGGGACTCAGAAGTCTGCTAGTTATCTTCAAGGTTTG  
209 nAspIeAlaGlyIeGluProGlyAlaPheAspSerAlaValPheGlnSerLeuAsnPheGlyGlyThrGlnAsnLeuLeuValIePheLysGlyLeu  
2401 AAGAAGTCTACGATCCAGTCTCTTGGCTGGGACATTTGAGGACATGGATGACGAAGATATTAGTCTGCCGTGTTGAGGGTCTCTGTGAAATGTCTG  
243 LysAsnSerThrIeGlnSerLeuTrpLeuGlyThrPheGluAspMetAspAspGluAspIeSerProAlaValPheGluGlyLeuCysGluMetSerV

XcmI (2563)  
2501 TGGAGAGCATCAACCTACAGAAGCATTATTTCTTCAACATTTCTCCAACACATTCATTGCTTCAAGTGGCCTCCAGGAAGTGGACCTAACAGCCACTCA  
276 aIeGluSerIeAsnLeuGlnLysHisTyrPhePheAsnIeSerSerAsnThrPheHisPheSerGlyLeuGlnGluLeuAspLeuThrAlaThrHi  
2601 CCTGAGTGAATTCCTTCTGGACTTGTGGACTAAGCACACTTAAGAAATTAGTTCTCAGTGCAATAAGTTTGGAGAATTTGTCCAAATCAGTGCTTCC  
309 sLeuSerGluLeuProSerGlyLeuValGlyLeuSerThrLeuLysLysLeuValLeuSerAlaAsnLysPheGluAsnLeuCysGlnIeSerAlaSer  
2701 AATTTCCCTCCCTTACTCACCTTTCCATCAAGGGCAACACGAAAGACTTGAAGTCTGGTACTGGCTGTTTGAAGAACTAGAAAATCTCCGCGAAGTTG  
343 AsnPheProSerLeuThrHisLeuSerIeLysGlyAsnThrLysArgLeuGluLeuGlyThrGlyCysLeuGluAsnLeuGluAsnLeuArgGluLeuA  
2801 ACCTCAGCCATGATGACATTGAACTTCTGATTGTTGCAACCTGCAACTCCGAAACCTGTCTCACTTACAGAGCCTGAACCTTGAAGTCAACATGAACCCCT  
376 spLeuSerHisAspAspIeGluThrSerAspCysCysAsnLeuGlnLeuArgAsnLeuSerHisLeuGlnSerLeuAsnLeuSerTyrAsnGluProLe  
2901 GAGCCTCAAGACGGAGGCAATCAAGAATGCCCTCAGCTAGAACTCCTAGATTTGGCATTACTCGACTAAAGGTGAAGATGCACAGAGTCCCTTCCAG  
409 uSerLeuLysThrGluAlaPheLysGluCysProGlnLeuGluLeuLeuAspLeuAlaPheThrArgLeuLysValLysAspAlaGlnSerProPheGln  
3001 AACCTCCATCTTTGAAGTGTGAATCTCTCCACAGCCTCTTGACATCAGCAGTGGAGCAGCTCTCGATGGCCTGCCAGCACTCCAGACTTTGAAT  
443 AsnLeuHisLeuLysValLeuAsnLeuSerHisSerLeuLeuAspIeSerSerGluGlnLeuPheAspGlyLeuPheAlaLeuGlnHisLeuAsnL  
3101 TACAGGGAAATCACTTTCAAAGGGAATATCAAAGACCAACTCACTTCCAGACACTGGGAAGACTAGAAATCCTGGTTTTATCTTTTGTGATCTCTC  
476 euGlnGlyAsnHisPheProLysGlyAsnIeGlnLysThrAsnSerLeuGlnThrLeuGlyArgLeuGluIeLeuValLeuSerPheCysAspLeuSe

**PshAI (3249)**

3201 CTCCATTGACCAGCACGCCTTACCAGTCTGAAGATGATGAATCATGTAGACCTGAGTCACAACAGGCTGACATCCAGTAGCATTGAGGCTCTTAGTCAT  
509▶ rSerI leAspGlnHisAlaPheThrSerLeuLysMetMetAsnHisValAspLeuSerHisAsnArgLeuThrSerSerSerI leGluAlaLeuSerHis  
3301 CTTAAGGGGATCTACCTCAATCTGGCCTCCAATCACATCAGCATCATCTACCAGTCTCTCCCATCTTGTCCCAGCAGAGGACCATTAATTTAAGAC  
543▶ LeuLysGlyI leTyrLeuAsnLeuAlaSerAsnHisI leSerI leI leLeuProSerLeuLeuProl leLeuSerGlnGlnArgThrI leAsnLeuArgG

**XhoI (3469)**

3401 AAAATCCCCTTGACTGCACTTGTCTCAAACTTTACTTTTTAGAAATGGTACAAAAGAAAACATGCAAAAACCTCGAGGACACAGAGGACACTCTTTGCGAAAA  
576▶ InAsnProLeuAspCysThrCysSerAsnI leTyrPheLeuGluTrpTyrLysGluAsnMetGlnLysLeuGluAspThrGluAspThrLeuCysGluAs  
3501 TCCCCATTGCTGAGGGGAGTCAGGCTCTCCGATGTGACGCTATCTGTAGTATGGCAGCTGTGGGCATTTCTTTCTTATTGTATTCTTGCTCGTGTTT  
609▶ nProProLeuLeuArgGlyValArgLeuSerAspValThrLeuSerCysSerMetAlaAlaValGlyI lePhePheLeuI leValPheLeuLeuValPhe

**SspI (3628)**

**NheI (3683)**

3601 GCTATTTTGTGATTTTTGCAGTGAAATTTTTCTCAGATGAAATACCAACACATTTAGTCAAGGTTTCCAGAGAAGGCAGCTAGCTGGCCAGACATG  
643▶ AlaI leLeuLeuI lePheAlaValLysTyrPheLeuArgTrpLysTyrGlnHisI le•••  
3701 ATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCA

**HpaI (3823)**

**MfeI (3832)**

3801 TTATAAGCTGCAATAAAACAAGTTAAACAACAACAATTGCATTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCT

**PacI (3927)**

3901 CTACAAATGTGGTATGGAATGTTAATTAACCTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCTGTCCACTGAGCGTCAGACCCCGTAGAAAAGATCA

4001 AAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGTTTTGTTTCCGGATCAAGAGCT

4101 ACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCCTTCAAGAACTCT

4201 GTAGACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGT

**ApaLI (4341)**

4301 TACCGGATAAAGCGCAGCGGTGCGGCTGAACGGGGGTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA

4401 GCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGGAACAGGAGAGCGCAGAGGGAGCTTCCAGGG

4501 GGAAACGCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTGACTTGAGCGTCGATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGAAAA

**PacI (4667) SdaI (4676)**

4601 ACGCCAGCAACGCGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCCTTTTGTCCACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGA

4701 GGAACTTGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTA

**SphI (4811)**

**SphI (4883)**

4801 TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGC

**BssHII (4928)**

**NotI (4946)**

**SmaI (4973)**

4901 AACCATAGTCCCACTAGTTCGCCAGAGCGCGGAGGGCCTCCAGCGCGCCCTCCCCACAGCAGGGGCGGGTCCCAGCGCCACCGGAAGGAGCGG

5001 GCTCGGGGCGGGCGGCGTATTGGCCGGGGCGGCTGACGCCGACGCGGTATAAGAGACCACAAGCAGCCGCAGGGCCAGACGTTCTTCGCCAAG

5101 CTTGCCGTCAGAACGCAGgtgagggcggggtgtgcttccgcgggccgagctggaggtcctgctccgagcgggccccgctgctgctcggcggg

5201 gattagctgcgagcattccgcttcgagttgcggcgccgggagggagagtgcgaggcctagcggcaaccctgtagcctgcctcgtgctcggcctga

5301 ggcttagcgtggtgctccgcccgcgctgctactccggccgactcgtgctttttttttttgttgtgttgctcctgctgcttgcattgcccgtt

5401 cagcaataggggctaacaaggagggtgctggggcctgctcggcggagcccggagaggtatggttggggaggaatggaggacaggagtgccggcctgg

5501 ggccccccgcttcggagcactgctccgacccacctggatggggcaggcctggggttttcccgaagcaaccaggctggggttagcgtgcccaggcc

5601 atgtggccccagcaccggcagcactgctgcttggcggcgccgcttgcctcctcctaactaggtgagggcatccgctccggcaccagttgctgctg

**SmaI (5719)**

5701 tggaaagatggccgctcccgggcccctggtgcaaggagctcaaatggaggacgcccagcccgggtggagcgggcccgggtgagtcacccacacaaggaaga

5801 gggcctggtccctcaccgctgctgcttctgtagcccggtgcttatcggcgcaatagtcacctgggcttttgagcacggctagtcgcccggggg

**XcmI (5965)**

5901 gaggggatgtaatggcgttggagtttgtcacatttgggtgggtggagactagtcaggccagcctggcgtggaagtcatTTTTGGaatttcccccttga

**BspHI (6095)**

6001 gttttgagcggagcatttctcgggcttcttagcgggtcaaaggtatcttttaacccttttttagGTGTTGTGAAAACCACCGCTAATTCAAAGCAATC

**NcoI (6160)**

6101 ATGAATGGTGTGCGAGCTGCCCTCTTGTGTGATTCTGACTTCTCCGAGCAGCAGTGACCATGGCAGCGAAAATGGTTGGCCAAGCACACGGCCTGCA

1▶ MetAsnGlyValAlaAlaAlaLeuLeuValTrpI leLeuThrSerProSerSerSerAspHisGlySerGluAsnGlyTrpProLysHisThrAlaCysA  
6201 ACAGTGGGGCTTGAAGTAGTCTACCAGAGCTGTGATCCCTTACAGGATTTTGGCCTTTCCATTGACCAGTGTCCAAAGCAGATCCAATCAAATCTCAA

34▶ snSerGlyGlyLeuGluValValTyrGlnSerCysAspProLeuGlnAspPheGlyLeuSerI leAspGlnCysSerLysGlnI leGlnSerAsnLeuAs

EcoRV (6331)

6301 CATTAGATTTGGCATCATTCTGAGACAGGATATCAGAAAGCTGTTTCTGGACATAACTCTGATGGCAAAGGCTCTTCTATTCTGAACTACTCTATCCC

67▶ nl leArgPheGlyI leI leLeuArgGlnAspl leArgLysLeuPheLeuAspl leThrLeuMetAlaLysGlySerSerI leLeuAsnTyrSerTyrPro  
6401 CTTTGTGAGGAGGACCAGCCCAAGTTCATTCTGTGGAAGAAGAAAAGGAGAACAGATATACTATGCCGGCCCTGTCAATAACCCTGGACTTGATGTTC

101▶ LeuCysGluGluAspGlnProLysPheSerPheCysGlyArgArgLysGlyGluGlnI leTyrTyrAlaGlyProValAsnAsnProGlyLeuAspValP

BsrGI (6530)

6501 CACAGGGAGAATATCAGCTCTTGCTGGAACGTGACAATGAAAACCGTCTACTGTGGCTTGTCCAATGCCACTGTCACCTCCTCTGAGCATGGTCTGC

134▶ roGlnGlyGluTyrGlnLeuLeuLeuGluLeuTyrAsnGluAsnArgAlaThrValAlaCysAlaAsnAlaThrValThrSerSer•••

XbaI (6674)

6601 AAGGAAATGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGACTGCG

6701 TTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCTGAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGTAACAAGGAC

6801 CCACGGGGCCAAAAGCCACGCCACACGGGCCGTGTCATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGG

Eco47III (701)

6901 TACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGC

BstEII (7032)

7001 GCTCGGTTTAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTGCGCACCTTTCCTTTGCAATTACTGACCCTATGAATACACTGACTGTTTGACAATT

7101 AATCATCGGCATAGTATATCGGCATAGTATAATCGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGG

1▶ MetLysThrPheAsnI leSerGlnGlnAspLeuGluLeuValG

7201 AGGTCGCCACTGAGAAGATCACCATGTCTATGAGGACAACAAGCACCATGTGCGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCA

15▶ luValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI leSerAlaValHi

7301 CATTGAGGCTACATTGGCAGGGTCACTGTCTGTGTAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAAGACTTTGACACCATTGTGGCT

48▶ sl leGluAlaTyrI leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrl leValAla

7401 GTCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCT

82▶ ValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeul leSerAspTyrAlaProAspCysP

7501 TTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAACACCATTGAGGAACCTATCCCCCTCAAGTACACAGGAACCTAAACCTGAATTAATTTCGCTAGG

115▶ heValLeul leGluMetAsnGlyLysLeuValLysThrThrl leGluGluLeul leProLeuLysTyrThrArgAsn•••

MfeI (7669)

7601 ATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGGAAAGACGGTCTCAGAAGTGTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGAC

7701 TGGTTAATGATAACAATGCATCGTAAACCTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTA

ScaI (7818)

7801 TTAGTTTTTAAAATCAGTACTTTTTAATGAAACAACCTTGACAAAAATTTGTCACAGAATTTTGAGACCCATTAATAAAGTTAAATGAGAAACCTGTGT

HpaI (7993)

7901 GTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGTTTTACGAATCTGGAACTTCTTGAAAATGTAATTCTTGAGTTAACACTT

8001 CTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTCTTTGATTACAACACTGGAGAG

8101 AAATGCAGCATGTTGCTGATTGCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAAGCTG