

pDUO-hMD1/RP105

A plasmid coexpressing the human MD1 and RP105 genes

Catalog code: pduo-hmd1rp105

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

For research use only

Version 20H26-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-hMD1/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

• **Human MD1 (486 bp) / Human 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 proliferation¹. 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 RP105². When induced by LPS, the RP105/MD1 complex regulates B-cell proliferation, antibody production, and B7.2/CD86 up-regulation³.

• **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.

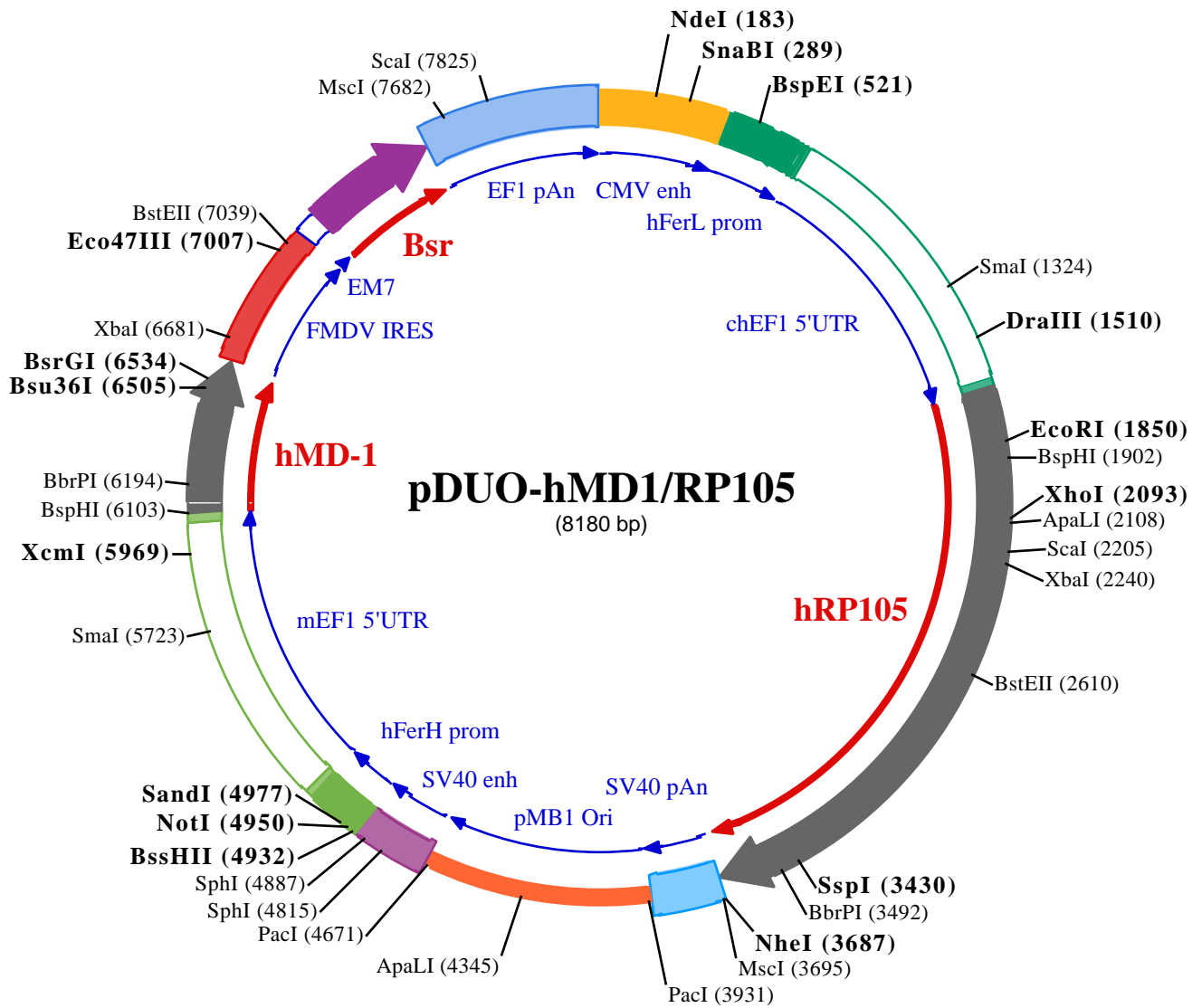
References

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.
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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.
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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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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (183)

101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

SnaBI (289)

201 TATTGACGTCAATGACGGTAAATGGCCCCCTGGCATTATGCCAGTACATGACCTTATGGGACTTCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGCTGGATAGCGGTTTACTCACGGGATTTCGAAGTCTCCACCCATTGACGTCAATG

401 GGAGTTTGTCTTACTAGTACAGGGCCCAACCCCCCAAGCCCCATTTCAACAACGCTGGCGCTACAGGCGCTGACTTCCCTTGTCTTGGGGCGGG

BspEI (521)

501 GGGCTGAGACTCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCGAGCGCCCTGCC

601 TCCGAGGGCCGGCGCACCATAAAGAAGCCGCCCTAGCCACGTCCTCGCAGTTCGGCGGTCGCCGGGCTCTGTCTCAAGCTTCCGCCAGAACACAGg

701 taagtgccgtgtgtggttcccggggcctggcctctttacgggttatggccttgcgtgcctgaattacttccatgccctggctgcagtacgtgattc

801 ttgatcccagccttccgggttgggaagtgggtgggagagttcgaggccttgcgcttaaggagcccttgcctcgtgcttgagttgaggcctggctgggagc

901 ctggggccgcgcgtgtaaatctggtggcaccttgcgcctgtctgcctgcttgcctaaagtctctagccatttaaatttttgataaccagctgcgacg

1001 cttttttctggcgagatagttctgtaaatcgggccaggatctgcacactggtatttcggtttttggggccggggggcgacggggccctgctgccc

1101 agcgcacatggtcggcgagggcggggcctgcgagcggccaccgagaatcggacgggggtagtctcaaaactggccgctgctctggtgctggcctgcg

1201 gccgcctgtatcgccccccctggcggaaggtggccggctggcaccagttgctgagcggaaagatggccgcttcccggcctgctgcagggagc

SmaI (1324)

1301 tcaaaatggaggacgcggcgcccgggagagcgggggggtgagtcacccacacaaggaaaaggccttctctcctcatccgtcgcttcatgtgactcca

1401 cggagtaccgggcccgtccaggcacctcgattagttgtcgagctttggagtagctgctttaggttggggggagggttttatgcatggagtttcc

DraIII (1510)

1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgaattctccttggaaatttgcctttttgagtttggatcttgcctcattc

1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAACACTACCCCTAAAAGCCACCATGGCGTTTACGTACAGTGTCTTCT

1701 TTGGTGTGCTGTTTTCTCGCGCTGTAAGTCACTACCTCCTGGGATCAGATGTGCATTGAGAAGAAGCCAAACAAACATATAACTGTGAAAATTTA
9 eTrpValValLeuPheSerAlaGlyCysLysVal I leThrSerTrpAspGlnMetCysI leGluLysGluAlaAsnLysThrTyrAsnCysGluAsnLeu

EcoRI (1850)

1801 GGTCTCAGTGAATCCCTGACACTCTACCAAACACAACAGAATTTTGGAAATCAGTTTAATTTTTTGCCCTACAATTCACAATAGAACCCTCAGCAGAC
43 GlyLeuSerGluI leProAspThrLeuProAsnThrThrGluPheLeuGluPheSerPheAsnPheLeuProThrI leHisAsnArgThrPheSerArgL

BspHI (1902)

1901 TCATGAATCTTACCTTTTTGGATTAACTAGGTGCCAGATTAAGTGGATACATGAAGACACTTTTCAAAGCCATCATCAATTAAGCACACTTGTGTAAAC
76 euMetAsnLeuThrPheLeuAspLeuThrArgCysGlnI leAsnTrpI leHisGluAspThrPheGlnSerHisHisGlnLeuSerThrLeuValLeuTh

XhoI (2093)

2001 TGAAATCCCCTGATATTCATGGCAGAAACATCGCTTAATGGGCCAAGTCACTGAAGCATCTTTCTTAATCCAACGGGAATATCCAATCTCGAGTTT
109 rGlyAsnProLeuI lePheMetAlaGluThrSerLeuAsnGlyProLysSerLeuLysHisLeuPheLeuI leGlnThrGlyI leSerAsnLeuGluPhe

ApaI (2108)

2101 ATTCCAGTGCACAATCTGGAAAACCTTGAAAAGCTTGTATCTTGAAGCAACCATATTTCTCCATTAAGTTCCCAAAGACTTCCAGCACGGAATCTGA
143 I leProValHisAsnLeuGluAsnLeuGluSerLeuTyrLeuGlySerAsnHisI leSerSerI leLysPheProLysAspPheProAlaArgAsnLeuL

Scal (2205)

XbaI (2240)

2201 AAGTACTGGATTTTCAAGAATAATGCTATACACTACATCTCTAGAGAAGACATGAGGTCTCTGGAGCAGGCCATCAACCTAAGCCTGAACCTCAATGGCAA
176 ysValLeuAspPheGlnAsnAsnAlaI leHisTyrI leSerArgGluAspMetArgSerLeuGluGlnAlaI leAsnLeuSerLeuAsnPheAsnGlyAs

2301 TAATGTTAAAGTATTGAGCTTGGGCTTTTGTATCAACGGTCTTCAAAGTTTGAACCTTGGAGGAACCTCAAATTTGTCTGTATATTCAATGGTCTG
209 nAsnValLysGlyI leGluLeuGlyAlaPheAspSerThrValPheGlnSerLeuAsnPheGlyGlyThrProAsnLeuSerVal I lePheAsnGlyLeu

2401 CAGAACTCTACTACTCAGTCTCTCTGGCTGGGAACATTTGAGGACATTGATGACGAAGATATTAGTTTACGCCATGCTCAAGGACTCTGTGAAATGTCTG
243 GlnAsnSerThrThrGlnSerLeuTrpLeuGlyThrPheGluAspI leAspAspGluAspI leSerSerAlaMetLeuLysGlyLeuCysGluMetSerV

2501 TTGAGAGCCTCAACCTGCAGGAACACCGCTTCTCTGACATCTCATCCACCACATTTCAAGTGTTCACCCAACTCCAAGAATTGGATCTGACAGCAACTCA
276 aIleGluSerLeuAsnLeuGlnGluHisArgPheSerAspI leSerSerThrThrPheGlnCysPheThrGlnLeuGlnGluLeuAspLeuThrAlaThrHi

BstEII (2610)

2601 CTTGAAAGGGTTACCCTCTGGGATGAAGGTCTGAACTTGTCTCAAGAAATTAGTTCTCAGTGAATTCATTTTCGATCAATTTGTGTCAAATCAGTGTCTGCC
309 sLeuLysGlyLeuProSerGlyMetLysGlyLeuAsnLeuLeuLysLysLeuValLeuSerValAsnHisPheAspGlnLeuCysGlnI leSerAlaAla

2701 AATTTCCCTCCCTTACACACTCTACATCAGAGGCAACGTGAAGAACTTCACTTGGTGTGGTCTGGGAGAACTAGGAAACCTTCCAGACTTGG
343 AsnPheProSerGlnThrHisLeuTyrI leArgGlyAsnValLysLysLeuHisLeuAlaPheValGlyCysLeuGluLysLeuGlyAsnLeuGlnThrLeuA

2801 ATTTAAGCCATAATGACATAGAGGCTTCTGACTGCTGAGTCTGCAACTCAAAAACCTGTCCACTTGCAAACTTAAACCTGAGCCACAATGAGCCTCT
376 splLeuSerHisAsnAspI leGluAlaSerAspCysCysSerLeuGlnLeuLysAsnLeuSerHisLeuGlnThrLeuAsnLeuSerHisAsnGluProLe

2901 TGGTCTCCAGAGTCAGGCATTCAAAGAATGCTCCTCAGTGAACCTTCTGATTTGGCATTACCCGCTTACACATTAATGCTCCACAAAGTCCCTTCAA
409 uGlyLeuPheSerGlnAlaPheLysGluCysProGlnLeuAspLeuLeuAspLeuLeuAlaPheThrArgLeuHisI leAsnAlaProGlnSerProPheGln

3001 AACCTCCATTTCTTCCAGTTCTGAATCTCACTTACTGCTTCTTGGATACCAGCAATCAGCATCTTCTAGCAGGCTACCAGTTCTCCGGCATCTCAACT
443 AsnLeuHisPheLeuGlnValLeuAsnLeuThrTyrCysPheLeuAspThrSerAsnGlnHisLeuLeuAlaGlyLeuProValLeuArgHisLeuAsnL

3101 TAAAAGGGAATCACTTCAAGATGGGACTATCAGGAAGCAACCTACTTCAAGCCTGGGAGCTTGGAGGTTCTGATTTTGTCTTGTGGTCTCTCT
476 euLysGlyAsnHisPheGlnAspGlyThrI leThrLysThrAsnLeuGlnThrValGlySerLeuGluValLeuI leLeuSerSerCysGlyLeuLe

3201 CTCATAGACCAGCAAGCATCCACAGCTTGGGAAAATGAGCATTGAGACTTAAGCCACAACAGCCTGACATCGCAGCAGCATTTGATCTTCAAGCAT
509 uSerI leAspGlnGlnAlaPheHisSerLeuGlyLysMetSerHisValAspLeuSerHisAsnSerLeuThrCysAspSerI leAspSerLeuSerHis

3301 CTTAAGGGAATCTACCTCAATCTGGCTGCCAACAGCATTAAACATCATCTCACCCGCTCTCTCCCTATCTTGTCCCAGCAGAGCACCATTAATTTAAGTC
543▶ LeuLysGlyI leTyrLeuAsnLeuAlaAlaAsnSerI leAsnI leI leSerProArgLeuLeuProl leLeuSerGlnGlnSerThrI leAsnLeuSerH
SspI (3430) BbrPI (3492)
3401 ATAACCCCTGGACTGCACCTTGTCTCGAATATTCATTTCTTAACATGGTACAAAGAAAACCTGCACAACTTGAAGGCTCGGAGGAGACCACGTGTGCAAA
576▶ isAsnProLeuAspCysThrCysSerAsnI leHisPheLeuThrTrpTyrLysGluAsnLeuHisLysLeuGluGlySerGluGluThrThrCysAlaAs
3501 CCCGCCATCTCTAAGGGGAGTTAAGCTATCTGATGTCAAGCTTTCCTGTGGGATTACAGCCATAGGCATTTTCTTCTCATAGTATTTCTATTATTGTTG
609▶ nProProSerLeuArgGlyValLysLeuSerAspValLysLeuSerCysGlyI leThrAlaI leGlyI lePhePheLeuI leValPheLeuLeuLeu
MscI (3695) NheI (3687)
3601 GCTATTCTGCTATTTTTGTCAGTTAAATACCTTCTCAGGTGAAAATACCAACACATTTAGTGTGAAGGTTTCCAGAGAAAAGCAAGCTAGCTGGCCAGAC
643▶ AlaI leLeuLeuPhePheAlaValLysTyrLeuLeuArgTrpLysTyrGlnHisI le•••
3701 ATGATAAGATACATTGATGAGTTTGGACAAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTGTAA
3801 CCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATGTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAA
PacI (3931)
3901 CCTCTACAAATGTGGTATGAAAATGTTAATTAACCTAGCCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGA
4001 TCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGTGCTTGCACAAAAAAAACCACCCTACCAGCGGTGGTTTTGTTGCCGGATCAAGA
4101 GCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAC
4201 TCTGTAGCACCGCTACATACCTCGCTCTGTAATCCTGTTACCAGTGGCTGTGCCAGTGGCGATAAGTCTGTCTTACCGGTTGGACTCAAGACGAT
ApaLI (4345)
4301 AGTTACCGGATAAGGCGCAGCGGTCCGGCTGAACGGGGGTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCG
4401 TGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCCA
4501 GGGGAAACGCTGGTATCTTTATAGTCTTCTCGGGTTTTGCCACCTCTGACTTGGAGCGTCAATTTTTGTGATGCTCGTACGGGGGGCGGAGCCTATGGA
PacI (4671)
4601 AAAACGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGCGCCTTTTGTCTCACATGTTCTTAATTAACCTGCAGGGCTGAAATAACCTCTGAA
4701 AGAGGAACCTGGTTAGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCAAGGCTCCCAAGCAGGCGAGAA
SphI (4815) SphI (4887)
4801 GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAAGGCTCCCAAGCAGGCGAGAAGTATGCAAAGCATGCATCTCAATTAGTC
BssHIII (4932) NotI (4950) SandI (4977)
4901 AGCAACCATAGTCCCACTAGTTCCCGCAGAGCGCGCGAGGGGCTCCAGCGCGCCCTCCCCACAGCAGGGGCGGGGTCCCGCGCCACCAGGAGGAG
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SmaI (5723)
5701 gcgtgaaagatggccgctcccgggctgtgcaaggagctcaaatggaggacgcccagcccgtggagcgggcggtgagtcacccacacaagga
5801 agagggctggtccctcaccggtgctgcttctgtgacccgctggtcctatcgggcgcaatagtcacctgggcttttgagcacggctagtcgcgccg
XcmI (5969)
5901 ggggaggggatgtaaatggcgttggagttgttcacatttgggtgggtggagactagtcaggccagcctggcgctggaagtcattttggaaattgtcccct
6001 tgagttttgagcggagctaatctcgggctccttagcgggtcaaggtatcttttaacccttttttagGTGTTGTGAAAACCACCGCTAATTTCAAAGCA
BspHI (6103) BbrPI (6194)
6101 ATCATGAAGGTTTACAGCCACTCTCTTCTCTGGACTCTGATTTTTCCAGCTGCAGTGGAGGCGCGGTGGAAAAGCCTGGCCACACACGTGGTCT
▶ MetLysGlyPheThrAlaThrLeuPheLeuTrpThrLeuI lePheProSerCysSerGlyGlyGlyGlyLysAlaTrpProThrHisValValC
6201 GTAGCGACAGCGCTTGAAGTGTCTTACCAGAGTTGCGATCCATTACAAGATTTTGGCTTTTCTGTGAAAAGTGTCCAAAGCAATTAATCAATAT
33▶ ysSerAspSerGlyLeuGluValLeuTyrGlnSerCysAspProLeuGlnAspPheGlyPheSerValGluLysCysSerLysGlnLeuLysSerAsnI
6301 CAACATTAGATTTGGAATTAATCTGAGAGAGGACATCAAAGAGCTTTTTCTTGACCTAGCTCTCATGTCTCAAGGCTCATCTGTTTTGAATTTCTCTAT
66▶ eAsnI leArgPheGlyI leI leLeuArgGluAspI leLysGluLeuPheLeuAspLeuAlaLeuMetSerGlnGlySerSerValLeuAsnPheSerTyr
6401 CCCATCTGTAGGCGGCTCTGCCAAGTTTTCTTCTGTGGAAGAAGGAAAGGAGAGCAGATTTACTATGCTGGCCCTGTCAATAATCCTGAATTTACTA
100▶ Prol leCysGluAlaAlaLeuProLysPheSerPheCysGlyArgArgLysGlyGluGlnI leTyrTyrAlaGlyProValAsnAsnProGluPheThrI
Bsu36I (6505) BsrGI (6534)
6501 TTCCTCAGGGAGAATACCAGTTTTGCTGGAACGTACACTGAAAAACGGTCCACCGTGGCCTGTGCCAATGCTACTATCATGTCTCTGACTGTGGCC
133▶ leProGlnGlyGluTyrGlnValLeuLeuGluLeuTyrThrGluLysArgSerThrValAlaCysAlaAsnAlaThrI leMetCysSer•••

XbaI (6681)

6601 TGTAGCAAAAATCAGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTCCAGGTCTAGAGGGGTAACACTTTGT

6701 ACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTGGTAAC

6801 AAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCGTCATGTGTGCAACCCAGCAGCGGCACTTTACTGCGAAACCACTTAAAGTGACATTGA

6901 AACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCGAGGTAACACGGGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTAT

Eco47III (7007) BstEII (7039)

7001 AAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTGGCACCTTTCCTTTGCAATTAAGTACCTGACTGTTTG

7101 ACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGC
1▶MetLysThrPheAsnI leSerGlnGlnAspLeuGluL

7201 TGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGC
13▶euValGluValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI leSerAl

7301 TGTCACATTGAGGCTACATTGGCAGGGTCACTGCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATT
46▶aValHisI leGluAlaTyrI leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrI le

7401 GTGGCTGCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTG
80▶ValAlaValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyrAlaProA

7501 ACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGGAACATCCCCCTCAAGTACACCAGGAATAAACCTGAATTAATTC
113▶spCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgAsn•••

MscI (7682)

7601 GCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTA

7701 AAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTGTGGACCACTTTGGTTTCTTTTTTGCGTGTGGCAGTTTT

ScaI (7825)

7801 AAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAATAAGTTAAATGAGAAAC

7901 CTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTCTTGAAAATGTAATTCTTGAGTTA

8001 ACACTTCTGGGTGAGAAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGGTTTCTTTGATTACAACACT

8101 GGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAAACAGGCCAAAAAAGTGAAGCTTGGGTTGCATAGAAAGCTG