pDUO-mTLR1/TLR2

A plasmid coexpressing the murine TLR1 and TLR2 genes

Catalog code: pduo-mtlr1tlr2

https://www.invivogen.com/pduo-tlr1-tlr2

For research use only

Version 20H25-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-mTLR1/TLR2 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 TLR1** (2388 bp) **/ Murine TLR2** (2355 bp)

TLR1, the first member of the Toll-Like Receptor family was identified by the presence of a domain homology found in both Drosophila Toll and human IL-1 receptors. So far, no direct ligands for TLR1 have been identified however it seems to act as a coreceptor. TLR1 has been shown to associate with TLR2 in response to triacylated lipopeptides[1], but not diacylated lipopeptides[2]. These observations suggest that TLR1 is able to discriminate among lipoproteins by recognizing the lipid configuration.

- **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[3]. 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[4].
- **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[5].
- **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 expression[7].

• **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_2O. 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. **Takeuchi O. *et al.*, 2002.** Cutting edge: Role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol, 169(1):10-14..
2. **Takeuchi O. *et al.*, 2001.** Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol, 13(7): 933-40.
3. **Eisenstein RS. & 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. & 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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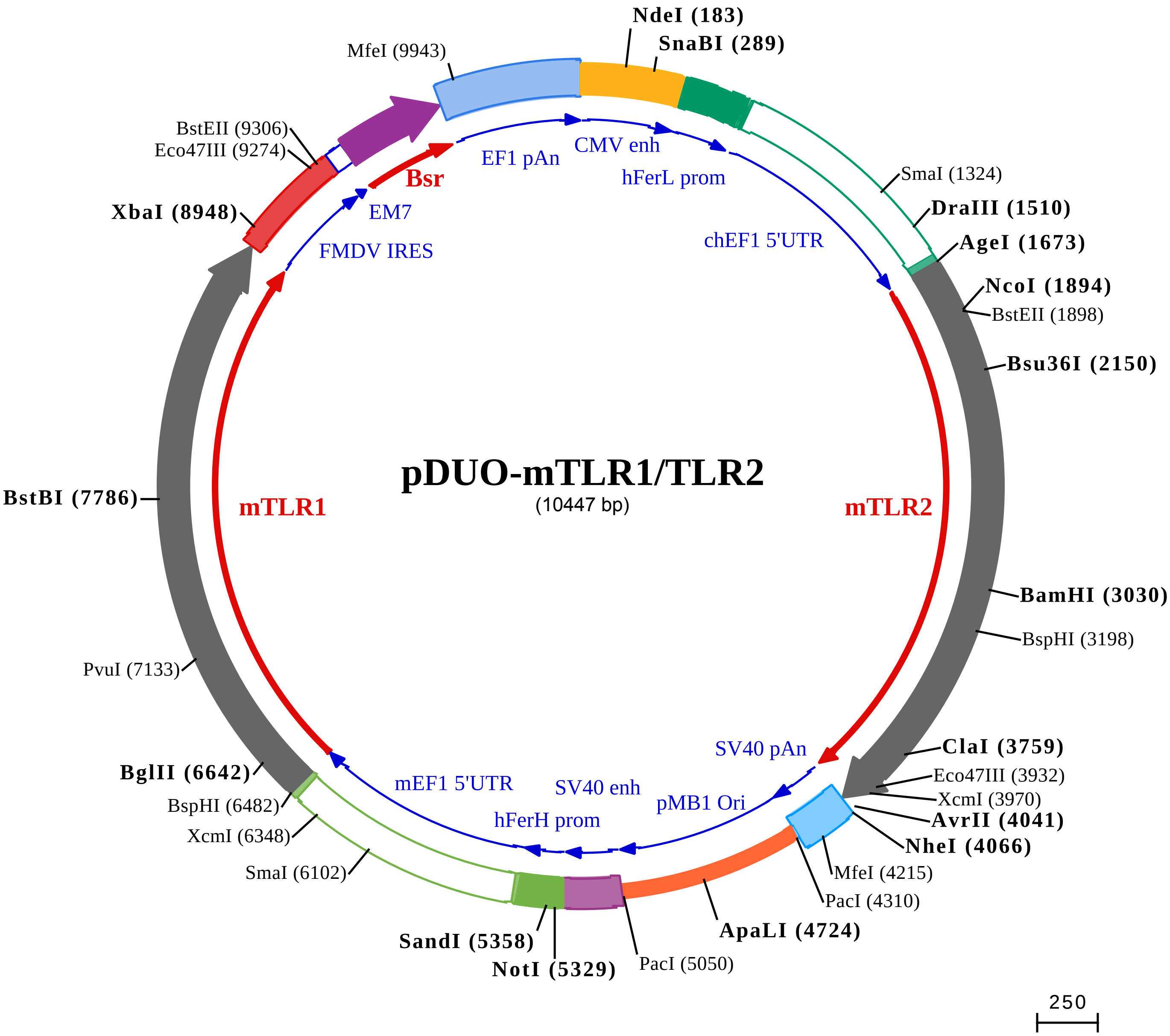
pDUO-mTLR1/TLR2
(10447 bp)
NdeI (183)
SnaBI (289)
SmaI (1324)
DraIII (1510)
AgeI (1673)
NcoI (1894)
BstEII (1898)
Bsu36I (2150)
BamHI (3030)
BspHI (3198)
ClaI (3759)
Eco47III (3932)
XcmI (3970)
AvrII (4041)
NheI (4066)
MfeI (4215)
PacI (4310)
ApaLI (4724)
PacI (5050)
NotI (5329)
SandI (5358)
SmaI (6102)
XcmI (6348)
BspHI (6482)
BglII (6642)
PvuI (7133)
BstBI (7786)
XbaI (8948)
Eco47III (9274)
BstEII (9306)
MfeI (9943)
EF1 pAn
CMV enh
hFerL prom
chEF1 5'UTR
mTLR2
SV40 pAn
pMB1 Ori
SV40 enh
hFerH prom
mEF1 5'UTR
mTLR1
FMDV IRES
EM7
Bsr
250

1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (183)

101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC

SnaBI (289)

201 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG

401 GGAGTTTGTTTTGACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGG

501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCC

601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGg

701 taagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc

801 ttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcg

901 ctggggccgccgcgtgctaatctggtggcaccttcgcgcctgtctcgctgctttcgctaagtctctagccatttaaaatttttgataaccagctgcgacg

1001 cttttttctggcgagatagtcttgtaaatgcgggccaggatctgcacactggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtccc

1101 agcgcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaaactggccggcctgctctggtgcctggcctcgc

1201 gccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagc

SmaI (1324)

1301 tcaaaatggaggacgcggcgcccgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccttcctcatccgtcgcttcatgtgactcca

1401 cggagtaccgggcgccgtccaggcacctcgattagttgtcgagcttttggagtacgtcgtctttaggttggggggaggggttttatgcgatggagtttcc

DraIII (1510)

1501 ccacactgagtgggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttgcctcattc

AgeI (1673)

1601 tcaagcctcagacagtggttcaaagtttttttcttccatttcagGTGTCGTGAAAACTACCCCTAAAAGCCACCGGTAGGAGGGCCAGCATGCTACGAGC
1 MetLeuArgAl

1701 TCTTTGGCTCTTCTGGATCTTGGTGGCCATAACAGTCCTCTTCAGCAAACGCTGTTCTGCTCAGGAGTCTCTGTCATGTGATGCTTCTGGGGTGTGTGAT
4 aLeuTrpLeuPheTrpIleLeuValAlaIleThrValLeuPheSerLysArgCysSerAlaGlnGluSerLeuSerCysAspAlaSerGlyValCysAsp

BstEII (1898)
NcoI (1894)

1801 GGCCGCTCCAGGTCTTTCACCTCTATTCCCTCCGGACTCACAGCAGCCATGAAAAGCCTTGACCTGTCTTTCAACAAGATCACCTACATTGGCCATGGTG
38 GlyArgSerArgSerPheThrSerIleProSerGlyLeuThrAlaAlaMetLysSerLeuAspLeuSerPheAsnLysIleThrTyrIleGlyHisGlyA

1901 ACCTCCGAGCGTGTGCGAACCTCCAGGTTCTGATTTTGAAGTCCAGCAGAATCAATACAATAGAGGGAGACGCCTTTTATTCTCTGGGCAGTCTTGAACA
71 spLeuArgAlaCysAlaAsnLeuGlnValLeuIleLeuLysSerSerArgIleAsnThrIleGluGlyAspAlaPheTyrSerLeuGlySerLeuGluHi

2001 TTTGGATTTGTCTGATAATCACCTATCTAGTTTATCTTCCTCCTGGTTCGGGCCCCTTTCCTCTTTGAAATACTTAAACTTAATGGGAAATCCTTACCAG
104 sLeuAspLeuSerAspAsnHisLeuSerSerLeuSerSerSerTrpPheGlyProLeuSerSerLeuLysTyrLeuAsnLeuMetGlyAsnProTyrGln

Bsu36I (2150)

2101 ACACTGGGGGTAACATCGCTTTTTCCCAATCTCACAAATTTACAAACCCTCAGGATAGGAAATGTAGAGACTTTCAGTGAGATAAGGAGAATAGATTTTG
138 ThrLeuGlyValThrSerLeuPheProAsnLeuThrAsnLeuGlnThrLeuArgIleGlyAsnValGluThrPheSerGluIleArgArgIleAspPheA

2201 CTGGGCTGACTTCTCTCAATGAACTTGAAATTAAGGCATTAAGTCTCCGGAATTATCAGTCCCAAAGTCTAAAGTCGATCCGCGACATCCATCACCTGAC
171 laGlyLeuThrSerLeuAsnGluLeuGluIleLysAlaLeuSerLeuArgAsnTyrGlnSerGlnSerLeuLysSerIleArgAspIleHisHisLeuTh

2301 TCTTCACTTAAGCGAGTCTGCTTTCCTGCTGGAGATTTTTGCAGATATTCTGAGTTCTGTGAGATATTTAGAACTAAGAGATACTAACTTGGCCAGGTTC
204 rLeuHisLeuSerGluSerAlaPheLeuLeuGluIlePheAlaAspIleLeuSerSerValArgTyrLeuGluLeuArgAspThrAsnLeuAlaArgPhe

2401 CAGTTTTCACCACTGCCCGTAGATGAAGTCAGCTCACCGATGAAGAAGCTGGCATTCCGAGGCTCGGTTCTCACTGATGAAAGCTTTAACGAGCTCCTGA
238 GlnPheSerProLeuProValAspGluValSerSerProMetLysLysLeuAlaPheArgGlySerValLeuThrAspGluSerPheAsnGluLeuLeuL

2501 AGCTGTTGCGTTACATCTTGGAACTGTCGGAGGTAGAGTTCGACGACTGTACCCTCAATGGGCTCGGCGATTTCAACCCCTCGGAGTCAGACGTAGTGAG
271 ysLeuLeuArgTyrIleLeuGluLeuSerGluValGluPheAspAspCysThrLeuAsnGlyLeuGlyAspPheAsnProSerGluSerAspValValSe

2601 CGAGCTGGGTAAAGTAGAAACAGTCACTATCCGGAGGTTGCATATCCCCCAGTTCTATTTGTTTTATGACCTGAGTACTGTCTATTCCCTCCTGGAGAAG
304 rGluLeuGlyLysValGluThrValThrIleArgArgLeuHisIleProGlnPheTyrLeuPheTyrAspLeuSerThrValTyrSerLeuLeuGluLys

2701 GTGAAGCGAATCACAGTAGAGAACAGCAAGGTCTTCCTGGTTCCCTGCTCGTTCTCCCAGCATTTAAAATCATTAGAATTCTTAGACCTCAGCGAAAATC
338 ValLysArgIleThrValGluAsnSerLysValPheLeuValProCysSerPheSerGlnHisLeuLysSerLeuGluPheLeuAspLeuSerGluAsnL

2801 TGATGGTTGAAGAATATTTGAAGAACTCAGCCTGTAAGGGAGCCTGGCCTTCTCTACAAACCTTAGTTTTGAGCCAGAATCATTTGAGATCAATGCAAAA
371 euMetValGluGluTyrLeuLysAsnSerAlaCysLysGlyAlaTrpProSerLeuGlnThrLeuValLeuSerGlnAsnHisLeuArgSerMetGlnLy

2901 AACAGGAGAGATTTTGCTGACTCTGAAAAACCTGACCTCCCTTGACATCAGCAGGAACACTTTTCATCCGATGCCCGACAGCTGTCAGTGGCCAGAAAAG
404 sThrGlyGluIleLeuLeuThrLeuLysAsnLeuThrSerLeuAspIleSerArgAsnThrPheHisProMetProAspSerCysGlnTrpProGluLys

BamHI (3030)

3001 ATGCGCTTCCTGAATTTGTCCAGTACAGGGATCCGGGTGGTAAAAACGTGCATTCCTCAGACGCTGGAGGTGTTGGATGTTAGTAACAACAATCTTGACT
438 MetArgPheLeuAsnLeuSerSerThrGlyIleArgValValLysThrCysIleProGlnThrLeuGluValLeuAspValSerAsnAsnAsnLeuAspS

BspHI (3198)

3101 CATTTTCTTTGTTCTTGCCTCGGCTGCAAGAGCTCTATATTTCCAGAAATAAGCTGAAAACACTCCCAGATGCTTCGTTGTTCCCTGTGTTGCTGGTCAT
471 erPheSerLeuPheLeuProArgLeuGlnGluLeuTyrIleSerArgAsnLysLeuLysThrLeuProAspAlaSerLeuPheProValLeuLeuValMe

3201 GAAAATCAGAGAGAATGCAGTAAGTACTTTCTCTAAAGACCAACTTGGTTCTTTTCCCAAACTGGAGACTCTGGAAGCAGGCGACAACCACTTTGTTTGC
504 tLysIleArgGluAsnAlaValSerThrPheSerLysAspGlnLeuGlySerPheProLysLeuGluThrLeuGluAlaGlyAspAsnHisPheValCys

3301 TCCTGCGAACTCCTATCCTTTACTATGGAGACGCCAGCTCTGGCTCAAATCCTGGTTGACTGGCCAGACAGCTACCTGTGTGACTCTCCGCCTCGCCTGC
538 SerCysGluLeuLeuSerPheThrMetGluThrProAlaLeuAlaGlnIleLeuValAspTrpProAspSerTyrLeuCysAspSerProProArgLeuH

3401 ACGGCCACAGGCTTCAGGATGCCCGGCCCTCCGTCTTGGAATGTCACCAGGCTGCACTGGTGTCTGGAGTCTGCTGTGCCCTTCTCCTGTTGATCTTGCT
571 isGlyHisArgLeuGlnAspAlaArgProSerValLeuGluCysHisGlnAlaAlaLeuValSerGlyValCysCysAlaLeuLeuLeuIleLeuLe

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3501 CGTAGGTGCCCTGTGCCACCATTTCCACGGACTGTGGTACCTGAGAATGATGTGGGCGTGGCTCCAGGCCAAGAGGAAGCCCAAGAAAGCTCCCTGCAGG
 604 uValGlyAlaLeuCysHisHisPheHisGlyLeuTrpTyrLeuArgMetMetTrpAlaTrpLeuGlnAlaLysArgLysProLysLysAlaProCysArg
3601 GACGTTTGCTATGATGCCTTTGTTTCCTACAGTGAGCAGGATTCCCATTGGGTGGAGAACCTCATGGTCCAGCAGCTGGAGAACTCTGACCCGCCCTTTA
 638 AspValCysTyrAspAlaPheValSerTyrSerGluGlnAspSerHisTrpValGluAsnLeuMetValGlnGlnLeuGluAsnSerAspProProPheL
                                                                                   ClaI (3759)
3701 AGCTGTGTCTCCACAAGCGGGACTTCGTTCCGGGCAAATGGATCATTGACAACATCATCGATTCCATCGAAAAGAGCCACAAAACTGTGTTCGTGCTTTC
 671 ysLeuCysLeuHisLysArgAspPheValProGlyLysTrpI leI leAspAsnI leI leAspSerI leGluLysSerHisLysThrValPheValLeuSe
3801 TGAGAACTTCGTACGGAGCGAGTGGTGCAAGTACGAACTGGACTTCTCCCACTTCAGGCTCTTTGACGAGAACAACGACGCGGCCATCCTTGTTTTGCTG
 704 rGluAsnPheValArgSerGluTrpCysLysTyrGluLeuAspPheSerHisPheArgLeuPheAspGluAsnAsnAspAlaAlaI leLeuValLeuLeu
                                         Eco47III (3932)                        XcmI (3970)
3901 GAGCCCATTGAGAGGAAAGCCATTCCCCAGCGCTTCTGCAAACTGCGCAAGATAATGAACACCAAGACCTACCTGGAGTGGCCCTTGGATGAAGGCCAGC
 738 GluProI leGluArgLysAlaI leProGlnArgPheCysLysLeuArgLysI leMetAsnThrLysThrTyrLeuGluTrpProLeuAspGluGlyGlnG
                                                AvrII (4041)            NheI (4066)
4001 AGGAAGTGTTTTGGGTAAATCTGAGAACTGCAATAAAGTCCTAGGTTCTCCACCCAGTTCCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAG
 771 InGluValPheTrpValAsnLeuArgThrAlaI leLysSer•••
4101 TTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAC

                  MfeI (4215)
4201 AAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGA

      PacI (4310)
4301 AATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC

4401 CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA

4501 GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC

4601 CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC

                       ApaLI (4724)
4701 GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCAC

4801 GCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTT

4901 TATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCT

                                                  PacI (5050)
5001 TTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACC

5101 TTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA

5201 ATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGT

                         NotI (5329)                     SandI (5358)
5301 TCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGGGCGGGGTCCCGCGCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGC

5401 TGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAG

5501 gtgaggggcgggtgtggcttccgcgggccgccgagctggaggtcctgctccgagcgggccgggccccgctgtcgtcggcggggattagctgcgagcattc

5601 ccgcttcgagttgcgggcggcgcgggaggcagagtgcgaggcctagcggcaaccccgtagcctcgcctcgtgtccggcttgaggcctagcgtggtgtccg

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5901 gcacatgtccgacgccacctggatggggcgaggcctggggtttttcccgaagcaaccaggctggggttagcgtgccgaggccatgtggccccagcacccg

                                                                                                 SmaI (6102)
6001 gcacgatctggcttggcggcgccgcgttgccctgcctccctaactagggtgaggccatcccgtccggcaccagttgcgtgcgtggaaagatggccgctcc

6101 cgggccctgttgcaaggagctcaaaatggaggacgcggcagcccggtggagcgggcgggtgagtcacccacacaaaggaagagggcctggtccctcaccg

6201 gctgctgcttcctgtgaccccgtggtcctatcggccgcaatagtcacctcgggcttttgagcacggctagtcgcggcgggggaggggatgtaatggcgt

                                          XcmI (6348)
6301 tggagtttgttcacatttggtgggtggagactagtcaggccagcctggcgctggaagtcattttggaatttgtccccttgagttttgagcggagctaat

                                                                                  BspHI (6482)
6401 tctcgggcttcttagcggttcaaaggtatcttttaaacccttttttagGTGTTGTGAAAACCACCGCTAATTCAAAGCAATCATGACTAAACCAAATTCC
   1                                                                                MetThrLysProAsnSer
6501 CTCATCTTCTACTGTATCATTGTTTTAGGACTGACACTTATGAAAATCCAATTATCTGAGAAATGTGAGCTTATCATAAAGAGGCCAAACGCAAACCTTA
   7 LeuI lePheTyrCysI leI leValLeuGlyLeuThrLeuMetLysI leGlnLeuSerGluLysCysGluLeuI leI leLysArgProAsnAlaAsnLeuT
                                            BglII (6642)
6601 CCAGAGTGCCCAAGGACCTACCCTTGCAAACAACTACTTTAGATCTATCACAAAACAATATATCTGAGCTTCAGACTTCTGACATCCTCTCATTGTCCAA
  40 hrArgValProLysAspLeuProLeuGlnThrThrThrLeuAspLeuSerGlnAsnAsnI leSerGluLeuGlnThrSerAspI leLeuSerLeuSerLy
6701 GCTGAGGGTCCTGATAATGTCCTACAACAGACTCCAGTATCTTAATATCAGTGTTTTCAAATTCAACACAGAGCTGGAATATTTGGATTTGTCCCACAAT
  73 sLeuArgValLeuI leMetSerTyrAsnArgLeuGlnTyrLeuAsnI leSerValPheLysPheAsnThrGluLeuGluTyrLeuAspLeuSerHisAsn
6801 GAGCTAAAGGTGATCTTGTGCCACCCAACAGTCAGCCTCAAGCATTTGGACCTCTCCTTTAATGCCTTTGATGCCCTGCCTATATGCAAAGAATTTGGCA
 107 GluLeuLysValI leLeuCysHisProThrValSerLeuLysHisLeuAspLeuSerPheAsnAlaPheAspAlaLeuProI leCysLysGluPheGlyA
6901 ACATGTCCCAACTACAGTTCCTGGGGTTGAGCGGTTCTCGGGTACAAAGTTCAAGTGTGCAGCTGATTGCTCATTTGAACATCAGTAAGGTTTTGCTGGT
 140 snMetSerGlnLeuGlnPheLeuGlyLeuSerGlySerArgValGlnSerSerSerValGlnLeuI leAlaHisLeuAsnI leSerLysValLeuLeuVa
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7001 GTTAGGAGATGCTTATGGGGAAAAAGAAGACCCCGAATCTCTTCGGCACGTTAGCACTGAGACTCTGCATATTGTTTTCCCGTCGAAAAGAGAATTCCGT
 173 lLeuGlyAspAlaTyrGlyGluLysGluAspProGluSerLeuArgHisValSerThrGluThrLeuHisl leValPheProSerLysArgGluPheArg
                                   PvuI (7133)
7101 TTTCTTCTGGATGTGTCCGTCAGCACTACGATCGGTTTGGAACTGTCTAACATCAAGTGTGTGCTTGAAGACCAGGGCTGCTCTTATTTCTTACGTGCTT
 207 PheLeuLeuAspValSerValSerThrThrl leGlyLeuGluLeuSerAsnl leLysCysValLeuGluAspGlnGlyCysSerTyrPheLeuArgAlaL
7201 TGTCAAAGCTTGGAAAGAATCTGAAGCTCTCAAATCTTACCCTGAACAATGTGGAAACAACGTGGAATTCCTTCATTAATATCCTCCAGATAGTTTGGCA
 240 euSerLysLeuGlyLysAsnLeuLysLeuSerAsnLeuThrLeuAsnAsnValGluThrThrTrpAsnSerPhel leAsnl leLeuGlnl leValTrpHi
7301 TACGCCAGTCAAATATTTCTCAATTTCAAATGTGAAGCTACAAGGTCAACTTGCCTTCAGGATGTTCAATTATTCTGACACTTCTCTGAAGGCTTTGTCG
 273 sThrProValLysTyrPheSerl leSerAsnValLysLeuGlnGlyGlnLeuAlaPheArgMetPheAsnTyrSerAspThrSerLeuLysAlaLeuSer
7401 ATACATCAAGTTGTCACTGATGTCTTCAGCTTCCCCCAAAGTTACATATACAGTATCTTTGCCAATATGAACATCCAAAACTTTACAATGTCTGGAACAC
 307 I leHisGlnValValThrAspValPheSerPheProGlnSerTyrl leTyrSerl lePheAlaAsnMetAsnl leGlnAsnPheThrMetSerGlyThrH
7501 ACATGGTCCACATGCTGTGCCCGTCCCAAGTTAGCCCATTTCTGCATGTGGACTTTACAGATAACCTTTTAACAGACATGGTTTTTAAAGACTGTAGAAA
 340 isMetValHisMetLeuCysProSerGlnValSerProPheLeuHisValAspPheThrAspAsnLeuLeuThrAspMetValPheLysAspCysArgAs
7601 CTTAGTTAGATTGAAAACACTTAGTTTACAAAAGAATCAGTTAAAAAACCTTGAGAATATAATCCTCACATCTGCAAAGATGACATCCCTACAAAAACTA
 373 nLeuValArgLeuLysThrLeuSerLeuGlnLysAsnGlnLeuLysAsnLeuGluAsnl lel leLeuThrSerAlaLysMetThrSerLeuGlnLysLeu
                                                                                           BstBI (7786)
7701 GACATTAGCCAGAATTCTCTAAGGTACAGCGATGGGGGAATCCCATGCGCCTGGACCCAGAGTTTGTTAGTTTTAAATTTGTCTTCGAATATGCTTACAG
 407 Aspl leSerGlnAsnSerLeuArgTyrSerAspGlyGlyl leProCysAlaTrpThrGlnSerLeuLeuValLeuAsnLeuSerSerAsnMetLeuThrG
7801 GCTCTGTCTTCAGATGCTTACCTCCCAAAGTCAAGGTCCTTGACCTTCACAACAACAGGATAATGAGCATCCCTAAAGATGTCACCCACCTGCAGGCTTT
 440 lySerValPheArgCysLeuProProLysValLysValLeuAspLeuHisAsnAsnArgl leMetSerl leProLysAspValThrHisLeuGlnAlaLe
7901 GCAGGAACTCAATGTAGCATCCAACTCCTTAACTGACCTTCCTGGGTGCGGGGCCTTCAGCAGCCTTTCTGTGCTGGTCATCGACCATAACTCAGTTTCC
 473 uGlnGluLeuAsnValAlaSerAsnSerLeuThrAspLeuProGlyCysGlyAlaPheSerSerLeuSerValLeuVal I leAspHisAsnSerValSer
8001 CATCCCTCTGAGGATTTCTTCCAGAGCTGTCAGAATATTAGATCCCTAACAGCGGGAAACAACCCATTCCAATGCACATGTGAGCTGAGGGACTTTGTCA
 507 HisProSerGluAspPhePheGlnSerCysGlnAsnl leArgSerLeuThrAlaGlyAsnAsnProPheGlnCysThrCysGluLeuArgAspPheValL
8101 AGAACATAGGCTGGGTAGCAAGAGAAGTGGTGGAGGGCTGGCCTGACTCTTACAGGTGTGACTACCCAGAAAGCTCTAGGGGAACTGCACTGAGGGACTT
 540 ysAsnl leGlyTrpValAlaArgGluValValGluGlyTrpProAspSerTyrArgCysAspTyrProGluSerSerArgGlyThrAlaLeuArgAspPh
8201 CCACATGTCTCCACTATCCTGTGATACTGTTCTGCTGACTGTCACCATCGGGGCCACTATGCTGGTGCTGGCTGTCACTGGGGCTTTCCTCTGTCTCTAC
 573 eHisMetSerProLeuSerCysAspThrValLeuLeuThrValThrl leGlyAlaThrMetLeuValLeuAlaValThrGlyAlaPheLeuCysLeuTyr
8301 TTTGACCTGCCCTGGTATGTGAGGATGCTGTGTCAGTGGACACAGACCAGGCACAGGGCCAGGCACATCCCCTTAGAGGAACTCCAGAGAAACCTCCAGT
 607 PheAspLeuProTrpTyrValArgMetLeuCysGlnTrpThrGlnThrArgHisArgAlaArgHisl leProLeuGluGluLeuGlnArgAsnLeuGlnP
8401 TCCATGCTTTTGTCTCATACAGTGGGCATGATTCTGCCTGGGTGAAGAACGAATTACTACCCAACCTAGAGAAAGATGACATCCAGATTTGCCTCCATGA
 640 heHisAlaPheValSerTyrSerGlyHisAspSerAlaTrpValLysAsnGluLeuLeuProAsnLeuGluLysAspAspl leGlnl leCysLeuHisGl
8501 GAGGAACTTTGTCCCTGGCAAGAGCATTGTGGAGAACATCATCAATTTCATTGAGAAGAGTTACAAGTCCATCTTTGTGCTGTCTCCCCACTTCATCCAG
 673 uArgAsnPheValProGlyLysSerl leValGluAsnl lel leAsnPhel leGluLysSerTyrLysSerl lePheValLeuSerProHisPhel leGln
8601 AGTGAGTGGTGTCATTATGAACTCTATTTTGCCCATCACAATCTCTTCCATGAAGGCTCTGATAACTTAATCCTCATCTTGCTGGCACCCATTCCCCAGT
 707 SerGluTrpCysHisTyrGluLeuTyrPheAlaHisHisAsnLeuPheHisGluGlySerAspAsnLeul leLeul leLeuLeuAlaProl leProGlnT
8701 ACTCCATCCCTACCAATTACCACAAGCTCAAAACTCTCATGTCACGAAGGACCTATCTGGAATGGCCCACAGAGAAGAACAAGCATGGACTTTTTGGGC
 740 yrSerl leProThrAsnTyrHisLysLeuLysThrLeuMetSerArgArgThrTyrLeuGluTrpProThrGluLysAsnLysHisGlyLeuPheTrpAl
8801 AAACCTAAGAGCATCCATTAATGTTAAGCTGGTTAACCAGGCAGAAGGAACGTGTTACACACAGCAATAAGAATATCCACCGCTAGGAGCAGGTTTCCCC
 773 aAsnLeuArgAlaSerl leAsnValLysLeuValAsnGlnAlaGluGlyThrCysTyrThrGlnGln•••
                                                    XbaI (8948)
8901 AATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCG
9001 AGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACAC
9101 GGGCCCGTCATGTGTGCAACCCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGG
                                                                           Eco47III (9274)
9201 ATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTG
        BstEII (9306)
9301 AATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAG
9401 TATAATACGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGC
                                   1 MetLysThrPheAsnl leSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysl leThrMetL
9501 TCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCAC
  24 euTyrGluAspAsnLysHisHisValGlyAlaAlal leArgThrLysThrGlyGlul lel leSerAlaValHisl leGluAlaTyrl leGlyArgValTh
9601 TGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTG
  57 rValCysAlaGluAlal leAlal leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrl leValAlaValArgHisProTyrSerAspGluVal
9701 GACAGATCCATCAGGGTGGTCAGCCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGC
  91 AspArgSerl leArgValValSerProCysGlyMetCysArgGluLeul leSerAspTyrAlaProAspCysPheValLeul leGluMetAsnGlyLysL
9801 TGGTCAAAACCACCATTGAGGAACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACT
 124 euValLysThrThrl leGluGluLeul leProLeuLysTyrThrArgAsn•••
                                                  MfeI (9943)
9901 CTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAA
10001 ACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAA
10101 TGGAAACAACTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTT
10201 AGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCC
10301 CCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGT
10401 CACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG
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