# pDUO-mTLR6/TLR2

A plasmid coexpressing the murine TLR6 and TLR2 genes

Catalog code: pduo-mtlr6tlr2

https://www.invivogen.com/pduo-tlr6-tlr2

For research use only

Version 20H25-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pDUO-mTLR6/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 TLR6** (2418 bp) / **Murine TLR2** (2352 bp)

TLR6 is expressed in spleen and BPL and, similarly to TLR1, acts as a co-receptor. Studies with dominant negative receptors have shown that TLR6 cooperates with TLR2 to recognize peptidoglycan and the yeast cell wall particle, zymosan[1]. Furthermore, TLR6- and TLR2-deficient mice were reported to be hyporesponsive to mycoplasma macrophage-activating lipopeptide-2 kD (MALP-2), a diacylated lipoprotein, suggesting that TLR2 and TLR6 coordinate the response to this ligand. By contrast, TLR2 is able to recognize bacterial lipoproteins triacylated at the N-terminus cysteine residue[2]. Thus TLR6 appears to discriminate between the N-terminal lipoylated structures of MALP-2 and lipopeptides derived from other bacteria.

- **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

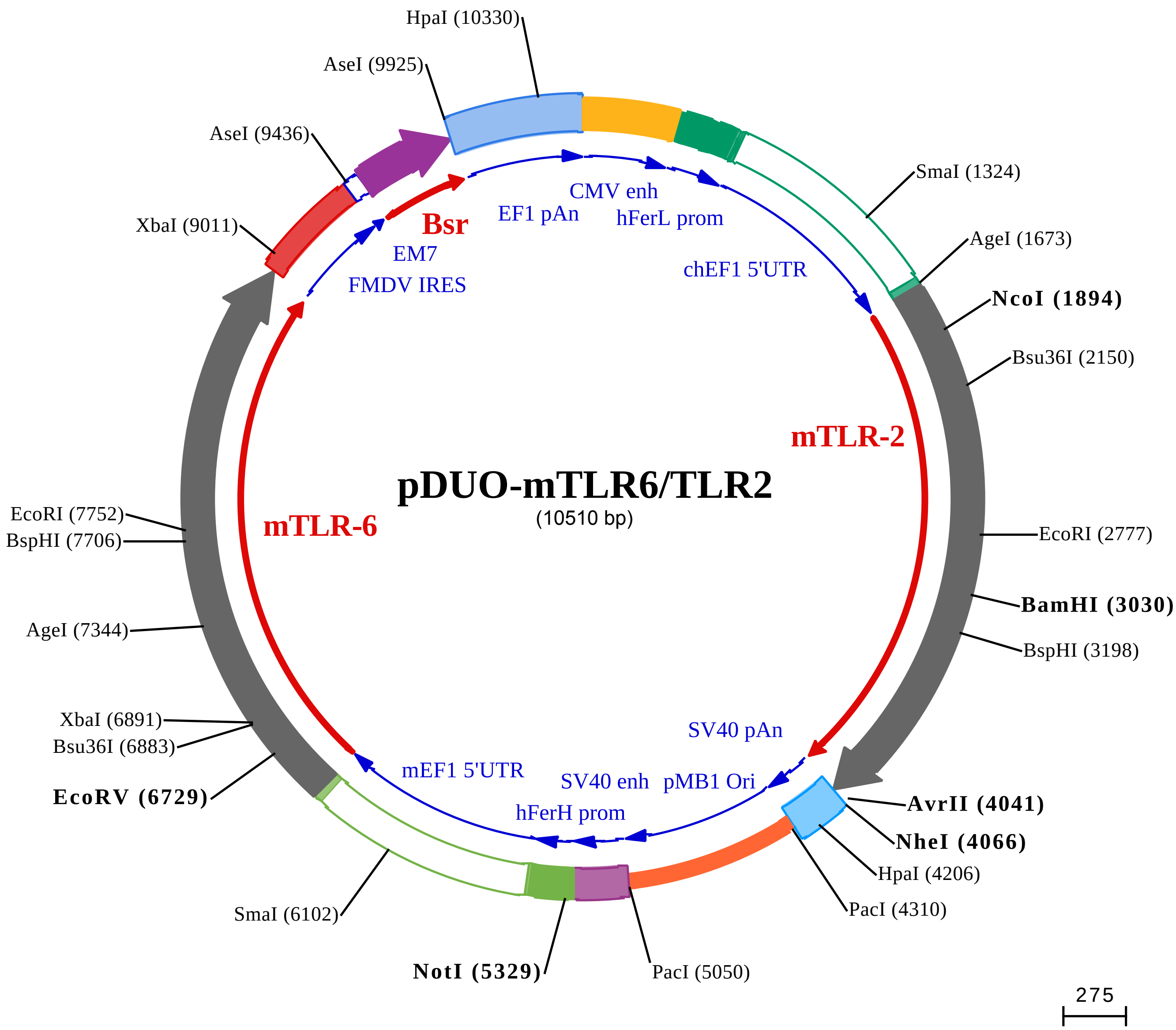
Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

### 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. **Ozinsky A. *et al.*, 2000.** The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. PNAS 97(25):13766-71.
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.



pDUO-mTLR6/TLR2
(10510 bp)
HpaI (10330)
AseI (9925)
AseI (9436)
XbaI (9011)
SmaI (1324)
AgeI (1673)
NcoI (1894)
Bsu36I (2150)
EcoRI (2777)
BamHI (3030)
BspHI (3198)
AvrII (4041)
NheI (4066)
HpaI (4206)
PacI (4310)
PacI (5050)
NotI (5329)
SmaI (6102)
EcoRV (6729)
Bsu36I (6883)
XbaI (6891)
AgeI (7344)
BspHI (7706)
EcoRI (7752)
Bsr
EF1 pAn
CMV enh
hFerL prom
chEF1 5'UTR
EM7
FMDV IRES
mTLR-2
mTLR-6
SV40 pAn
pMB1 Ori
SV40 enh
hFerH prom
mEF1 5'UTR
275

1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC

201 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG

401 GGAGTTTGTTTTGACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGG

501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCC

601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGg

701 taagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc

801 ttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcg

901 ctggggccgccgcgtgctaatctggtggcaccttcgcgcctgtctcgctgctttcgctaagtctctagccatttaaaatttttgataaccagctgcgacg

1001 ctttttttctggcgagatagtcttgtaaatgcgggccaggatctgcacactggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtccc

1101 agcgcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaaactggccggcctgctctggtgcctggcctcgc

1201 gccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagc

SmaI (1324)

1301 tcaaaatggaggacgcggcgcccgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccttcctcatccgtcgcttcatgtgactcca

1401 cggagtaccgggcgccgtccaggcacctcgattagttgtcgagcttttggagtacgtcgtctttaggttggggggaggggttttatgcgatggagtttcc

1501 ccacactgagtgggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttgcctcattc

AgeI (1673)

1601 tcaagcctcagacagtggttcaaagtttttttcttccatttcagGTGTCGTGAAAACTACCCCTAAAAGCCACCGGTAGGAGGGCCAGCATGCTACGAGC

1 MetLeuArgAl

1701 TCTTTGGCTCTTCTGGATCTTGGTGGCCATAACAGTCCTCTTCAGCAAACGCTGTTCTGCTCAGGAGTCTCTGTCATGTGATGCTTCTGGGGTGTGTGAT

4 aLeuTrpLeuPheTrpIleLeuValAlaIleThrValLeuPheSerLysArgCysSerAlaGlnGluSerLeuSerCysAspAlaSerGlyValCysAsp

**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

EcoRI (2777)

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 isGlyHisArgLeuGlnAspAlaArgProSerValLeuGluCysHisGlnAlaAlaLeuValSerGlyValCysCysAlaLeuLeuLeuLeuIleLeLe

3501 CGTAGGTGCCCTGTGCCACCATTTCCACGGACTGTGGTACCTGAGAATGATGTGGGCGTGGCTCCAGGCCAAGAGGAAGCCCAAGAAAGCTCCCTGCAGG
604 uValGlyAlaLeuCysHisHisPheHisGlyLeuTrpTyrLeuArgMetMetTrpAlaTrpLeuGlnAlaLysArgLysProLysLysAlaProCysArg
3601 GACGTTTGCTATGATGCCTTTGTTTCCTACAGTGAGCAGGATTCCCATTGGGTGGAGAACCTCATGGTCCAGCAGCTGGAGAACTCTGACCCGCCCTTTA
638 AspValCysTyrAspAlaPheValSerTyrSerGluGlnAspSerHisTrpValGluAsnLeuMetValGlnGlnLeuGluAsnSerAspProProPheL
3701 AGCTGTGTCTCCACAAGCGGGACTTCGTTCCGGGCAAATGGATCATTGACAACATCATCGATTCCATCGAAAAGAGCCACAAAACTGTGTTCGTGCTTTC
671 ysLeuCysLeuHisLysArgAspPheValProGlyLysTrpIleIleAspAsnIleIleAspSerIleGluLysSerHisLysThrValPheValLeuSe
3801 TGAGAACTTCGTACGGAGCGAGTGGTGCAAGTACGAACTGGACTTCTCCCACTTCAGGCTCTTTGACGAGAACAACGACGCGGCCATCCTTGTTTTGCTG
704 rGluAsnPheValArgSerGluTrpCysLysTyrGluLeuAspPheSerHisPheArgLeuPheAspGluAsnAsnAspAlaAlaIleLeuValLeuLeu
3901 GAGCCCATTGAGAGGAAAGCCATTCCCCAGCGCTTCTGCAAACTGCGCAAGATAATGAACACCAAGACCTACCTGGAGTGGCCCTTGGATGAAGGCCAGC
738 GluProIleGluArgLysAlaIleProGlnArgPheCysLysLeuArgLysIleMetAsnThrLysThrTyrLeuGluTrpProLeuAspGluGlyGlnG

**AvrII (4041)** **NheI (4066)**

4001 AGGAAGTGTTTTGGGTAAATCTGAGAACTGCAATAAAGTCCTAGGTTCTCCACCCAGTTCCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAG
771 lnGluValPheTrpValAsnLeuArgThrAlaIleLysSer•••
4101 TTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAC

HpaI (4206)

4201 AAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGA

PacI (4310)

4301 AATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
4401 CTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
4501 GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC
4601 CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC
4701 GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCAC
4801 GCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTT
4901 TATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCT

PacI (5050)

5001 TTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACC
5101 TTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA
5201 ATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGT

**NotI (5329)**

5301 TCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGGGCGGGGTCCCGCGCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGC
5401 TGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAG
5501 gtgaggggcgggtgtggcttccgcgggccgccgagctggaggtcctgctccgagcgggccgggccccgctgtcgtcggcggggattagctgcgagcattc
5601 ccgcttcgagttgcgggcggcgcgggaggcagagtgcgaggcctagcggcaaccccgtagcctcgcctcgtgtccggcttgaggcctagcgtggtgtccg
5701 cgccgccgccgcgtgctactccggccgcactctggtctttttttttttttgttgttgttgccctgctgccttcgattgccgttcagcaataggggctaaca
5801 aagggagggtgcggggcttgctcgcccggagcccggagaggtcatggttggggaggaatggagggacaggagtggcggctggggcccgcccgccttcgga
5901 gcacatgtccgacgccacctggatggggcgaggcctggggtttttcccgaagcaaccaggctggggttagcgtgccgaggccatgtggccccagcacccg

SmaI (6102)

6001 gcacgatctggcttggcggcgccgcgttgccctgcctccctaactagggtgaggccatcccgtccggcaccagttgcgtgcgtggaaagatggccgctcc
6101 cgggccctgttgcaaggagctcaaaatggaggacgcggcagcccggtggagcgggcgggtgagtcacccacacaaaggaagagggcctggtccctcaccg
6201 gctgctgcttcctgtgaccccgtggtcctatcggccgcaatagtcacctcgggcttttgagcacggctagtcgcggcgggggggaggggatgtaatggcgt
6301 tggagtttgttcacatttggtgggtggagactagtcaggccagcctggcgctggaagtcattttggaatttgtccccttgagttttgagcggagctaat
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1 MetValLysSerLeuTrp
6501 GATAGCCTCTGCAACATGAGCCAAGACAGAAAACCCATCGTGGGGAGTTTCCACTTTGTTTGCGCCCTGGCCTTAATAGTCGGAAGCATGACCCCGTTCT
7 AspSerLeuCysAsnMetSerGlnAspArgLysProIleValGlySerPheHisPheValCysAlaLeuAlaLeuIleValGlySerMetThrProPheS
6601 CTAATGAACTTGAGTCTATGGTAGACTATTCAAACAGGAACCTTACTCATGTCCCCAAAGACCTGCCACCAAGAACAAAAGCCCTGAGTCTGTCTCAAAA
40 erAsnGluLeuGluSerMetValAspTyrSerAsnArgAsnLeuThrHisValProLysAspLeuProProArgThrLysAlaLeuSerLeuSerGlnAs

**EcoRV (6729)**

6701 CTCTATATCTGAGCTTCGGATGCCTGATATCAGCTTTCTGTCAGAGCTGAGAGTTCTGAGACTCTCCCACAACAGGATACGGAGCCTTGATTTCCATGTA
73 nSerIleSerGluLeuArgMetProAspIleSerPheLeuSerGluLeuArgValLeuArgLeuSerHisAsnArgIleArgSerLeuAspPheHisVal

XbaI (6891)
Bsu36I (6883)

6801 TTCTTGTTCAATCAGGACTTAGAATACCTGGATGTCTCACACAATCGGTTGCAAAACATCTCTTGCTGCCCTATGGCGAGCCTGAGGCATCTAGACCTCT
107 PheLeuPheAsnGlnAspLeuGluTyrLeuAspValSerHisAsnArgLeuGlnAsnIleSerCysCysProMetAlaSerLeuArgHisLeuAspLeuS
6901 CATTCAATGACTTTGATGTACTGCCTGTGTGTAAGGAATTTGGCAACCTGACGAAGCTGACTTTCCTGGGATTAAGTGCTGCCAAGTTCCGACAACTGGA
140 erPheAsnAspPheAspValLeuProValCysLysGluPheGlyAsnLeuThrLysLeuThrPheLeuGlyLeuSerAlaAlaLysPheArgGlnLeuAs

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7001  TCTGCTCCCAGTTGCTCACTTGCATCTAAGCTGCATTCTTCTGGACTTAGTGAGTCATCATATAAAAGGCGGGGAAACAGAAAGTCTTCAGATTCCCAAT
 173▸ pLeuLeuProValAlaHisLeuHisLeuSerCysI leLeuLeuAspLeuValSerHisHisI leLysGlyGlyGluThrGluSerLeuGlnI leProAsn
7101  ACCACCGTTCTCCATTTGGTCTTTCATCCAAATAGCTTGTTCTCTGTTCAAGTGAACATGTCTGTAAACGCTTTAGGACATTTACAACTGAGTAATATTA
 207▸ ThrThrValLeuHisLeuValPheHisProAsnSerLeuPheSerValGlnValAsnMetSerValAsnAlaLeuGlyHisLeuGlnLeuSerAsnI leL
7201  AATTGAATGATGAAAACTGTCAAAGGTTAATGACATTTTTATCAGAACTCACCAGAGGTCCAACCTTATTGAATGTGACCCTCCAGCACATAGAAACAAC
 240▸ ysLeuAsnAspGluAsnCysGlnArgLeuMetThrPheLeuSerGluLeuThrArgGlyProThrLeuLeuAsnValThrLeuGlnHisI leGluThrTh
                                                     AgeI (7344)
7301  CTGGAAGTGCTCGGTTAAACTTTTCCAATTCTTTTGGCCCCGACCGGTGGAGTACCTCAATATTTACAACTTAACGATAACTGAGAGAATCGACAGGGAA
 273▸ rTrpLysCysSerValLysLeuPheGlnPhePheTrpProArgProValGluTyrLeuAsnI leTyrAsnLeuThrI leThrGluArgI leAspArgGlu
7401  GAATTTACTTACTCGGAGACAGCACTGAAGTCACTGATGATAGAGCACGTCAAAAACCAAGTGTTCCTCTTTTCAAAGGAGGCGCTATACTCGGTGTTTG
 307▸ GluPheThrTyrSerGluThrAlaLeuLysSerLeuMetI leGluHisValLysAsnGlnValPheLeuPheSerLysGluAlaLeuTyrSerValPheA
7501  CTGAGATGAACATCAAGATGCTCTCTATCTCAGACACCCCTTTCATCCACATGGTGTGCCCGCCATCCCCAAGCTCATTTACATTTCTGAACTTTACCCA
 340▸ laGluMetAsnI leLysMetLeuSerI leSerAspThrProPheI leHisMetValCysProProSerProSerSerPheThrPheLeuAsnPheThrGl
7601  GAATGTTTTTACTGACAGTGTTTTTCAAGGCTGTTCCACCTTAAAGAGATTGCAGACACTTATCTTACAAAGGAATGGTTTGAAGAACTTTTTTAAAGTA
 373▸ nAsnValPheThrAspSerValPheGlnGlyCysSerThrLeuLysArgLeuGlnThrLeuI leLeuGlnArgAsnGlyLeuLysAsnPhePheLysVal
          BspHI (7706)                                           EcoRI (7752)
7701  GCTCTCATGACTAAGAATATGTCCTCTCTGGAAACTTTGGATGTTAGTTTGAATTCTTTGAACTCTCATGCATATGACAGGACATGCGCCTGGGCTGAGA
 407▸ AlaLeuMetThrLysAsnMetSerSerLeuGluThrLeuAspValSerLeuAsnSerLeuAsnSerHisAlaTyrAspArgThrCysAlaTrpAlaGluS
7801  GCATATTGGTGTTGAATTTGTCTTCGAATATGCTTACAGGCTCTGTCTTCAGATGCTTACCTCCCAAGGTCAAGGTCCTTGACCTTCACAACAACAGGAT
 440▸ erI leLeuValLeuAsnLeuSerSerAsnMetLeuThrGlySerValPheArgCysLeuProProLysValLysValLeuAspLeuHisAsnAsnArgI l
7901  AATGAGCATCCCTAAAGATGTCACCCACCTGCAGGCTTTGCAGGAACTCAATGTAGCATCCAACTCCTTAACTGACCTTCCTGGGTGTGGGGCCTTCAGC
 473▸ eMetSerI leProLysAspValThrHisLeuGlnAlaLeuGlnGluLeuAsnValAlaSerAsnSerLeuThrAspLeuProGlyCysGlyAlaPheSer
8001  AGCCTTTCTGTGCTGGTCATCGACCATAACTCAGTTTCCCATCCCTCTGAGGATTTCTTCCAGAGCTGTCAGAATATTAGATCCCTAACAGCGGGAAACA
 507▸ SerLeuSerValLeuVal I leAspHisAsnSerValSerHisProSerGluAspPhePheGlnSerCysGlnAsnI leArgSerLeuThrAlaGlyAsnA
8101  ACCCATTCCAATGCACATGTGAGCTGAGGGACTTTGTCAAGAACATAGGCTGGGTAGCAAGAGAAGTGGTGGAGGGCTGGCCTGACTCTTACAGGTGTGA
 540▸ snProPheGlnCysThrCysGluLeuArgAspPheValLysAsnI leGlyTrpValAlaArgGluValValGluGlyTrpProAspSerTyrArgCysAs
8201  CTACCCAGAAAGCTCTAAGGGAACTGCACTGAGGGACTTCCACATGTCTCCACTGTCCTGTGATACTGTTCTGCTGACTGTCACCATCGGGGCCACTATG
 573▸ pTyrProGluSerSerLysGlyThrAlaLeuArgAspPheHisMetSerProLeuSerCysAspThrValLeuLeuThrValThrI leGlyAlaThrMet
8301  CTGGTGCTGGCTGTCACTGGGGCTTTCCTCTGTCTCTACTTTGACCTGCCCTGGTATGTGAGGATGCTGTGTCAGTGGACACAGACCAGGCACAGGGCCA
 607▸ LeuValLeuAlaValThrGlyAlaPheLeuCysLeuTyrPheAspLeuProTrpTyrValArgMetLeuCysGlnTrpThrGlnThrArgHisArgAlaA
8401  GGCACATCCCCTTAGAGGAACTCCAGAGAAACCTCCAGTTCCATGCTTTTGTCTCATACAGTGAGCATGATTCTGCCTGGGTGAAGAACGAATTACTACC
 640▸ rgHisI leProLeuGluGluLeuGlnArgAsnLeuGlnPheHisAlaPheValSerTyrSerGluHisAspSerAlaTrpValLysAsnGluLeuLeuPr
8501  CAACCTAGAGAAAGATGACATCCGGGTTTGCCTCCATGAGAGGAACTTTGTCCCTGGCAAGAGCATTGTGGAGAACATCATCAATTTCATTGAGAAGAGT
 673▸ oAsnLeuGluLysAspAspI leArgValCysLeuHisGluArgAsnPheValProGlyLysSerI leValGluAsnI leI leAsnPheI leGluLysSer
8601  TACAAGGCCATCTTTGTGCTGTCTCCCCACTTCATCCAGAGTGAGTGGTGCCATTATGAACTCTATTTTGCCCATCATAATCTCTTCCATGAAGGCTCTG
 707▸ TyrLysAlaI lePheValLeuSerProHisPheI leGlnSerGluTrpCysHisTyrGluLeuTyrPheAlaHisHisAsnLeuPheHisGluGlySerA
8701  ATAACTTAATCCTCATCTTGCTGGAACCCATTCTACAGAACAACATTCCCAGTAGATACCACAAGCTGCGGGCTCTCATGGCACAGCGGACTTACTTGGA
 740▸ spAsnLeuI leLeuI leLeuLeuGluProI leLeuGlnAsnAsnI leProSerArgTyrHisLysLeuArgAlaLeuMetAlaGlnArgThrTyrLeuGl
8801  ATGGCCTACTGAGAAGGGCAAACGTGGGCTGTTTTGGGCCAACCTTAGAGCTTCATTTATTATGAAGTTAGCCTTAGTCAATGAGGATGATGTGAAAACT
 773▸ uTrpProThrGluLysGlyLysArgGlyLeuPheTrpAlaAsnLeuArgAlaSerPheI leMetLysLeuAlaLeuValAsnGluAspAspValLysThr
8901  TGAAACTTGGGTTTCTAACTTAATAAACTGTCAACCTGGGCTCTGCTAGGAGCAGGTTTCCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGT
 807▸ •••
                XbaI (9011)
9001  CTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCA

9101  TGACGGCCGTGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCCGTCATGTGTGCAACCCCAGCACGGCGACTTT

9201  ACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTC

9301  GGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAAT

                                           AseI (9436)
9401  TACTGACCCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGA
                                                                                                   1▸ MetLysT
9501  CCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCAT
   3▸ hrPheAsnI leSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI l
9601  CAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTG
  36▸ eArgThrLysThrGlyGluI leI leSerAlaValHisI leGluAlaTyrI leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaVal
9701  AGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCCTGTGGCATGT
  70▸ SerAsnGlyGlnLysAspPheAspThrI leValAlaValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetC
9801  GCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGGAACTCATCCCCCTCAA
 103▸ ysArgGluLeuI leSerAspTyrAlaProAspCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLy
                               AseI (9925)
9901  GTACACCAGGAACTAAACCTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTT
 136▸ sTyrThrArgAsn•••
10001 GTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACT

10101 TTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAAACAACTTGACCAAAAATTTGTCACAGAATTTT

10201 GAGACCCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGG

                                    HpaI (10330)
10301 AAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAG

10401 CTATGGGAGGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCA

10501 TAGAAAGCTG
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