

pDUO-hTLR6/TLR2

A plasmid coexpressing the human TLR6 and TLR2 genes

Catalog code: pduo-htrl6tlr2

<https://www.invivogen.com/pduo-trl6-trl2>

For research use only

Version 20H25-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-hTLR6/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

- **Human TLR6** (2388 bp) / **Human 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, zymosan¹. 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 residue². 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 protein³. 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 plasmids⁴.

- **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 enhancer⁵.

- **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.

TECHNICAL SUPPORT

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- **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 expression⁷.
- **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.

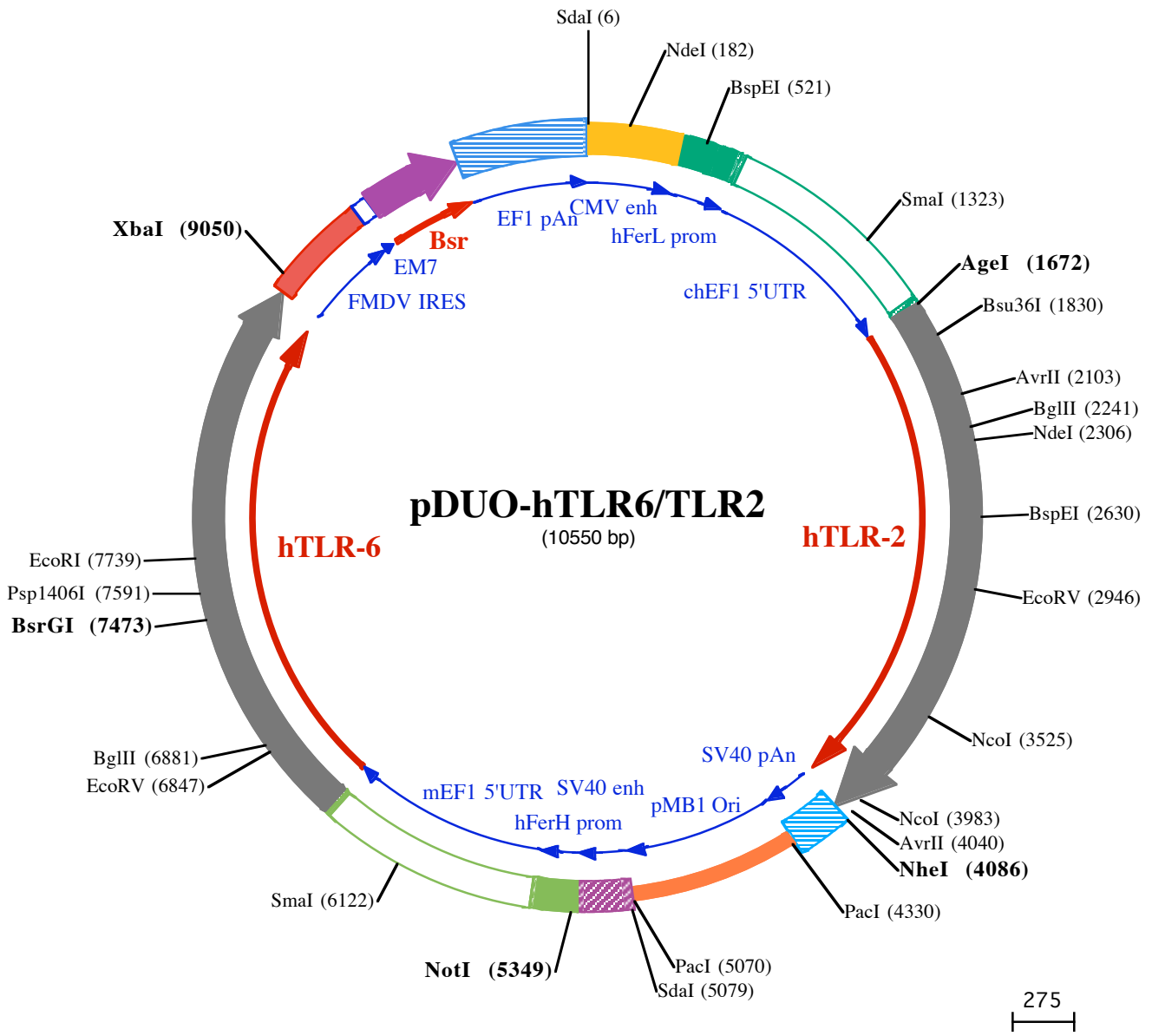
References

1. **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.

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SdaI (6)
1 CCTGCAGGCGTTACATAA...
NdeI (182)
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTCCAAAGTCTCCACCCCAATTGACGTCAATG
401 GGAGTTTGTGTTGACTAGTCAGGGCCCCAACCCCCCAAGCCCCATTTACAACACGCTGGCGCTACAGGCGCGTGACTTCCCTTGTCTTTGGGCGGG
BspEI (521)
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTGGCCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCTCCCGAGCGCCCTGCC
601 TCCGAGGGCGGGCCACCATAAAAGAAGCCGCCCTAGCCACGTCCCTCGCAGTTCGGCGGTCCCGCGGTCTGTCTCAAGCTTGGCCGAGAACAACAGg
701 taagtgcctgtgtggttcccgcggcctggcctctttacgggttatggccttgctgcttgaattacttccatgcccctggctgcagtacgtgattc
801 ttgatcccagacttcgggttgaagtgggtgggagagttcaggccttgctgcttaaggagccccttcgctcgtgcttgagttgaggcctggcttgggag
901 ctggggcccgctgctaatctggtggcacccttcgctcgtctcgtctctcgtcttaagtctctagccatttaaaatgttataaccagctgcgagc
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1201 gccgctgtatgcgccgctggcggcagggtggccggtggcaccagttgctgagcggaaagatggcggctcccggcctgctgcaggggagc
SmaI (1323)
1301 tcaaaatggaggacgccccgggagagcgggggggtgagtcacccacacaaaggaaaggcctttctctctcatcctgcttcatgtgactcca
1401 cggagtaccgggcccgtccaggcaccctgattagttgctgagcctttggagtagctgctcttaggttgggggagggggtttatgcatggagtttcc
1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggatttgcctttttgagttggacttgcctcattc
AgeI (1672)
1601 tcaagcctcagacagtggttcaaggttttttcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGTAGGAGGGCCAGCATGCCACATAC
1701 TTTGTGGATGGTGTGGGTCTTGGGGTTCATCATCAGCCTCTCAAGGAAGAATCCTCCAATCAGGCTTCTCTGTCTTGTGACCGCAATGGTATCTGCAAG
4rLeuTrpMetValTrpValLeuGlyValIleIleSerLeuSerLysGluGluSerSerAsnGlnAlaSerLeuSerCysAspArgAsnGlyIleCysLys
Bsu36I (1830)
1801 GGCAGCTCAGGATCTTTAACTCCATTCCCTCAGGGCTCACAGAAGCTGAAAAAGCCTTGACCTGTCCAACAACAGGATCACCTACATTAGCAACAGTG
38GlySerSerGlySerLeuAsnSerIleProSerGlyLeuThrGluAlaValLysSerLeuAspLeuSerAsnAsnArgIleThrTyrIleSerAsnSerA
1901 ACCTACAGAGGTGTGTGAACCTCAGGCTCTGGTGTGACATCCAATGGAATTAACACAATAGAGGAAGATTCTTTTCTCCCTGGGCACTTTGAACA
71spLeuGlnArgCysValAsnLeuGlnAlaLeuValLeuThrSerAsnGlyIleAsnThrIleGluGluAspSerPheSerSerLeuGlySerLeuGluHi
2001 TTTAGACTTATCCTATAATTACTTATCTAATTTATCGTCTTCTGGTTCAGGCCCTTTCTTTTAACTTCTTAACTTACTGGGAAATCCTTACAAA
104sLeuAspLeuSerTyrAsnTyrLeuSerAsnLeuSerSerSerTrpPheLysProLeuSerSerLeuThrPheLeuAsnLeuLeuGlyAsnPProTyrLys
AvrII (2103)
2101 ACCCTAGGGGAAACATCTCTTTTTCTCATCTCACAAAATTGCAATCCTGAGAGTGGGAAATATGGACACCTTCACTAAGATTCAAAGAAAAGATTTTG
138ThrLeuGlyGluThrSerLeuPheSerHisLeuThrLysLeuGluNileuArgValGlyAsnMetAspThrPheThrLysIleGluNArgLysAspPheA
BglIII (2241)
2201 CTGGACTTACCTTCTTGGAGAACTTGGATTGATGCTTCAGATCTACAGACTATGAGCAAAAAGTTTGAAGTCAATTCAGAATGTAAGTCACTCTGAT
171IleGlyLeuThrPheLeuGluGluLeuGluIleAspAlaSerAspLeuGlnSerTyrGluProLysSerLeuLysSerIleGluNAsnValSerHisLeuI
NdeI (2306)
2301 CCTTCATATGAAGCAGCATATTTACTGCTGGAGATTTTGTAGATGTTACAAGTTCGTTGGAATGTTTGAACCTGCGAGACTGATTGGACACTTTC
204eLeuHisMetLysGluNHisIleLeuLeuLeuGluIlePheValAspValThrSerSerValGluCysLeuGluLeuArgAspThrAspLeuAspThrPhe
2401 CATTHTTCAGAACTATCCACTGGTGAACAATTCATTGATTAATAAAGTTTACATTTAGAAATGTGAAAATCACCGATGAAAGTTTGTTCAGGTTATGA
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2501 AACTTTGAATCAGATTTCTGGATTGTTAGAATTAGAGTTTGTAGCTGTACCCCTAATGGAGTTGTAATTTTAGAGCATCTGATAATGACAGAGTTAT
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BspEI (2630)
2601 AGATCCAGGTAAGTGAACCGTAAACAATCCGGAGGCTGCATATTCGAAGTTTACTTATTTATGATCTGAGCACTTTATATTCACTTACAGAAAAG
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2701 GTTAAAAGAATCACAGTAGAAAACAGTAAAGTTTCTGTTCTTGTACTTTCACAACATTTAAAATCATTAGAATCTGGATCTCAGTGAAAAT
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EcoRV (2946)
2901 AACCGGAGAGACTTGTCTCACTCTGAAAACTTACTAACAATGATATCAGTAAGAAGTATTTTACTTCTATGCTGAAACTTGTCAAGTGGCCAGAAAAG
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3001 ATGAAATATTTGAACCTATCCAGCACACGAATACACAGTGAACAGGCTGCATCCCAAGACTGGAAAATTTAGATGTTAGCAACAACAATCTCAATT
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3101 TATTTTCTTGAATTTGCCGCAACTCAAAGAATTTATATTTCCAGAATAAGTTGATGACTCTACCAGATGCCTCCCTCTTACCATGTTACTAGTATT
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538SerCysGluPheLeuSerPheThrGluNgluGluNgluAlaLeuAlaLysValLeuIleAspTrpProAlaAsnTyrLeuCysAspSerProSerHisValA
3401 GTGCCAGCAGTTTCAAGATGTCGGCTCTCGGTGTCGGAATGTCACAGGACAGCACTGGTGTCTGGCATGTGCTGTCTGCTCTGCTGATCTGCT
571rGlyGluNgluValGluAspValArgLeuSerValSerGluCysHisArgThrAlaLeuValSerGlyMetCysCysAlaLeuPheLeuLeuIleLeuLe

NcoI (3525)

3501 CACGGGGTCTGTGCCACCGTTTCCATGGCCTGTGGTATATGAAAATGATGTGGCCTGGCTCCAGGCCAAAAGGAAGCCAGGAAAGCTCCAGCAGG
 604 uThr GlyVal LeuCysHis sArgPheHis sGlyLeuTrpTyrMetLysMetMetT rPAl aTrpLeuGlnAl aLysArgLysP rArgLysAl aP rSerArg
 3601 AACATCTGCTATGATGCATTTGTTTCTTACAGTGAGCAGGATGCCTACTGGGTGGAGAACCCTATGGTCCAGGAGCTGGAGAACCCTCAATCCCCCTTCA
 638 AsnI leCysTyrAspAl aPheVal Ser TyrSer Gl uGlnAspAl aTyrTrpVal Gl uAsnLeuMetVal Gl nGl uAsnPheAsnP rP rPheL
 3701 AGTTGTGTCTTCATAAGCGGGACTTCATTCTGGCAAGTGGATCATTGACAATATCATTGACTCCATTGAAAAGAGCCAAAACTGTCTTTGTGCTTTC
 671 y sLeuCysLeuHis sLysArgAspPheI leP rOgl yLysTrpI leI leAspAsnI leI leAspSer I leGl uLysSer His sLysThr Val PheVal LeuSe
 3801 TGAACACTTTGTGAAGAGTGAGTGGTGAAGTATGAAGTGGACTTCTCCATTTCCGCTTTTGTGATGAGAACAATGATGCTGCCATTCTCATTCTCTG
 704 r Gl uAsnPheVal LysSer Gl uTrpCysLysTyrGl uLeuAspPheSer His sPheArgLeuPheAspGl uAsnAsnAspAl aAl aI leLeuI leLeuLeu

NcoI (3983)

3901 GAGCCCATTGAGAAAAAGCCATTTCCACAGCGTTCTGCAAGCTGCGGAAGATAATGAACACCAAGACCTACCTGGAGTGGCCATGGACGAGGCTCAGC
 738 Gl uP rOl leGl uLysLysAl aI leP rOgl nArgPheCysLysLeuArgLysI leMe tAsnThr Lys Thr TyrLeuGl uTrpP rOme tAspGl uAl aGl nA

AvrII (4040)

NheI (4086)

4001 GGGAAGGATTTTGGGTAACTCTGAGAGCTGCGATAAAGTCTAGTTCATATTTAAGACCAAGTCTTTGTCTAGTGGGATCTTGCTAGCTGGCCAGC
 771 r gGl uGl uPheTrpVal AsnLeuArgAl aAl aI leLysSer ●●●

4101 ATGATAAGATACATTGATGAGTTTGGACAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA
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PacI (4330)

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4401 TCAAAGGATCTTCTGAGATCCTTTTTCTGCGCGTAATCTGCTGCTTGC AAAACAAAAAACCACCGCTACCAGCGGTGGTTGTTTCCGGATCAAGA

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4701 AGTTACC GGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCG

4801 TGAGCTATGAGAAAAGCGCCACGCTTCCGAAGGGAGAAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAAACAGGAGAGCGACGAGGGAGCTTCCA

4901 GGGGGAACGCTGATCTTTATAGTCTCTGCGGTTTCGCCACTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGGGGAGCCTATGGA

PacI (5070) SdaI (5079)

5001 AAAACGCCAGCAACGCGGCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAA

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NotI (5349)

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5601 ggggattagctgcgagcattccgcctcaggttgcgggccccgagctggaggcttagcggcaacccccgtagcctcgcctcgtgtccggct

5701 tgaggcctagcgtggtgtccgcgccgccccgcgctgctactccggccgcactctggtctttttttttttggtgtgtgcccctgctgccttcgattgccc

5801 gttcagcaatagggtctaacaaggagggtgccccgtgctgcggagccccggagaggtcatggttgggaggaaatggaggacagaggtggcggc

5901 tggggccccgccccttcggagcacatgtccgagccccctggatggggcgaggcctggggtttttcccgaagcaaccaggctggggttagctgcccag

6001 gccatgtggccccagcaccggcacgatctggcctggcggcggcggcctgcctccctaactagggtgaggccatccccgtccggaccaggttgcgt

SmaI (6122)

6101 gcgtgaaagatggcgcctccgggccccgttgcaaggagctcaaatggagagcggcagccccggtggagcgggccccgtgagtcaccacacaaga

6201 agagggcctggtcccctaccggctgctgcttccctgtgaccccggtgctcctcggccgaatagtcacctcgggcttttgagcacggctagtcggggcg

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6501 ATCATGACCAAAGACAAAGAACCTATTGTTAAAGCTTCCATTTGTTGCCTTATGATCATAATAGTTGGAACAGAATCCAGTCTCCGACGGAATG

6601 AATTTGACAGTACAGCAAGTCAAAAAGAGGTCTTATTTCATGTTCAAAAAGACTACCGCTGAAAACCAAAGTCTTAGATATGTCTCAGAACTACATCGCTGA

6701 GCTTCAGTCTCTGACATGAGCTTTCTATCAGAGTGTGACAGTGTGAGACTTTCCATAACAGAATCCAGTACTTGATTTAAGTGTTCAGTTCAAC

6801 CAGGATTTAGAAATTTGGATTATCTCATAATCAGTTGCAAAAAGATATCTGCCATCTTATTGTGAGTTCAGGCATTTAGATCTCTCATTCAATGATT

6901 TCAAGGCCCTGCCATCTGTAAGGAATTTGGCAACTTATCACAACCTGAATTTCTGGGATTGAGTGTATGAAGCTGCAAAAATTAGATTTGCTGCCAAT

7001 TGCTCACTTGCATCTAAGTTATATCTTCTGGATTTAAGAAATTTATATAAAAAGAAAATGAGACAGAAAGTCTACAAATTTCTGAATGCAAAAACCCCT

7101 eAl aHi sLeuHis sLeuSer TyrI leLeuLeuAspLeuArgAsnTyrTyrI leLysGl uAsnGl uThr Gl uSer LeuGl nI leLeuAsnAl aLysThrLeu

100 Gl nAspLeuGl uTyrLeuAspLeuSer His sAsnGl nLeuGl nLysI leSer CysHis sP rOl leVal Ser PheArgHis sLeuAspLeuSer PheAsnAspP

133 heLysAl aLeuP rOl leCysLysGl uPheGl yAsnLeuSer Gl nLeuAsnPheLeuGl yLeuSer Al aMetLysLeuGl nLysLeuAspLeuLeuP rOl l

166 eAl aHi sLeuHis sLeuSer TyrI leLeuLeuAspLeuArgAsnTyrTyrI leLysGl uAsnGl uThr Gl uSer LeuGl nI leLeuAsnAl aLysThrLeu

7101 CACCTTGTTCACCCAAGTATGTTTATTCGCTATCCAAGTGAACATATCAGTTAATACTTTAGGGTGCTTACAAGTACTAATATTAATGAATGATG
200▶ Hi sLeuVal PheHi sP roThr Ser LeuPheAl a l eGl nVal AsnI l eSer Val l AsnThr LeuGl yCysLeuGl nLeuThr AsnI l eLysLeuAsnAspA
7201 ACAACTGTCAAGTTTTTCATTAATTTTTATCAGAACTCACCAGAGGTTCAACCTTACTGAATTTTACCTCAACCACATAGAAACGACTTGAAATGCCT
233▶ spAsnCysGl nVal Phe l eLysPheLeuSer Gl uLeuThr ArgGl ySer Thr LeuLeuAsnPheThr LeuAsnHi s l eGl uThr Thr TrpLysCysLe
7301 GGTCAGAGTCTTTCAATTTCTTTGGCCAAACCTGTGGAAATATCTCAATTTTACAATTTAAACAATAATTGAAAGCATTCTGTGAAGAAATTTACTTAT
266▶ uVal l ArgVal l PheGl nPheLeuTrpP roLysP roVal Gl uTyrLeuAsnI l eTyrAsnLeuThr l l e l l eGl uSer l eArgGl uGl uAspPheThr Tyr

BsrGI (7473)

7401 TCTAAAACGACATTGAAAGCATTGACAATAGAACATATCACGAACCAAGTTTTCTGTTTTTACAGACAGCTTTGTACACCGTGTTCCTGAGATGAACA
300▶ Ser LysThr Thr LeuLysAl aLeuThr l l eGl uHi s l l eThrAsnGl nVal l PheLeuPheSer Gl nThrAl aLeuTyrThr Val l PheSer Gl uMetAsnI

Psp1406I (7591)

7501 TTATGATGTTAACCATTTTTCAGATACACCTTTTATACACATGCTGTGCTCCTCATGCACCAAGCACATTCAAGTTTTTGAACCTTACCAGAACGTTTTTAC
333▶ l eMetMetLeuThr l l eSer AspThr P roPhe l l eHi sMetLeuCysP roHi sAl aP roSer Thr PheLysPheLeuAsnPheThr Gl nAsnVal l PheTh
7601 AGATAGTATTTTTGAAAAATGTTCCACGTTAGTTAAATTTGGAGACACTTATCTTACAAAAGAATGGATTAAGACCTTTTCAAAGTAGGTCATGACG
366▶ r AspSer l l ePheGl uLysCysSer Thr LeuVal l LysLeuGl uThr Leu l l eLeuGl nLysAsnGl yLeuLysAspLeuPheLysVal l Gl yLeuMetThr

EcoRI (7739)

7701 AAGGATATGCCTTCTTTGGAAACTGGATGTTAGCTGGAATTCCTTTTGGAACTCTGGTAGACATAAAGAAAAGTGCACCTGGGTTGAGAGTATAGTGGTGT
400▶ LysAspMetP roSer LeuGl u l l eLeuAspVal Ser TrpAsnSer LeuGl uSer Gl yArgHi sLysGl uAsnCysThr TrpVal l Gl uSer l l eVal Val l L
7801 TAAATTTGCTTCAAATATGCTTACTGACTCTGTTTTTTCAGATGTTTACCTCCAGGATCAAGTACTTGTATCTTACAGCAATAAAAAAAGAGCGTTTCC
433▶ euAsnLeuSer Ser AsnMetLeuThr AspSer Val l PheArgCysLeuP roP roArg l l eLysVal l LeuAspLeuHi sSerAsnLys l l eLysSer Val l P r
7901 TAAACAAGTCGTAAGAACTGGAAGCTTTCGCAAGAACTCAATGTTGCTTTCAATCTTTAACTGACCTTCTGGATGTGGCAGCTTTAGCAGCCTTTCTGTA
466▶ oLysGl nVal l Val l LysLeuGl uAl aLeuGl nGl uLeuAsnVal l Al aPheAsnSer LeuThr AspLeuP roGl yCysGl ySer PheSer Ser LeuSer Val l
8001 TTGATCATTGATCACAAATTCAGTTTCCACCCATCGGCTGATTTCTTCCAGAGCTGCCAGAAGATGAGGTCAATAAAGCAGGGGACAATCCATTCCAAT
500▶ Leu l l e l l eAspHi sAsnSer Val Ser Hi sP roSer Al aAspPhePheGl nSer CysGl nLysMetArgSer l l eLysAl aGl yAspAsnP roPheGl nC
8101 GTACCTGTGAGCTAAGAGAATTTGTCAAAAATATAGACCAAGTATCAAGTGAAGTGTAGAGGCTGGCCTGATCTTATAAGTGTGACTACCCAGAAAG
533▶ ysThr CysGl uLeuArgGl uPheVal l LysAsn l l eAspGl nVal Ser Ser Gl uVal l LeuGl uGl yTrpP roAspSer TyrLysCysAspTyrP roGl uSe
8201 TTATAGGAAGCCCACTAAAGACTTTCACATGCTGAATATCTGCAACATAACTCTGCTGATCGTCACCATCGGTGCCACCTGTTGTTGGCT
566▶ r TyrArgGl ySer P roLeuLysAspPheHi sMetSer Gl uLeuThr CysAsn l l eThr LeuLeu l l eVal Thr l l eGl yAl aGl yMetLeuVal l LeuAl a
8301 GTGACTGTGACCTCCCTCTGCATCTACTTGGATCTGCCTGGTATCTCAGGATGGTGTGCCAGTGGACCCAGACTCGGCGCAGGGCCAGGAACATACCT
600▶ Val Thr Val Thr Ser LeuCys l l eTyrLeuAspLeuP roTrpTyrLeuArgMetVal CysGl nTrpThr Gl nThr ArgArgAl aArgAsn l l eP roL
8401 TAGAAGAAGCTCCAAAGAACTCCAGTTTTCAGCTTTTATTTTATCATATAGTGAACATGATTCTGCCTGGGTGAAAAGTAAATGTTGACTTACCTAGAAAA
633▶ euGl uGl uLeuGl nArgAsnLeuGl nPheHi sAl aPhe l l eSer TyrSer Gl uHi sAspSer Al aTrpVal l LysSer Gl uLeuVal l P roTyrLeuGl uLy
8501 AGAAGATATACAGATTTGCTTTCATGAGAGAACTTTTGCCTGGCAAGAGCATTGTGAAAAATATCATCAACTGCATTGAGAAGAGTTACAAGTCCATC
666▶ sGl uAsp l l eGl n l l eCysLeuHi sGl uArgAsnPheVal l P roGl yLysSer l l eVal l Gl uAsn l l e l l eAsnCys l l eGl uLysSer TyrLysSer l l e
8601 TTTGTTTTGCTCCCAACTTTTGTCCAGAGTGGTGGCCATTACGAACCTATTTTTGCCCATCACAACTCTTTTCCCATCACAACTCTTAACTAACTAACTC
700▶ PheVal l LeuSer P roAsnPheVal l Gl nSer Gl uTrpCysHi sTyrGl uLeuTyrPheAl aHi sHi sAsnLeuPheHi sGl uGl ySerAsnAsnLeu l l eL
8701 TCATCTTACTGGAACCCATTCACAGAACAGCATTCCCAACCAAGTACCACAAGCTGAAGGCTCTCATGACGCAGCGGACTTATTTGCAGTGGCCCAAGGA
733▶ e l l eLeuLeuGl uP roGl u l l eP roGl nAsnSer l l eP roAsnLysTyrHi sLysLeuLysAl aLeuMetThr Gl nArgThr TyrLeuGl nTrpP roLysGl
8801 GAAAAGCAAAAGCTGTTTGGGCTAACATTAGAGCCGCTTTTAAATAGAAATTAACACTAGTCACTGAAAAGTAAATGTTGAAATCTTAAAAAAAT
766▶ uLysSer LysArgGl yLeuPheTrpAl aAsn l l eArgAl aAl aPheAsnMetLysLeuThr LeuVal l Thr Gl uAsnAsnAspVal l LysSer ●●●
8901 TTAGGAAATTCAACTTAAGAAACCATTTACTTGGATGATGGTGAATAGTACAGTCGTAAGTNACTGTCTGGAGTGCCTCCGCTAGGAGCAGGTTTT

XbaI (9050)

9001 CCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTTCCAGTCTAGAGGGTAACTTTGTACTGCGTTTGGCTCCAGCTCGATCCACTG
9101 GCGAGTGTTAGTAACAGCACTGTTGCTTCTGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAAACAAGGACCCACGGGGCCAAAAGCCACGCCCA
9201 CACGGGCCGTCATGTGTGCAACCCAGCAGCGGCACTTTACTGCGAAACCACTTTAAAGTGACATTGAAACTGGTACCCACACTGGTGACAGGCTA
9301 AGGATGCCCTTACGGTACCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGC
9401 CTGAATAGGTGACCGGAGTCCGACCTTTCTTTGCAATTACTGACCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCA
9501 TAGTATAATCGACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGTGCCTGAGAAGATCACCA
1▶ Me tLysThr PheAsn l l eSer Gl nGl nAspLeuGl uLeuVal l Gl uVal l Al aThr Gl uLys l l eThr M
9601 TGCTCTATGAGGACAACAAGCACCATGTGGGGCGGCCATCAGGACCAAGCTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGT
23▶ e tLeuTyrGl uAspAsnLysHi sHi sVal l Gl yAl aAl a l l eArgThr LysThr Gl yGl u l l e l l eSer Al aVal l Hi s l l eGl uAl aTyr l l eGl yArgVal
9701 CACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAG
56▶ l Thr Val CysAl aGl uAl a l l eAl a l l eGl ySer Al aVal SerAsnGl yGl nLysAspPheAspThr l l eVal l Al aVal l ArgHi sP roTyrSerAspGl u
9801 GTGGACAGATCCATCAGGTGGTCCAGCCCTGTGGCATGTGCAGAGAGCTATCTGACTATGCTCCTGACTGCTTTGTGCTATTGAGATGAATGGCA
90▶ Val l AspArgSer l l eArgVal l Val Ser P roCysGl yMetCysArgGl uLeu l l eSer AspTyrAl aP roAspCysPheVal l Leu l l eGl uMetAsnGl yL
9901 AGCTGGTCAAACCACTTGGAGAACTCATCCCCTCAAGTACACAGGAAGTAACTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCC
123▶ ysLeuVal l LysThr Thr l l eGl uGl uLeu l l eP roLeuLysTyrThrArgAsn●●●
10001 ACTCTAATCAGTGGTGAAGAAGCGTCTCAGAAGTGTGTTTCAATTTGGCCATTAAGTTTAGTAGTAAAAGACTGGTAAATGATAACAATGCATCGT
10101 AAAACCTTCAGAAGGAAAGGAGAAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAAGTATTAGTTTTTAAATCAGTACTTTT
10201 TAATGGAACAACCTTGACCAAAAATTTGTACAGAATTTTGTAGACCCATTAAGAAAGTAAATGAGAACCCTGTGTTCCTTTGGTCAACACCGAGACA
10301 TTTAGGTGAAAGACATCTAATCTGGTTTTACGAATCTGGAACCTCTTGAAAATGTAATCTTGTAGTTAACACTTCTGGGTGGAGAATAGGTTGTTTT
10401 CCCCCACATAATTGGAAGGGGAAAGGAATATCATTAAAGCTATGGAGGGTTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCC
10501 TGTCATAAAACAGGCCAAAACCTGAGTCCTTGGGTTGCATAGAAAGCTG