

pDUO-hCD14/TLR4A

A plasmid coexpressing the human CD14 and TLR4 genes

Catalog code: pduo-hcd14tlr4a

<https://www.invivogen.com/pduo-cd14-tlr4>

For research use only

Version 20H25-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-hCD14/TLR4A 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 CD14 (1125 bp) / Human TLR4A (2517 bp)**

TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS¹. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS². TLR4 physically associates with MD2, and together with a third protein called CD14, this complex is responsible for LPS recognition and signaling³.

- **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.

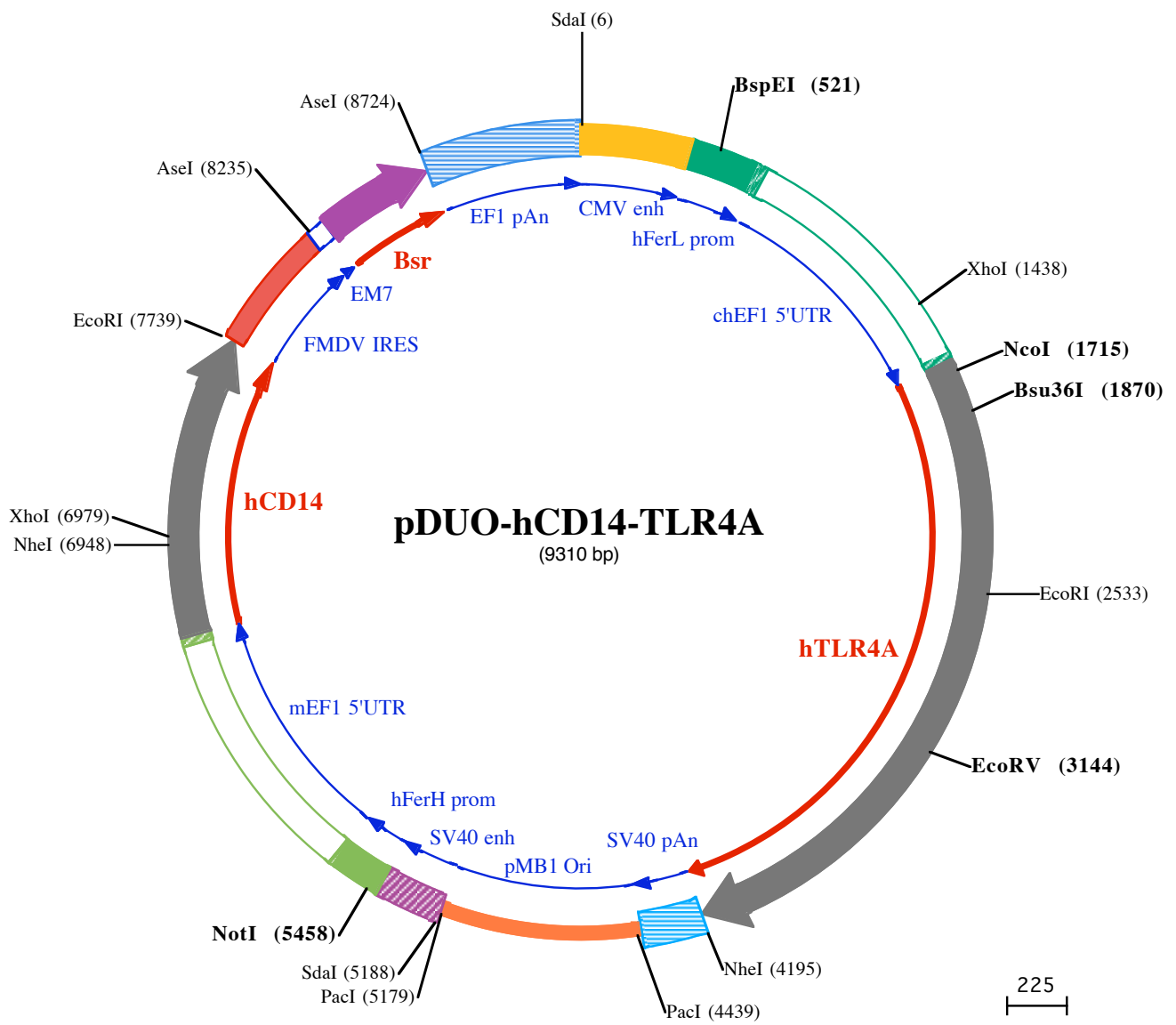
References

1. Poltorak A. *et al.*, 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282(5396):2085-8.
2. Nagai Y. *et al.*, 2002. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol.* 3(7):667-72.
3. da Silva Correia J. *et al.*, 2001. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex transfer from CD14 to TLR4 and MD-2. *J Biol Chem.* 276(24):21129-35.
4. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
5. Dean DA. *et al.*, 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22.
6. Boshart M. *et al.*, 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141(2):521-30.
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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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SdaI (6)
1 CCTGCAGCGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
101 CGCCAATAGGGACTTTCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGATTTCGAAGTCTCCACCCATTGACGTCAATG
401 GGAGTTTGTGTTGACTAGTCAGGCCCAACCCCAAGCCCATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCTTGTCTTGGGCGGG
BspEI (521)
501 GGGCTGAGACTCTATGTGCTCCGATTGGTCAGGCACGGCCTTGGCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCCCTCCCGAGCGCCCTGCC
601 TCCGAGGGCCGGCACCATAAAGAAGCCGCCCTAGCCACGTCCTCGCAGTTGCGCGGTCCCGGGTCTGTCTCAAGCTTGCCCCAGAACACAGG
701 taagtgcctgtgtggttcccgcgggcctggcctctttacgggttatggccttgcgtgcttgaattacttccatgccctggctgcagtacgtgattc
801 ttgatcccgagcttcgggttgaagtggtgggagagttcgaggccttgcgcttaaggagcccttcgctcgtgcttgagttgagcctggcttgggagc
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1201 gccgctgtatgccccccctggcggaaggtggccggctggcaccagttgctgagcggaaagatggcggcttccggccctgctgcagggagc
1301 tcaaaatggaggagcggcgccgggagagcggcggtgagtcaccacacaaaggaagggccttcttctcatcctgcttcatgtgactcca
XhoI (1438)
1401 cggagtaccggcgccgtccaggcacctcgattagttctcgagctttggagtagctgctcttaggttggggggggggttttatgcatggagtttc
1501 ccacactgagtggtggagactgaagagttaggccagcttggcacttgcgtgtaattctccttgaatttgcctttttgagttggatcttgcctcattc
1601 tcaagcctcagacagtggttcaagttttttcttccatttcagGTGCTGTAAGAACTACCCCTAAAAGCCACCATGATGTCTCGCCTGGCTGGCTGG
MetMetSerAl aSerArgLeuAl aGI
NcoI (1715)
1701 GACTCTGATCCAGCCATGGCCTTCTCTCTCGGTGAGACCCGAAAGCTGGGAGCCCTCGGTGGAGGTGGTTCTTAATATTACTTATCAATGCATGGAG
9 yThr LeuI l eP roAl aMe tAl aPheLeuSer CysVal A r gP roGl uSer T r pGl uP roCysVal Gl uVal Val P roAsnI l eThr TyrGl nCysMetGl u
Bsu36I (1870)
1801 CTGAATTTCTACAAAATCCCGACAACCTCCCTTCTCAACCAAGAAGCTGGACCTGAGCTTTAATCCCTGAGGCATTAGGCAGTATAGCTTCTTCA
43 LeuAsnPheTyrLysI l eP roAspAsnLeuP roPheSer Thr LysAsnLeuAspLeuSer PheAsnP roLeuArgH i sLeuGl ySer TyrSer PhePheS
1901 GTTCCAGAACTGCAGGTGCTGGATTATCCAGGTGTGAAATCCAGACAATTGAAGATGGGCATATCAGAGCCTAAGCCACCTCTACCTTAATATT
76 er PheP roGl uLeuGl nVal l eLeuAspLeuSer ArgCysGl u l eGl nThr l l eGl uAspGl yAl aTyrGl nSer LeuSer H i sLeuSer Thr LeuI l eLe
2001 GACAGGAAACCCATCCAGAGTTTAGCCCTGGGAGCCTTTCTGGACTCAAGTTTACAGAAGCTGGTGGCTGGAGACAAATCTAGCATCTCTAGAG
109 uThr Gl yAsnP ro l l eGl nSer LeuAl aLeuGl yAl aPheSer Gl yLeuSer Ser LeuGl nLysLeuVal Al aVal Gl uThrAsnLeuAl aSer LeuGl u
2101 AACTTCCCATTGGACATCTCAAACTTTGAAAGAACTTAATGTGGCTACAATCTTCAATCTTCAAATACCTGAGTATTTTCTAATCTGACCA
143 AsnPheP ro l l eGl yH i sLeuLysThr LeuLysGl uLeuAsnVal Al aH i sAsnLeu l l eGl nSer PheLysLeuP roGl uTyrPheSerAsnLeuThrA
2201 ATCTAGACTTGGACCTTCCAGCAACAAGATCAAAGTATTATTGCACAGACTTGGGGTCTACATCAAAATGCCCTCACTCAATCTCTCTTTAGA
176 snLeuGl uTyrLeuAspLeuSer SerAsnLys l l eGl nSer l l eTyrCysThrAspLeuArgVal l eLeuH i sGl nMe tP roLeuLeuAsnLeuSer LeuAs
2301 CCTGTCCCTGAACCTATGAACCTTATCCAACAGGTGCATTTAAAGAAATTAGGCTTATAAGCTGACTTAAAGAAATAATTTGATAGTTAAATGTA
209 pLeuSer LeuAsnP roMe tAsnPhe l l eGl nP roGl yAl aPheLysGl u l l eArgLeuH i sLysLeuThr LeuArgAsnAsnPheAspSer LeuAsnVal
2401 ATGAAAACCTGTATTCAAGGCTGGCTGGTTTAGAAGTCCATCGTTGGTTCTGGGAGAAATTTGAAAATGAAGGAAACTTGGAAAAGTTTGACAAATCTG
243 Me tLysThr Cys l l eGl nGl yLeuAl aGl yLeuGl uVal H i sArgLeuVal l eLeuGl yGl uPheArgAsnGl uGl yAsnLeuGl uLysPheAspLysSerA
EcoRI (2533)
2501 CTCTAGAGGGCTGTGCAATTTGACCATTGAAGAATTCGATTAGCATACTTAGACTACTACCTCGATGATATTATTGACTTATTTAATTGTTTGACAAA
276 l aLeuGl uGl yLeuCysAsnLeuThr l l eGl uGl uPheArgLeuAl aTyrLeuAspTyrTyrLeuAspAsp l l e l l eAspLeuPheAsnCysLeuThrAs
2601 TGTTTCTTCAATTTCCCTGGTGGAGTGTGACTATTGAAAGGTAAGAACTTTCTTATAATTTCCGATGGCAACATTTAGAATTAGTTAACTGTAATTT
309 nVal Ser Ser PheSer LeuVal Ser Val Thr l l eGl uArgVal l LysAspPheSer TyrAsnPheGl yTrpGl nH i sLeuGl uLeuVal Al AsnCysLysPhe
2701 GGACAGTPTCCACATTGAACTCAAATCTCTCAAAAGGCTTACTTTCACTTCCAACAAAGGTGGGAATGCTTTTTCAGAAGTTGATCTACCAAGCCTTG
343 Gl yGl nPheP roThr LeuLysLeuLysSer LeuLysArgLeuThr PheThr SerAsnLysGl yGl yAsnAl aPheSer Gl uVal AspLeuP roSer LeuG
2801 AGTTTCTAGATCTCAGTAGAAAATGGCTTGGTTCGTTTCAAAGGTTGCTGTTCTCAAAGTATTTTGGGACAACAGCCTAAAGTATTTAGATCTGAGCTTCAA
376 l uPheLeuAspLeuSer ArgAsnGl yLeuSer PheLysGl yCysCysSer Gl nSer AspPheGl yThr Thr Ser LeuLysTyrLeuAspLeuSer PheAs
2901 TGGTGTATTACCATGAGTTCAAACCTTCTGGGCTTAGAACAACCTAGAACATCTGGATTTCCAGCATTCCAATTTGAAACAAATGAGTGAGTTTTCAGTA
409 nGl yVal l l eThr Me tSer SerAsnPheLeuGl yLeuGl uGl nLeuGl uH i sLeuAspPheGl nH i sSerAsnLeuLysGl nMe tSer Gl uPheSer Val
3001 TTCCTATCACTCAGAACTCATTACCTTACATTTCTCATACTCACACCAGAGTTGCTTTCAATGGCATCTTCAATGGCTTGTCCAGTCTCGAAGTCT
443 PheLeuSer LeuArgAsnLeu l l eTyrLeuAsp l l eSer H i sThr H i sThr ArgVal Al aPheAsnGl y l l ePheAsnGl yLeuSer Ser LeuGl uVal l
EcoRV (3144)
3101 TGAAAATGGCTGGCAATTTCTTCCAGGAAAACCTCCTTCCAGATATCTTCCACAGAGCTGAGAAAATGACCTTCTGGACCTCTCTCAGTGTCAACTGGA
476 euLysMe tAl aGl yAsnSer PheGl nGl uAsnPheLeuP roAsp l l ePheThr Gl uLeuArgAsnLeuThr PheLeuAspLeuSer Gl nCysGl nLeuGl
3201 GCAGTTGTCTCAACAGCATTAACTCACTCTCCAGTCTTCCAGTACTAAATATGAGCCACAACAACCTTCTTTTCAATGGATACGTTTCTTATAAGTGT
509 uGl nLeuSer P roThr Al aPheAsnSer LeuSer Ser LeuGl nVal l eLeuAsnMe tSer H i sAsnAsnPhePheSer LeuAspThr PheP roTyrLysCys
3301 CTGAACCTCCCTCAGGTTCTTGATTACAGTCTCAATCACATAATGACTTCCAAAAACAGGAACCTACAGCATTTCGAAGTAGTCTAGCTTTCTTAAATC
543 LeuAsnSer LeuGl nVal l eLeuAspTyrSer LeuAsnH i s l l eMe tThr Ser LysLysGl nGl uLeuGl nH i sPheP roSer Ser LeuAl aPheLeuAsnL
3401 TTAATCAGAATGACTTTGCTTGTACTTGTGAACCCAGAGTTTCTGCAATGGATCAAGGACAGAGGAGCTCTTGGTGAAGTTGAACGAATGAATG
576 euThr Gl nAsnAspPheAl aCysThr CysGl uH i sGl nSer PheLeuGl nTrp l l eLysAspGl nArgGl nLeuLeuVal l eGl uVal Gl uArgMe tGl uCy

3501 TCGCACACCTTCAGATAAGCAGGGCATGCCTGTGCTGAGTTTGAATATCACCTGTGAGTGAATAAGACCATCATTGGTGTGTCGGTCCCTCAGTGTGCTT
609 ▶ sAl aThr ProSerAspLysGlnGlyMetProValLeuSerLeuAsnIleThrCysGlnMetAsnLysThrIleIleGlyValSerValLeuSerValLeu
3601 GTAGTATCTGTTGTAGCAGTTCTGGTCTATAAGTTCTATTTTCCACCTGATGCTTCTGCTGGCTGCATAAAGTATGGTAGAGGTGAAAAATCTATGATG
643 ▶ ValValSerValValAlaValLeuValTyrLysPheTyrPheHisLeuMetLeuLeuAlaGlyCysIleLysTyrGlyArgGlyGlyuAsnIleTyrAspA
3701 CCTTTGTTATCTACTCAAGCCAGGATGAGGACTGGTAAAGGAATGAGCTAGTAAAGAATTTAGAAGAAGGGGTGCCCTCATTTCAGCTCTGCCCTCACTA
676 ▶ IaPheValIleTyrSerSerGlnAspGluAspTrpValArgAsnGluLeuValLysAsnLeuGluGlyValIleProPheGlnLeuCysLeuHisTy
3801 CAGAGACTTTATCCCGGTGTGGCCATTGCTGCCAACATCATCCATGAAGGTTTCCATAAAAAGCCGAAAGGTGATTGTTGGTGTGCCAGCACTTCATC
709 ▶ rArgAspPheIleProGlyValAlaIleAlaAlaAsnIleIleHisGluGlyPheHisLysSerArgLysValIleValValValSerGlnHisPheIle
3901 CAGAGCCGCTGGTGTATCTTTGAATATGAGATTGCTCAGACCTGGCAGTTTCTGAGCAGTCTGCTGGTATCATCTTCATTGCTCAGAAAGGTGGAGA
743 ▶ GlnSerArgTrpCysIlePheGluTyrGluIleAlaGlnThrTrpGlnPheLeuSerSerArgAlaGlyIleIlePheIleValLeuGlnLysValGluL
4001 AGACCTGCTCAGGCAGCAGGTGGAGCTGTACCGCTTCTCAGCAGGAACACTTACCTGGGTGGGAGGACAGTGTCTGGGGCGGCACATCTCTGGAG
776 ▶ ysThrLeuLeuArgGlnGlnValGluLeuTyrArgLeuLeuSerArgAsnThrTyrLeuGlyTrpGluAspSerValLeuGlyArgHisIlePheTrpAr
NheI (4195)
4101 ACGACTCAGAAAAGCCCTGCTGGATGGTAAATCATGGAATCCAGAAGGAACAGTGGGTACAGGATGCAATTGGCAGGAAGCAACATCTATCTGAGCTAGC
809 ▶ gArgLeuArgLysAlaLeuLeuAspGlyLysSerTrpAsnProGluGlyThrValGlyThrGlyCysAsnTrpGlnGluAlaThrSerIle●●●
4201 TGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTT
4301 TATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAG
PacI (4439)
4401 CAAGTAAAACCTCTACAATGTGGTATGAAATGTTAATTAACCTAGCCATGACCAAAATCCCTAACCTGAGTGTTCCTTCCACTGAGCGTCAGACCCCG
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4701 TTCAAGAACTCTGTAGCACCGCTACATAGCTGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACT
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PacI (5179) SdaI (5188)
5101 GCCTATGAAAAACGCCAGCAACCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCGCTTTTGTCTACATGTTCTTAATTAACCTGCAGGGCTGAAATA
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NotI (5458)
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5501 CGGAAGGAGCGGGCTCGGGCGGGCGGCGCTGATTGGCCGGGGCGGCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGACGT
5601 TCTTCGCCGAAGCTTGCCGTGAGAACGACGAGtgaggggcggtgtggcttccgccccgagctggaggtcctgctccgagcgggccccgct
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6601 TTCAAAGCAATCATGGAGCGCGCTCCTGCTGTTGCTGCTGCTGCTGCCCTGGTGCACGCTCTCTGCGACCACGCCAGAACCTTGTGAGCTGGACGATG
▶ MetGluArgAlaSerCysLeuLeuLeuLeuLeuLeuProLeuValHisValSerAlaThrThrProGluProCysGluLeuAspAspG
6701 AAGATTTCCGCTGCGTCTGCAACTTCTCGAACCTCAGCCGACTGGTCCGAAGCCTTCCAGTGTGTGCTGCAAGTAGAGGTGGAGATCCATGCCGGCGG
30 ▶ IAspPheArgCysValCysAsnPheSerGluProGlnProAspTrpSerGluAlaPheGlnCysValSerAlaValGluValGluIleHisAlaGlyGly
6801 TCTCAACCTAGAGCCGTTTCTAAAGCGCTCGATGCGGACGCCACCCGCGGAGTATGCTGACACGGTCAAGGCTCTCCCGGTGCGGGCGGCTCACAGTG
63 ▶ yLeuAsnLeuGluProPheLeuLysArgValAspAlaAspAlaAspProArgGlnTyrAlaAspThrValLysAlaLeuArgValArgArgLeuThrVal
NheI (6948) XhoI (6979)
6901 GGAGCCGCACAGTTCTGCTCAGTACTGGTAGGCGCCCTGCTGTGCTGCTGCTGCCCTGAGCGTACTCCCGCTCAAGGAACCTGACCTCGAGGACCTAAGATAACCG
97 ▶ GlyAlaAlaGlnValProAlaGlnLeuLeuValGlyAlaLeuArgValLeuAlaTyrSerArgLeuLysGluLeuThrLeuGluAspLeuLysIleThrG
7001 GCACCATGCCTCCGCTGCTCTGGAAGCCACAGGACTTGACATTTCCAGCTTGGCCCTACGCAACGTGTGCTGGGCGACAGGGCGTCTTGGCTCGCCGA
130 ▶ IyThrMetProProLeuProLeuGluAlaThrGlyLeuAlaLeuSerSerLeuArgLeuArgAsnValSerTrpAlaThrGlyArgSerTrpLeuAlaGly

7101 GCTGCAGCAGTGGCTCAAGCCAGGCCTCAAGGTACTGAGCATTGCCAAGCACACTCGCTGCTTTTCTCTGCGAACAGGTTGCGCCTTCCCGCCCTT
163▶ uLeuGlnGlnTrpLeuLysProGlyLeuLysValLeuSerIleAlaGlnAlaHisSerProAlaPheSerCysGluGlnValArgAlaPheProAlaLeu
7201 ACCAGCCTAGACCTGTCTGACAATCCTGGACTGGGCGAACGCGGACTGATGGCGGCTCTCTGTCCCCACAAGTCCCGGCCATCCAGAATCTAGCGCTGC
197▶ ThrSerLeuAspLeuSerAspAsnProGlyLeuGlyGluArgGlyLeuMetAlaAlaLeuCysProHisLysPheProAlaIleGlnAsnLeuAlaLeuA
7301 GCAACACAGGAATGGAGACGCCACAGGCGTGTGCGCCGACTGGCGGCGGCAGGTGTGCAGCCCCACAGCCTAGACCTCAGCCACAACCTCGCTGCGCC
230▶ rGAsnThrGlyMetGluThrProThrGlyValCysAlaAlaLeuAlaAlaAlaGlyValGlnProHisSerLeuAspLeuSerHisAsnSerLeuArgAl
7401 CACCGTAAACCTAGCGCTCCGAGATGCATGTGGTCCAGCGCCCTGAACTCCCTCAATCTGTCTGTTGCTGGGCTGGAACAGGTGCCATAAGGACTGCCA
263▶ aThrValAsnProSerAlaProArgCysMetTrpSerSerAlaLeuAsnSerLeuAsnLeuSerPheAlaGlyLeuGluGlnValProLysGlyLeuPro
7501 GCCAAGCTCAGAGTCTCGATCTCAGCTGCAACAGACTGAACAGGGCGCCGAGCTGACGAGCTGCCGAGGTGGATAACCTGACACTGGACGGGAATC
297▶ AlaLysLeuArgValLeuAspLeuSerCysAsnArgLeuAsnArgAlaProGlnProAspGluLeuProGluValAspAsnLeuThrLeuAspGlyAsnP
7601 CCTTCTGGTCCCTGGAACCTGCCCTCCCCACGAGGGCTCAATGAACTCCGGCGTGGTCCCAGCCTGTGCACGTTCCGACCTGTCGGTGGGGGTGTCGGG
330▶ rOpheLeuValProGlyThrAlaLeuProHisGluGlySerMetAsnSerGlyValValProAlaCysAlaArgSerThrLeuSerValGlyValSerGly
EcoRI (7739)
7701 AACCTGGTGTCTCCAAGGGCCCCGGGCTTTGCCTGAATTCGCTAGGAGCAGGTTTTCCCAATGACACAAAACGTGCAACTGAACTCCGCTGGT
363▶ yThrLeuValLeuLeuGlnGlyAlaArgGlyPheAla●●●
7801 CTTTCCAGGTCTAGAGGGTAACACTTTGTACTGCTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCA
7901 TGACGGCCGTGGAACTCCTCCTTGGTAACAAGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCCGTCATGTGTGCAACCCAGCACGGCGACTTT
8001 ACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTC
8101 GGGATCTGAGAAGGGGACTGGGGCTTCTATAAAGCGCTCGGTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGTGGCGACCTTCTTTGCAAT
AseI (8235)
8201 TACTGACCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGA
1▶ MetLysT
8301 CCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTGCGCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCAT
3▶ hrPheAsnIleSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysIleThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaIle
8401 CAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCATTTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTG
36▶ eArgThrLysThrGlyGluIleIleSerAlaValHisIleGluAlaTyrIleGlyArgValThrValCysAlaGluAlaIleAlaIleGlySerAlaVal
8501 AGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTGAGGACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCCGTGGCATGT
70▶ SerAsnGlyGlnLysAspPheAspThrIleValAlaValArgHisProTyrSerAspGluValAspArgSerIleArgValValSerProCysGlyMetC
8601 GCAGAGAGCTCATCTGACTATGCTCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACACCATTGAGGAACTCATCCCCCTCAA
103▶ ysArgGluLeuIleSerAspTyrAlaProAspCysPheValLeuIleGluMetAsnGlyLysLeuValLysThrThrIleGluGluLeuIleProLeuLys
AseI (8724)
8701 GTACACCAGGAACTAAACCTGAATTAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACTTAAATCAGTGGTGAAGAACGGTCTCAGAAGTGT
136▶ sTyrThrArgAsn●●●
8801 GTTTCATTGACCATTAAAGTTTAGTAGTAAAGACTGGTAAATGATAAACAATGCATCGTAAACCTCAGAAGGAAAGGAGAATGTTTTGTGGACCACT
8901 TTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGAAACAACCTGACCAAAAATTTGTCACAGAATTTT
9001 GAGACCCATTAATAAAGTTAAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATCTGGTTTTACGAATCTGG
9101 AAACCTCTGAAAATGTAATCTTGAGTTAACACTTCTGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAG
9201 CTATGGGAGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCTGTCACTAAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCA
9301 TAGAAAGCTG
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