

# pDUO2-hMD2/CD14

A plasmid coexpressing the human MD2 and CD14 genes

Catalog code: pduo2-hmd2cd14

<https://www.invivogen.com/pduo-md2-cd14>

For research use only

Version 19I24-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pDUO2-hMD2/CD14 provided as DNA
- 1 ml of Hygromycin B Gold at 100 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 Hygromycin B Gold 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.

pDUO2 is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other.

The genes cloned into pDUO2 comprise the coding sequence (without introns) from the ATG to the Stop codon.

## PLASMID FEATURES

- **Human MD2 (480 bp) / Human CD14 (1125 bp)**

MD2 and CD14 are necessary for proper LPS-induced TLR4 signaling. TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). TLR4 alone is not sufficient to confer LPS responsiveness. MD-2 is a secreted molecule that functionally interacts with LPS<sup>1,2</sup>. TLR4 physically associates with MD2 and CD14 to form the complex responsible for LPS recognition and signaling<sup>3</sup>.

- **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<sup>4</sup>. 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<sup>5</sup>.

- **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<sup>6</sup>.

- **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.*<sup>7</sup>

- **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<sup>8</sup>.

- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

## TECHNICAL SUPPORT

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- **Hph (hygromycin resistance gene):** confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* 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<sub>2</sub>O. 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.

### Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

## References

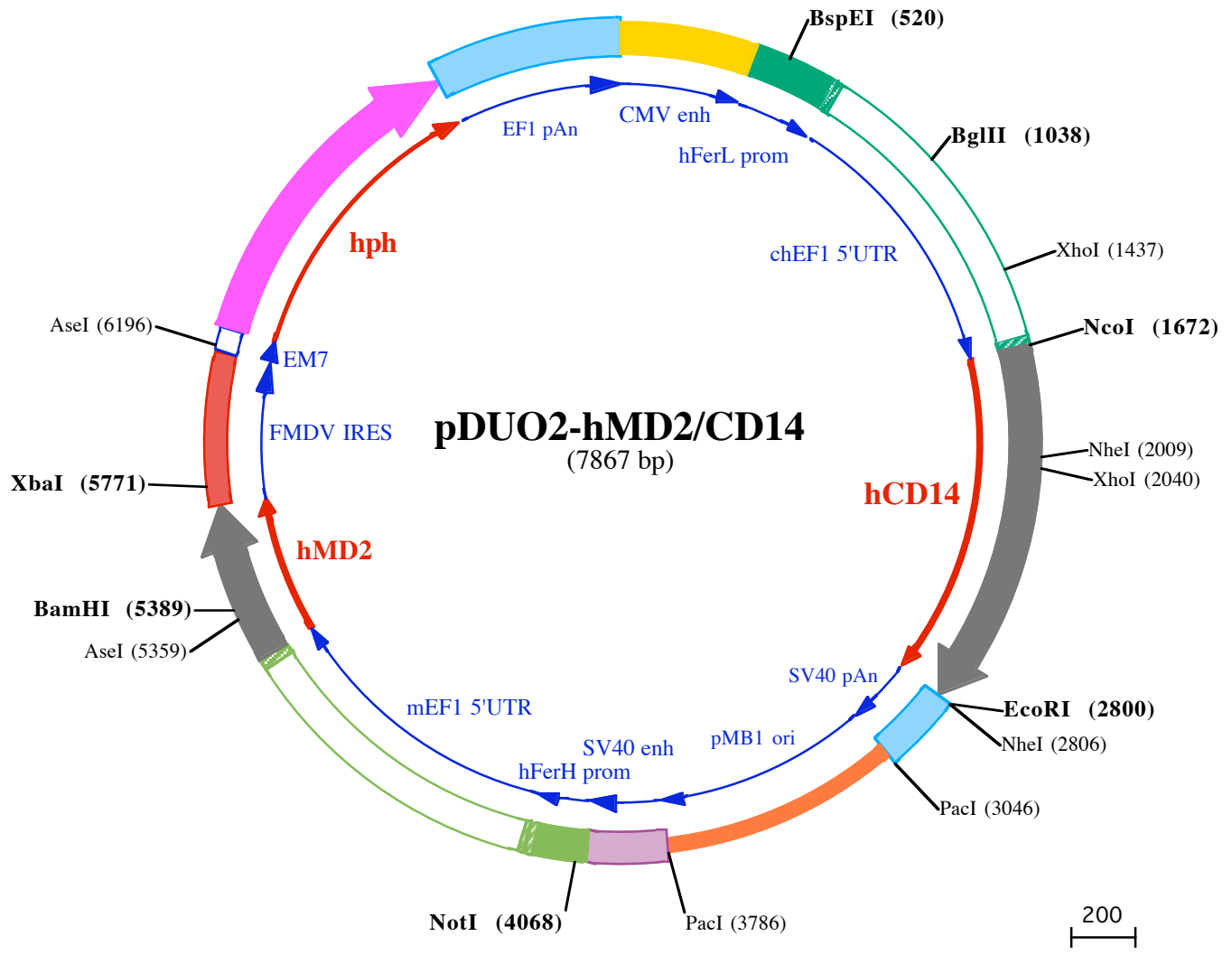
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1 CCTGCAGGCGTTACATAA...  
101 CGCCAATAGGGACTTTCC...  
201 TATTGACGTCAATGACGG...  
301 GCTATTACCATGATGATG...  
401 GGAGTTTGT...  
BspEI (520)  
501 GGGCTGAGACTCCTATGT...  
601 TCCGAGGGCCGGCGCACC...  
701 taagtccgtgtgtggttcc...  
801 ttgatcccagacttcgggt...  
901 ctggggccgcgcgtctaac...  
BglII (1038)  
1001 ctttttttctggcgagatag...  
1101 agcgcacatgttcggcgagg...  
1201 gccgcgctgtatgccccc...  
1301 tcaaatggaggagcgcggc...  
XhoI (1437)  
1401 cggagtagcggcgccgtcc...  
1501 ccacactgagtggggtgga...  
NcoI (1672)  
1601 tcaagcctcagacagtgg...  
1701 GCTGCTGCTGCCGCTGG...  
1801 CCCGACTGGTCCGAAGC...  
1901 ACGCCGACCCGGCAGTAT...  
NheI (2009) XhoI (2040)  
2001 CCTGCGTGTGCTAGCGT...  
2101 GCACTTTCCAGCTTGGC...  
2201 GCATTGCCAAGCACACT...  
2301 ACGCGACTGATGGCGCT...  
2401 GCACTGGCGGGCAGGTG...  
2501 GCGCCCTGAACTCCCTC...  
2601 GAACAGGGCGCCGAGCT...  
2701 TCAATGAACTCCGGCTG...  
NheI (2806) EcoRI (2800)  
2801 gaATTGCTAGCTGGCCAG...  
2901 ATGCTATTGCTTTATTT...  
PacI (3046)  
3001 GGTTTTTAAAGCAAGTAA...  
3101 CGTCAGACCCCGTAGAAA...  
3201 GGTTTGTGGCCGATCAAG...

3301 TTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTA  
3401 CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGCGCAGCGGTGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACAC  
3501 CGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGCAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGA  
3601 GAGCGCACGAGGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGGGTTTCCGCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGT

3701 CAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGCCTTTTTACGGTTCTGGCCTTTTGTGCCTTTTGTCTACATGTTCTTAATTAACCTGCA  
3801 GGGCTGAAATAACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCTGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCC  
3901 CAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCA

PacI (3786)

4001 AAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGGAGGGCTCCAGCGGCCGCCCTCCCCACAGCAGGGGCGGGG  
4101 TCCCGCGCCACCGAAGAGCGGGCTCGGGCGGGCGCGCTGATTGGCCGGGCGGGCTGACGCCAGCGGGCTATAAGAGACCACAAGCGACCCGC  
4201 AGGGCCAGACGTTCTTCGCCAAGCTTGGCTCAGAACGCAAGTgaggggggggtgtggttccgcgggcgcgagctggaggtcctgctccgagcggg  
4301 ccgggcccgcctgctgctggcggggattagctgcgagcattccgcttcgagttgcgggcgcgggaggcagagtgcgaggtcctagcggcaaccccg  
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4601 tggagggacagaggtggcggtggggcccgccttcggagcacatgtccgacgccacctggatggggcgaggcctggggttttccgaagcaacca  
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4801 ccgctccggcaccagttgctgctgctggaagatggcgctcccgggcccgttgaaggagctcaaatggaggcgcggcagccgggtggagcgggcg  
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5001 gagcacggctagtcgcccgggggggggaggtgtaattggcggttggagtttgcacatttgggtgggtggagactagtcaggccagcctggcgtggaagt  
5101 catttttggaatttgcctttagttttagcgggagcattctcgggcttcttagcgggtcaaggtatcttttaaaccttttttagGTGTGTGAA

NotI (4068)

5201 AACCAACGTAATTCAAAGCAATCATGTTACCATTCTGTTTTTCCACCCTGTTTTCTCCATATTTACTGAAGCTCAGAAGCAGTATTGGGTCTGCA  
MetLeuP roPheLeuPhePheSer Thr LeuPheSer Ser I lePheThr Gl uAl aGl nLysGl nTyrTrpVal CysA

AseI (5359)

BamHI (5389)

5301 ACTCATCCGATGCAAGTATTTTCATACACCTACTGTGATAAAATGCAATACCCAAATTTCAATTAATGTTAACCCCTGTATAGAATTGAAAGGATCCAAAGG  
26 snSer SerAspAl aSer I leSer TyrThr TyrCysAspLysMetGl nTyrProI leSer I leAsnValAsnP roCysI leGl uLeuLysGl ySer LysGl  
5401 ATTATTGCACATTTCTACATTTCAAGGAGAGATTTAAAGCAATTATATTTCAATCTCTATATAACTGTCAACACCATGAATCTTCAAAGCGCAAAGAA  
59 yLeuLeuHis I lePheTyr I leProArgArgAspLeuLysGl nLeuTyrPheAsnLeuTyrI leThr ValAsnThr MetAsnLeuP roLysArgLysGl u  
5501 GTTATTTGCCGAGGATCTGATGACGATTACTCTTTTGCAGAGCTCTGAAGGGAGAGACTGTGAATACAACAATATCATTCTCCTCAAGGGAATAAAAT  
93 Val I leCysArgGl ySer AspAspAspTyrSer PheCysArgAl aLeuLysGl yGl uThr ValAsnThr Thr I leSer PheSer PheLysGl yI leLysP  
5601 TTTCTAAGGAAAATACAAATGTGTTGTTGAAGCTATTTCTGGAGCCAGAAGAAATGCTTTTTGCTTGAGTGTGTCTACCTACCAACCTAATTC  
126 heSer LysGl yLysTyrLysCysVal Val Gl uAl aI leSer Gl ySer ProGl uGl uMetLeuPheCysLeuGl uPheVal I leLeuHis s Gl nP roAsnSe

XbaI (5771)

5701 AAATTAGCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTTCCAGGTCTAGAGGGTAACACTTTGTACTGCGTT  
159 rAsn  
5801 TGGCTCCACGCTCGATCCACTGGCGAGTGTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGTAACAAGGACCC  
5901 ACGGGGCCAAAAGCCACGCCACACGGGCCGTCATGTGTGCAACCCAGCAGCGGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTA  
6001 CCCACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGC

AseI (6196)

6101 TCGGTTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGGTGCGCACCTTTCTTTGCAATTACTGACCTATGAATACAACTGACTGTTTGACAATTA  
6201 ATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAACTGACAGCAACTTCTGTTGAGAAGTTTCT  
MetLysLysProGl uLeuThr Al aThr Ser Val Gl uLysPheLe  
6301 CATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTTGTGTTGGAGGAAGAGGTTATGTTCTG  
15 uI leGl uLysPheAspSer Val SerAspLeuMetGl nLeuSer Gl uGl yGl uGl uSer ArgAl aPheSer PheAspVal Gl yGl yArgGl yTyrVal Leu  
6401 AGGGTCAATTTCTGTCTGATGGTTTTACAAGACAGATATGTTTACAGACACTTTGCCTCTGCTCTGCCAATTCAGAAGTTCTGGACATTGGAG  
49 ArgValAsnSer CysAl aAspGl yPheTyrLysAspArgTyrVal TyrArgHis sPheAl aSer Al aAl aLeuP roI leP roGl uVal LeuAspI leGl yG  
6501 AATTTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCTGAAACTGAGCTGCCAGCTGTTCTGCAACTGT  
82 I uPheSer Gl uSer LeuThr TyrCysI leSer ArgArgAl aGl nGl yVal Thr LeuGl nAspLeuP roGl uThr Gl uLeuP roAl aVal LeuGl nProVa  
6601 TGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCAGTACACCCTTTGGAGG  
115 I Al aGl uAl aMetAspAl aI leAl aAl aAl aAspLeuSer Gl nThr Ser Gl yPheGl yProPheGl yProGl nGl yI leGl yGl nTyrThr Thr TrpArg

6701 GATTTTCATTTGTGCCATTGCTGATCCTCATGTCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAACTCA  
149▶ AspPheI l eCysAl a l l eAl aAspP roHi sVal TyrHi sTrpGl nThr Val l Me tAspAspThr Val Ser Al aSer Val Al aGl nAl aLeuAspGl uLeuM  
6801 TGCTGTGGGCAGAAGATTGTCTGAAGTCAGACACCTGGTCCATGCTGATTTTTGGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCAT  
182▶ e tLeuTrpAl aGl uAspCysP roGl uVal A rgHi sLeuVal l Hi sAl aAspPheGl ySerAsnAsnVal l LeuThrAspAsnGl yA rg l l eThrAl aVal l l  
6901 TGA CTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAACAACAACAAGATAT  
215▶ eAspTrpSer Gl uAl aMe tPheGl yAspSer Gl nTyrGl uVal Al aAsn l l ePhePheTrpArgP roTrpLeuAl aCysMe tGl uGl nGl nThrArgTyr  
7001 TTTGAAAGAAGACACCCAGAAGTGGCTGGTTCCCCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGGAA  
249▶ PheGl uArgArgHi sP roGl uLeuAl aGl ySer P roArgLeuArgAl aTyrMe tLeuArg l l eGl yLeuAspGl nLeuTyrGl nSer LeuVal AspGl yA  
7101 ACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGTCTGGTGTGGAAGTGTGGAAGAAGTCAAATTGCAAGAAGGTCTGCTGC  
282▶ snPheAspAspAl aAl aTrpAl aGl nGl yA rgCysAspAl a l l eVal A rgSer Gl yAl aGl yThr Val l Gl yA rgThr Gl n l l eAl aArgArgSer Al aAl  
7201 TGT TGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAACAGGAGACCCTCCACAAGACCAGCAAGGAATGAATATTAGCTAGATTATCC  
315▶ aVal l TrpThrAspGl yCysVal l Gl uVal l LeuAl aAspSer Gl yAsnArgArgP roSer ThrArgP roArgAl aLysGl u●●●  
7301 CTAATACCTGCCACCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAAGTGTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAA  
7401 TGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTT  
7501 TTAAAATCAGTACTTTTTAATGGAACAACCTTGACCAAAAATTTGTCACAGAATTTTGAGACCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCTT  
7601 TGGTCAACACCGAGACATTTAGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAACCTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTG  
7701 GAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTGCTTTGATTACAACACTGGAGAGAAATGC  
7800 AGCATGTTGCTGATTGCTGTCACTAAAACAGGCCAAAAACTGAGTCTTGGGTTGCATAGAAAGCTG