

pZERO-mTLR5-HA

A plasmid expressing a HA-tagged TIR-deleted murine TLR5 gene

Catalog # pzzero-mtlr5ha

For research use only

Version # 07K27-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT110 bacteria transformed with pZERO-mTLR5-HA.
- GT110 genotype is: *F*-, *mcrA*, $\Delta(mrr\text{-}hsdRMS\text{-}mcrBC)$, $\Delta\text{lacZ}\Delta M15$, $\Delta lacX74$, *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® Puro.

Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Puro at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

Toll-like receptors (TLRs) activate intracellular signaling pathways that share much in common with IL1-R signaling, owing to their conserved TIR (Toll/IL-1R) domains present in the cytoplasmic tails. The TIR family also includes the adapters MyD88, TIRAP (MAL) and a new member TRIF (TICAM). Upon ligand binding, TLRs recruit MyD88 via their TIR domains. The TLR/MyD88 complex then activates IRAK eliciting a signaling cascade leading to the activation of NF- κ B. Some TLRs also signal through a MyD88-independent pathway. Recently, TLR3 and TLR4 have been reported to interact with TRIF inducing the activation of the IRF-3 signaling pathway. Despite immense progress in the understanding of the TLR pathways, a lot remains unclear. To help you uncover the complex mechanisms governing TLR signaling, InvivoGen has engineered TIR domain-deleted TLR genes (TLR- Δ TIR). These mutant genes will serve as useful tools to study TLR signaling.

All ten human and nine murine TLR- Δ TIR genes are available in pZERO-TLR plasmids. Their expression is controlled by the strong EF1/HTLV composite promoter. pZERO-TLR plasmids are selectable with the potent antibiotic puromycin in both *E. coli* and mammalian cells.

PLASMID FEATURES

• Murine TLR5- Δ TIR-HA (2115 bp)

TLR5 is the Toll-like molecule that recognizes flagellin from both Gram⁺ and Gram⁻ bacteria. TLR5 was identified by the presence of the TIR domain and is expressed in spleen, PBL and epithelial cells. Activation of the receptor stimulates the production of proinflammatory cytokines, such as TNF- α , through signaling via the adaptor protein MyD88 and the serine kinase IRAK^{1,2}. TLR5 can generate a proinflammatory signal as a homodimer suggesting that it might be the only TLR required for flagellin recognition³. Recent data suggest that TLR5 forms heteromeric complexes with TLR4 in macrophages in response to flagellin induction³.

TLR5- Δ TIR-HA is a TIR-less form of the TLR5 gene generated by deleting the TIR domain (501 bp) and adding the influenza hemagglutinin epitope

tag (YPYDVPDYA) at the 3' end. The HA tag allows for simple and convenient detection of the expressed TLR- Δ TIR gene by Western blot using an HA primary antibody. InvivoGen offers an Anti-HA tag (catalog code: ab-hatag) that can be used to detect the expressed fusion protein.

- **EF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) core promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁴. EF-1 α is a 'housekeeping' gene ubiquitously expressed in eukaryotic cells. The EF-1 α promoter exhibits a strong activity, higher than viral promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*. The R-U5' has been coupled to the EF-1 α core promoter to enhance stability of DNA and RNA
- **SV40 pAn:** The SV 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **CMV prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Pac:** The *Pac* gene encodes a puromycin N-acetyl-transferase that confers resistance to the antibiotic puromycin. Mammalian cells are generally sensitive to concentrations ranging from 1 to 10 μ g/ml depending on the cell line.
- **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.

References

1. Gewirtz AT. *et al.* (2001). *J Immunol*, 167(4):1882-5.
2. Hayashi F. *et al.* (2001). *Nature*, 410(6832):1099-103.
3. Mizel SB. *et al.* (2003). *J Immunol*, 170(12):6217-23.
4. Kim *et al.* (1990). *Gene* 2: 217-223.
5. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

METHODS

Growth of pZERO-transformed bacteria:

Use sterile conditions to do the following:

- 1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
- 2- Streak bacteria taken from this suspension on a puromycin LB agar plate prepared with the *E. coli* Fast-Media® Puro agar provided (see below).
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with puromycin using the Fast-Media® Puro liquid provided (see below).
- 5- Extract the pZERO plasmid DNA using the method of your choice.

Note: For long-term storage of the pZERO-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

TECHNICAL SUPPORT

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Selection of bacteria with *E. coli* Fast-Media Puro:

E. coli Fast-Media® Puro is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave.

Method:

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask.
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

Preparation of cell extract for detection of HA-tagged TLR protein:

The buffer used to prepare the cell lysates is a modified RIPA buffer that is suitable for recovery of membrane receptors such as the TLRs.

- 1- Lift cells from 6-well plate and wash twice with PBS.
- 2- Add 100 µl of Cell Lysis Buffer and incubate 30 min. on ice.
- 3- Centrifuge 10 min @ 10,000 rpm (at 4°C if possible).
- 4- Transfer supernatant to a new tube and store @ -20°C.

Cell Lysis Buffer

- 5 ml of 100 mM Tris HCl, pH 7.4.
- 5 ml of 1M NaCl
- 100 µl of 0.5M EDTA
- 500 µl of 100mM NaF
- 500 µl of 10% SDS
- 2.5 ml of 10% Na-Deoxycholate
- 500 µl of 100% Triton-X 100
- 5 ml of 10% Glycerol
- H₂O to a final volume of 50 ml.

Store this stock buffer @ 4°C.

Determine amount of lysis buffer needed to make cell extracts and just before use add the following components at the final concentrations below:

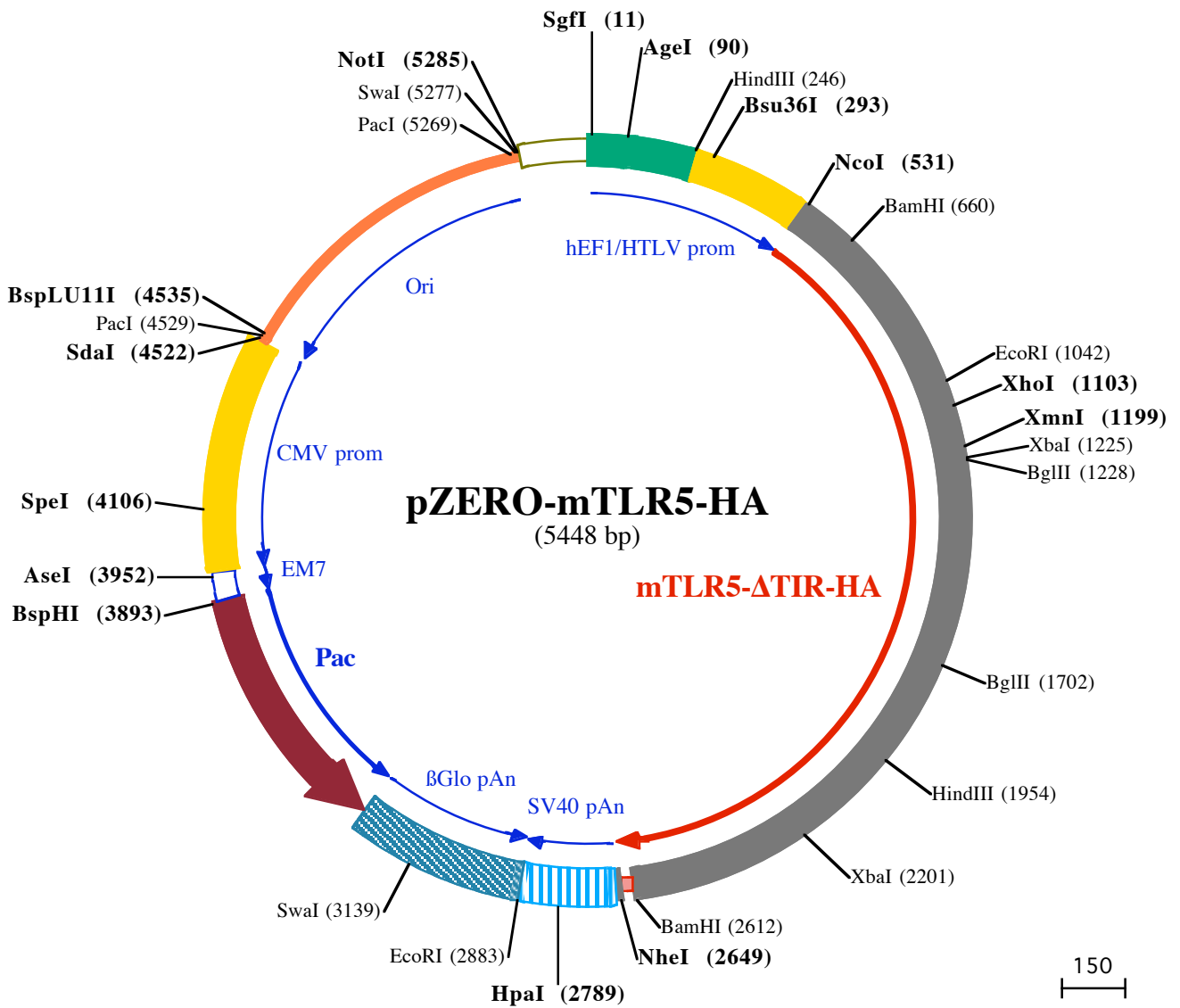
- 1 mM PMSF
- 1:100 Protease Inhibitor cocktail
- 2 mM of Na₃VO₄

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SgfI (11) AgeI (90)

1 GGATCTGCGATCGTCCGGTCCCGTCAGTGGCGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTGGCAATTGAACCGGTGCCTA

101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246) Bsu36I (293)

201 GTGAACGTTCTTTTTTCGAACGGGTTTCCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCCACGCGCCCGCCCTACCTGAGGGC

301 GCCATCCACGCGGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGCTTAGTAAAGTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCGGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCTGTTT

NcoI (531)

501 TCTGTTCTGCGCGTTACAGATCCAAGCCACCATGGCATGCAACTTGACTTGCTCATAGGTGTGATCTTCATGGCCAGCCCGTGGTAAATATCTCC

BamHI (660)

601 CTGTTCTCAGACGGCAGGATAGCCTTTTTCCGAGGCTGTAACCTACCCAGATTCCTGGATCCTCAATACTACCACTGAGAGGCTCCTGCTCAGCTTC

23▶ oCysSer SerAspGI yArgI l eAl aPhePheArgGI yCysAsnLeuThr GI nI l eProTrpI l eLeuAsnThr Thr Thr GI uArgLeuLeuLeuSer Phe

701 AACTATATCAGTATGTTGGTGGCCACATCATTCCACTCCTGGAGCAGCTCCAGTTGCTGGAGCTGGGACCCAGTATGCTAACTTGACCATTGTTCCAG

57▶ AsnTyrI l eSer MetVal ValAl aThr Ser PheProLeuLeuGI uGI nLeuGI nLeuLeuGI uLeuGI yThr GI nTyrAl aAsnLeuThr I l eGI yProG

801 GGGCTTTCCAGAACTGCCCAATCTTAGGACTTTGGACTTGGCCAAAGCCAGATCGAAGTCTTGAATCGAGATGCTTTCAAGGCTGCCCATCTCTT

90▶ yAl aPheArgAsnLeuProAsnLeuArgI l eLeuAspLeuGI yGI nSer GI nI l eGI uVal l eLeuAsnArgAspAl aPheGI nGI yLeuProHi l eLeuLe

901 GGAACCTCGGCTGTTTCTGTGGACTCTCCAGTGTGTAAAGTGACGGTACTTCAGAAATCTATATTATTAGCTCGTTAGACCTATCTGGCAAC

123▶ uGI uLeuArgLeuSer CysGI yLeuSer SerAl aVal l eLeuSerAspGI yTyrPheArgAsnLeuTyrSerLeuAl aLeuAspLeuSerGI yAsn

EcoRI (1042)

1001 CAGATTACAGCCTCCGCTCCATTCTTCCATTCGGGAAGTGAATTCCTTAAGCGACGTAATTTTCTTTCAACCAATATTCACTATATGTGAAGATG

157▶ GI nI l eHi sSer LeuArgLeuHi sSer Ser PheArgGI uLeuAsnSer LeuSerAspVal l eLeuAsnPheAl aPheAsnGI nI l ePheThr I l eCysGI uAspG

XhoI (1103) XmnI (1199)

1101 AACTCGAGCCTTCGAGGGCAAAACACTGTCTTTCTTTGGCCTCAAATTAAGCTGTTCCAGCAGAGTCTCTGTGGGCTGGGAGACATGCGAAGAACCC

190▶ l uLeuGI uProLeuGI nGI yLysThr LeuSer PhePheGI yLeuLysLeuThr LysLeuPheSer ArgVal l eGI yTrpGI uThr CysArgAsnPr

BglIII (1228)

1201 CTTCAGAGCGGTGAGGCTAGAACTCTAGATCTTTCTGAAATGGCTGGACGGTGGACATCACAAGAACTTCAGCAACATCATCCAGGGAAGCCAGATT

223▶ oPheArgGI yVal ArgLeuGI uThr LeuAspLeuSer GI uAsnGI yTrpThr Val AspI l eThr ArgAsnPheSerAsnI l eI l eGI nGI ySer GI nI l e

1301 TCCTCTTGTATCTTAAACACCATCATGGGTCCTGGCTTTGGCTCCAGAACATCAGAGATCCTGACCAGAGCACATTTGCCAGCCTGGCCAGAAGTT

257▶ Ser SerLeuI l eLeuLysHi sHi sI l eMetGI yProGI yPheGI yPheGI nAsnI l eArgAspProAspGI nSer Thr PheAl aSerLeuAl aArgSer S

1401 CGGTGCTGCAACTGGACCTTTCGCACGGCTTATCTTCTTGAATCCTCGACTGTTTGGACACTGAAGGATTTGAAGATGCTGAACCTTGCCTTCAA

290▶ erVal l eLeuGI nLeuAspLeuSer Hi sGI yPheI l ePheSer LeuAsnProArgLeuPheGI yThr LeuLysAspLeuLysMetLeuAsnLeuAl aPheAs

1501 CAAGATAAACCAAGATTGGAGAGAATGCCTTTATGGGCTTGACAGCCTCCAGGTTCTCAATCTATCTATAATCTTTTGGGGAACTCTATAATCTCAAAC

323▶ nLysI l eAsnLysI l eGI yGI uAsnAl aPheTyrGI yLeuAspSer LeuGI nVal l eLeuAsnLeuSer TyrAsnLeuLeuGI yGI uLeuTyrAsnSerAsn

1601 TTTATGGGCTTCTAGAGTAGCCTACGTTGACCTTCAAAGGAACACATTTGGGATCATTCAAGACCAACATTCAGATTATTAACAACTTCAACAACTT

357▶ PheTyrGI yLeuProArgValAl aTyrVal AspLeuGI nArgAsnHi sI l eGI yI l eI l eGI nAspGI nThr PheArgLeuLeuLysThrLeuGI nThrL

BglIII (1702)

1701 TAGATCTCCGTGACAATGCTCTTAAAGCCATTGGTTTTTCTTCAAGCATAAGATGTTCTCTGGGAGGCAATAAGTGGTCCATTTGCCACACATCCA

390▶ euAspLeuArgAspAsnAl aLeuLysAl aI l eGI yPheI l eProSer I l eGI nMetVal l eLeuLeuGI yGI yAsnLysLeuAl aHi sLeuProHi sI l eHi

1801 CTTTACTGCCAATCTCTAGAGTTATCTGAAAACAGGCTAGAAAACCTGTCGACCTCTACTTCTCTGCGAGTCCCCAGCTCCAGTTTCTCATCTTG

423▶ sPheThrAl aAsnPheLeuGI uLeuSer GI uAsnArgLeuGI uAsnLeuSerAspLeuTyrPheLeuLeuArgVal l eProGI nLeuGI nPheLeuI l eLeu

HindIII (1954)

1901 AATCAGAATCGCTTTCTGTCATGCAAGGCAGCCACACTCCCTCGGAGAACCAGCTTAGAACAGCTTTTCTTACAGAGAATATGCTGCAGCTGGCCT

457▶ AsnGI nAsnArgLeuSer Ser CysLysAl aAl aHi sThr ProSer GI uAsnProSer LeuGI uGI nLeuPheLeuThr GI uAsnMetLeuGI nLeuAl aT

2001 GGGAGACCGGCTCTGTTGGATGTTTTCAAGGCCTTCCGCGCTCCAGATCTTTACCTGAGTAATAACTACCTTAACTTCTTCCACCTGGGATATT

490▶ rPGI uThr GI yLeuCysTrpAspVal l PheGI nGI yLeuSer ArgLeuGI nI l eLeuTyrLeuSerAsnAsnTyrLeuAsnPheLeuProProGI yI l ePh

2101 TAACGACCTGGTTGCATTACGGATGCTTAGTCTAGTGCTAAACAGCTGACCGTCTCTCCGGGACAGTTTACCTGCTAATTTAGAGATTCTCGACATA

523▶ eAsnAspLeuValAl aLeuArgMetLeuSerLeuSerAl aAsnLysLeuThr Val l eLeuSerProGI ySerLeuProAl aAsnLeuGI uI l eLeuAspI l e

XbaI (2201)

2201 TCTAGAATCAGCTTTCTCTCCTGACCCTGCTTTGTTTTCTTCCGCTCGTGTGTTTGGACATAACTCATAACAGGTTCTGCTGCAACTGTGAACCTTAGCA

557▶ SerArgAsnGI nLeuPheSerProAspProAl aLeuPheSerSerLeuArgVal l eLeuAspI l eThr Hi sAsnGI nPheVal l eCysAsnCysGI uLeuSerT

2301 CTTTTATCTCTGGCTCAACCAACCAACGTCACCTGTTCCGCTCCTCGACAGCTGATTGCATGTACCCTAACTCACTGCTAGGGGGCTCCCTCTA

590▶ hrPheI l eSer TrpLeuAsnGI nThrAsnVal l ThrLeuPheGI ySerProAl aAspVal l TyrCysMetTyrProAsnSerLeuLeuGI yGI ySerLeuTy

2401 CAACATATCCACGAAGACTGCGATGAAGGAAGCCATGGGCTCCCTAAAGTTTTCCCTTTTCTATCTCTGTCACGGTCACTTTGACTTATCTCTGCTC

623▶ rAsnI l eSer Thr GI uAspCysAspGI uGI uGI uAl aMetArgSerLeuLysPheSerLeuPheI l eLeuCysThrVal l ThrLeuThrLeuPheLeuVal

2501 ATCACCCTGTAGTCATAAAGTTCCGGGAATCTGTTTCTGTGCTATAAGACCTCAGAAGCTGGTGTTCAGGACAAGGCTGGAGTTTGGAACTCG

657▶ I l eThrLeuVal l eI l eLysPheArgGI yI l eCysPheLeuCysTyrLysThrI l eGI nLysLeuVal l PheLysAspLysVal l TrpSerLeuGI uProG

BamHI (2612) NheI (2649)

2601 GTGCATATAGAGGATCTACCCCTATGATGTGCCAGACTACGCCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAA

690▶ l yAl aTyrArgGI ySerTyrProTyrAspVal l ProAspTyrAl a●●●

HpaI (2789)

2701 CTAGAATCGAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAAACAA

EcoRI (2883)

2801 TTGCATTCATTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCAAAATACAGCA

2901 TAGCAAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCT

3001 GTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTTCCCAAGGTTTGAACAGCTCTTCACTTCTTATGTTTAAATGCAGCTGACCT

SwaI (3139)

3101 CCCACATTCCTTTTAGTAAATATTCAGAAATAATTAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGC

3201 CCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGAAACAAAGAACCTTTAATAGAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTTAA

200▶●●●●

3301 GCTCCAGGCTTCTTGTGCATGCACCAAGTTCTGGCCTTCTGAACTCAACATCAGCTGTCCAGTGAATCCAGCTTTCTATAAAAAGGACAGGTTTCT

198▶ l aGI yProLysArgThrMetCysTrpThrArgProGI yGI uProVal l eGI uVal l AspAl aThrVal l ThrPheGI yLeuArgGI uTyrPheProLeuAsnAr

3401 TGGGAGCAGAAGTTCCAGAAAGGCAGGAAGCTCCAGCCCTTTCAGCAGCTTCACTCCAGGCAGAACACAGCAGATCCAGACCCTTCCCTGGTGGTC
165 gP roAl aSer Thr Gl uLeuPheAl aProVal Gl yAl aArgGl uAl aAl aGl uVal Gl yProLeuVal Val Al aSer Gl yLeuGl yLysGl yGl nHi sAsp
3501 AGGGCTCACTCCAACAGTTGCCAGAAACCAAGCTGGCTCTTTGGCCTGTGTGGTCCAGCAGACCTCCATTTGTTGTTGTGCTGCCAGCCTGCTTCCA
132 P roSer Val Gl yVal Thr Al aLeuPheTrpAl aProGl uLysProArgHisP roAl aLeuLeuGl yGl uMetGl nGl nGl nAl aAl aLeuArgSer Gl yS
3601 GAGAGCTCAGCATTCTTGGTCCAATTTCCAGCAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAAACTGCAACAGCAGCTCCATCATCTGCAA
98 er LeuGl uAl aMetArgProGl y l eGl uAl aPheVal Al aGl yAl aGl uVal Ser Gl uProThr Thr T rpVal Al aVal Al aAl aGl yAspAspAl aVa
3701 CCAAACCTTTTCCAAATGCCAGTCCACTCTGGTGGAGAGAGTCTTGCAGTCTGTCCACCTCTCAATGTGCCTGTCCAGGCTCAACTGTGTGCCTGT
65 l T rpVal LysGl y l eAspLeuGl yVal ArgThr LeuPheLeuGl uGl nLeuGl uThr Val ArgGl u l eHi sArgAspP roAspVal Thr Hi sArgThr
BspHI (3893)
3801 TGCAGGGTAGTCTGCAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGAAACATCATCTCTGGTTGCCAGCCTCACTGTGGGTTGTACTAGTCATGATG
32 Al aProTyrAspAl aPheAl aAl aAl aLeuThrArgVal Al aArgP roVal AspAspArgThr Al aLeuArgVal Thr ProLysTyrGl uThr Met ←
AseI (3952)
3901 GCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTC
4001 ACTAAACGAGCTCTGCTTATATAGACCTCCACCCTACACGCTACCGCCATTTGGCGTCAATGGGGCGGAGTTGTTACGACATTTTGAAAGTCCCGTT
SpeI (4106)
4101 GATTTACTAGTCAAACAAACTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCA
4201 AAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCAATGTACTGGGCATAATGCCAGGCGGGC
4301 CATTACCCTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCGAGTTTACCCTAAACTCCACCCATTGAC
4401 GTC AATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTAC
PaeI (4529)
SdaI (4522) BspLU11I (4535)
4501 CGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTGTGCGCTTTTCC
4601 ATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGCGGTTTCCCCTGG
4701 AAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGC
4801 TG TAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTTCCAGCCGACCGCTGCGCTTATCCGGTAACTATC
4901 GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTT
5001 CTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCT
5101 TGATCCGGCAAAACAAACACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCT
PaeI (5269) SwaI (5277) NotI (5285)
5201 TTTCTACGGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATA
5301 TCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAAAT
5401 AGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA