

pDUO2-mcs

A plasmid containing two multiple cloning sites and the hygromycin resistance gene

Catalog # pduo2-mcs

For research use only

Version # 08K04-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT110 *E. coli* bacteria transformed with the pDUO2-mcs plasmid.
- GT110 genotype is: *F*-, *mcrA*, $\Delta(mrr-hsdRMS-mcrBC)$, $\Delta\text{Ø80lacZ}\Delta M15$, $\Delta lacX74$, *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® Hygro

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® Hygro at room temperature. Fast-Media® is 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) 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- κ B which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

To date, ten toll-like receptors have been reported in humans (TLR1 to TLR10) and only nine in mice (TLR1 to TLR9). 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- κ B 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 pDUO comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

• **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.

MCS1 includes the following restriction sites:

Age I, *Eco* RV, *Bam* HI, *Sal* I and *Avr* II

- *Age* I is compatible with *Bsp* EI and *Sgr* AI.
- *Eco* RV is compatible with any blunt-end restriction enzymes.
- *Bam* HI is compatible with *Bgl* II, *Bst* YI and *Bcl* I.
- *Sal* I is compatible with *Ava* I and *Xho* I.
- *Avr* II is compatible with *Xba* I, *Spe* I and *Nhe* I.

MCS2 includes the following restriction sites:

Sgr AI, *Bgl* II, *Xho* I and *Nhe* I

- *Sgr* AI is compatible with *Bsp* EI and *Age* I.
- *Bgl* II is compatible with *Bam* HI, *Bst* YI and *Bcl* I.
- *Xho* I is compatible with *Ava* I and *Sal* I.
- *Nhe* I is compatible with *Xba* I, *Spe* I and *Avr* II.

• **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 several-fold 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*.
- **hph 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

Growth of pDUO2-transformed bacteria:

Use **sterile conditions** to do the following:

- 1- Resuspend the lyophilized *E. coli* strain 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 an hygromycin LB agar plate prepared with the *E. coli* Fast-Media® Hygro agar provided.
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with hygromycin using the Fast-Media® Hygro liquid provided.
- 5- Extract the pDUO2 plasmid DNA using the method of your choice.

Note: For long-term storage of the pDUO2-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

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

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

- 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.

References:

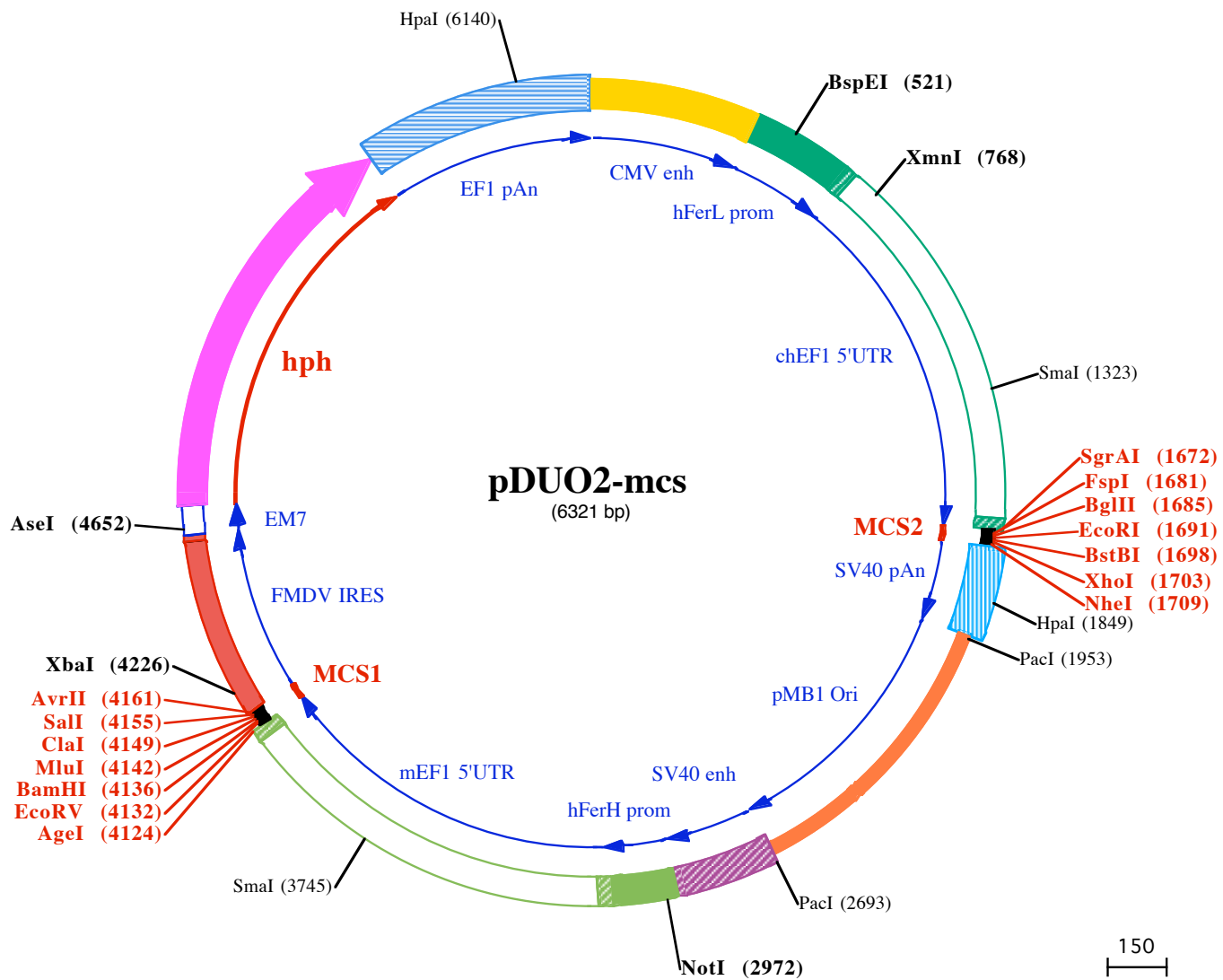
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1 CCTGCAGGCGTTACATAAAGTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATG
401 GGAGTTTGTGTTGACTAGT CAGGGCCCAACCCCAAGCCCAATTTACAACACGCTGGCGCTACAGGCGCGTGACTTCCCTTGTCTTGGGCGGG
BspEI (521)
501 GGGCTGAGACTCCTATGTGCTCCGATTGGTCAAGCACGGCCTTCGGCCCCGCTCCTGCCACCGCAGATTGGCCGCTAGCCCTCCCGAGCGCCTGCC
601 TCCGAGGCGCGGCACCATAAAAAGAAGCCGCTAGCCACGTCCCTCGCAGTTGCGGGTCCGCGGGTGTCTCAAGTTGCCGCCAGAACACAGG
XmnI (768)
701 taagtgcgctgtgtggttccgcgcccctggcctctttacgggtatggccttgctgccttgaattacttccatgcccctggctgcagtagctgattc
801 ttgatcccgagcttcgggttgaagtgggtgggagagttcgagccttgcttaaggagcccccttcgctcgtgcttgagttgagggctggctggggcg
901 ctggggcccgctgctaactggtggcaccttcgctcgtctcgtcttctgctaagtctctagccatttaaaatgataaacagctgcgacg
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1101 agcgcacatgttcggcgagggggcctgcgagcgcggccaccgagaatcggagcggggtagtctcaaaactggcggcctgctcgtgctggctgcgc
1201 gccgcgctgtatcggcgcccctggcggaaggctggcccggctggcaccagttgctgtagcggaaagatggcgcttcccggcctgctcagggagc
SmaI (1323)
1301 tcaaatggaggacgcggcgggggagagcgggctgagtcaccacacaaaggaaaggcccttctcctcatcgtcgttcatgtgactcca
1401 cggagtaccggcgccgctcaggcacctcgattagttgtcgagctttggagtagctcgtcttaggttgggggaggggtttatgcatggagtttcc
1501 ccacactgagtggtggagactgaagagttagccagcttggcactgtagtaattctccttggatattgccctttttagttggatcttgcctcattc
FspI (1681) EcoRI (1691)
SgrAI (1672) BglII (1685) BstBI (1698)
1601 tcaagcctcagacagtggttcaagttttttcttccatttcagGTGTCGTGAAAACACCCTAAAAGCACCGCGTGGCAAGATCTGAATTTCTCG
NheI (1709)
XhoI (1703)
1701 AACTCGAGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCAACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG
HpaI (1849)
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGTGAATAAAAGTTAACAACAATTGCATTCATTTTATGTTTCAGGTTGAGGGGAGGTGTGG
PacI (1953)
1901 GAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGAAATGTAATTAAGTACCCATGACCAAAATCCCTAACGTGAGTTTTCGTTCACCTG
2001 AGCGTCAGACCCGTAGAAAAGATCAAAGGATCTTCTTGTAGATCCTTTTTCTGCGGTAATCTGCTGCTTGCAAAACAAAAAACACCGCTACCAGCG
2101 GTGGTTTTTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGT
2201 AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCT
2301 TACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGAGCGAACGACCTAC
2401 ACCGAAGTGAATACCTACAGCGTGAATGAGAAAGCGCCAGCTTCCGAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGCGAGGTGGAACAG
2501 GAGAGCGCACGAGGAGCTTCCAGGGGAAACGCCTGGTATCTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTGTATTTTGTGATGCTC
PacI (2693)
2601 GTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGCCTTTTTACGTTCTGGCCTTTTGTGCGCTTTTGTGCTCATGTTCTTAATTAACCTG
2701 CAGGGCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACGACTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTC
2801 CCCAGGCTCCCGAGGAGGAGTATGCAAGATGCATCTCAATTAGTCAGCAACAGGTGTGAAAGTCCCAGGCTCCCGAGGAGGAGGATG
NotI (2972)
2901 CAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGGAGGGCTCCAGCGCCGCCCTCCCGACAGCAGGGGCGG
3001 GGTCCCGCCACCGAAGGAGCGGGCTCGGGCGGGCGGCTGATTGGCCGGGCGGGCTGACGCCGACGCGGTATAAGAGACCACAAGCAGCCC
3101 GCAGGGCCAGACTTCTTCGCCAAGCTTCCGCTCAGAACGCAAGtgaggggaggggtgagcttcgcccgggcccggagctggaggtcctgctccgagcg
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3401 ttgccctgctcctcattgcttgcggtcagcaataggggtaacaaggagggtgcccggcctgctcggcggagcccggagaggtcatggttggggagg
3501 aatggagggacaggagtgccggtggggcccggccttcggagcacatgtccgacccactggatggggcaggcctggggttttcccgaagcaac
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3701 atcccgctccggcaccagttgctgctggaagatggccgctcccgccctgttgcaaggagctcaaaatggaggacgcgagcccggtggagcggg
3801 gggtagtgcacccacacaaaggaagagggcctggctccctcaccggctgctgcttctgtgaccccggtgctctatcgccgcaatagtacctcgggctt
3901 ttgagcacggctagtcgcgccgggggggggagtgtaatggcgttgaggattgttccacatttggtgggtggagactagtcaggccagcctggcgtggaa
4001 gtcatttttggaaatttgccttgagttttgagcggagctaatctcgggcttcttagcgggttcaaggtatcttttaaaccttttttagGTGTGTG

EcoRV (4132) MluI (4142) SalI (4155)
AgeI (4124) BamHI (4136) ClaI (4149) AvrII (4161)
4101 AAAACCACCGCTAATTCAAAGCAACCGGTGATATCGGATCCACGCGTATCGATTGTCGACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT

XbaI (4226)
4201 TGAAACTCCGCTGGTCTTCCAGGTTCTAGAGGGTAACACTTTGACTGCGTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTT
4301 GCTTCGTAGCGGAGCATGACGGCCGTGGAACTCTCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCTCATGTGTGCAACC
4401 CCAGCACGGCGACTTTACTGCGAAACCCACTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCTTCAGGTACCCCGAG
4501 GTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGC

AseI (4652)
4601 ACCTTTCCTTTGCAATTAAGTACTGACCTATGAATACAAGTACTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAACGACTCACTATAG

4701 GAGGGCCACCATGAAGAACTGAACTGACAGCACTTCTGTTGAGAAGTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAA
MetLysLysProGluLeuThrAlaThrSerValGluLysPheLeuIleGluLysPheAspSerValSerAspLeuMetGlnLeuSerGlu
4801 GGTGAAGAAAGCAGAGCCTTTCTTTTGTATGTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATTCCTGTGCTGATGGTTTTTACAAGACAGATATGTTT
31> Gl yGl uGluSer ArgAl aPheSer PheAspVal Gl yGl yArgGlu yTyrVal lLeuArgVal AsnSer CysAl aAspGlu yPheTyrLysAspArgTyrVal T
4901 ACAGACACTTTGCTCTGCTGCTGCCAATCCAGAAGTTCTGGACATGGAGAATTTTCTGAATCTCTACCTACTGCATCAGCAGAAGAGCACAAAG
64> yr ArgHis sPheAl aSer Al aAl aLeuPro l l eP roGl uVal lLeuAsp l l eGl yGl uPheSer Gl uSer LeuThr TyrCys l l eSer ArgArgAl aGl nGl
5001 AGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCAACTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACC
97> yVal Thr LeuGl nAspLeuProGluThr Gl uLeuProAl aVal lLeuGl nProVal Al aGl uAl aMet AspAl a l l eAl aAl aAl aAspLeuSer Gl nThr
5101 TCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCAGTACACCCTGGAGGGATTTCAATTTGTCCATTGCTGATCCTCATGTCTATCACTGGCAGA
131> Ser Gl yPheGl yProPheGl yProGlnGl y l l eGl yGl nTyrThr Thr TrpArgAspPhe l l eCysAl a l l eAl aAspProHis sVal lTyrHis sTrpGl nT
5201 CTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAACCTCATGCTGTGGGCAGAAGATTGCTCTGAAGTCAGACACTGGTCCATGC
164> hr Val Met AspAspThr Val Ser Al aSer Val Al aGl nAl aLeuAspGl uLeuMetLeuTrpAl aGl uAspCysProGluVal lArgHis sLeuVal lHis Al
5301 TGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTGGAGATTCTCAATATGAGTTGCC
197> aAspPheGl ySer AsnAsnVal lLeuThr AspAsnGl yArg l l eThr Al aVal l l eAspTrpSer Gl uAl aMetPheGl yAspSer Gl nTyrGl uVal Al a
5401 AACATTTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAACAACAACAAGATATTTTGAAGAAGACACCCAGAAGCTGGCTGGTTCCTCCAGACTGAGAG
231> Asn l l ePhePheTrpArgProTrpLeuAl aCysMetGl uGlnGl nThr ArgTyrPheGl uArgArgHis sProGl uLeuAl aGl ySer ProArgLeuArgA
5501 CCTACATGCTCAGAATTTGGCTGGCAACTGTATCAATCTCTGGTTGATGGAACTTTGATGATGCTTGGGCACAAGGAAGATGTGATGCCATTGT
264> l aTyrMetLeuArg l l eGl yLeuAspGl nLeuTyrGl nSer LeuVal AspGl yAsnPheAspAl aAl aTrpAl aGl nGl yArgCysAspAl a l l eVa
5601 GAGGTCTGGTGTGAACTGTTGGAAGAACTCAAATTGCAAGAAGTCTGCTGCTGTTGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAAC
297> lArgSer Gl yAl aGl yThr Val Gl yArgThr Gl n l l eAl aArgArgSer Al aAl aVal lTrpThrAspGl yCysVal lGl uVal lLeuAl aAspSer Gl yAsn
5701 AGGAGACCTCCACAAGCCAGAGCCAGGAATGAATATTAGTAGATTATCCCTAATACCTGCCACCCACTTAAATCAGTGGTGAAGAAGCGTCT
331> ArgArgProSer Thr ArgProArgAl aLysGl u•••
5801 CAGAAGCTGTTGTTCAATTTGCCATTTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAAGGAAAGGAGAATGTTT

HpaI (6140)
5901 TGTGGACCACTTGGTTTTCTTTTTCGCTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGAAACAACCTTGACCAAAAATTTGT
6001 CACAGAATTTTGGAGACCATTAAAAAGTTAAATGAGAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTTCTGGTTT
6101 TACGAATCTGGAACCTTCTTGAATAATCTTGGTAAACACTCTGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAAT
6201 ATCATTAAAGCTATGGGAGGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTAAAACAGGCCAAAAACTGAGTC
6301 CTTGGGTTGCATAGAAAGCTG