

pVITRO2-neo-GFP/LacZ

A multigenic plasmid for high levels of expression of the GFP and LacZ reporter genes

Catalog code: pvitro2-ngfplacz

<https://www.invivogen.com/pvitro2-gfplacz>

For research use only

Version 19L18-MM

PRODUCT INFORMATION

Content

- 20 µg of pVITRO2-neo-GFP/LacZ provided as lyophilized DNA

Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA is stable for 12 months when stored at -20°C
- Resuspended DNA is stable for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

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

pVITRO is a new family of vectors with improved features. pVITRO1 and pVITRO2 allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and yield high levels of expression.

pVITRO2-neo-GFP/LacZ contains the reporter genes GFP and LacZ and can be used as a control vector.

pVITRO2-neo-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Bsp*HI/Avr II for GFP and *Nco*I/*Nhe*I for LacZ) that allow for convenient cloning of ORF's which can be selected from InvivoGen's extensive pORF and pBLAST lists.

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 protein¹. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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 plasmids².
- **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 enhancer³.
- **pMB1 ori:** A minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **GFP gene:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

- **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 expression⁴.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In bacteria, *neo* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *neo* 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.
- **LacZ gene:** The *E. coli lacZ* gene codes for the enzyme β -galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope.
- **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.*⁵

1. Eisenstein R.S. & Munro H.N. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
2. Dean D.A. *et al.* 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22.
3. 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.
4. 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.
5. Carswell S. & Alwine J.C., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258.

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 DH5 α .

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent <i>E. coli</i> cells	gt116-11
G418	Selection antibiotic	ant-gn-1

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

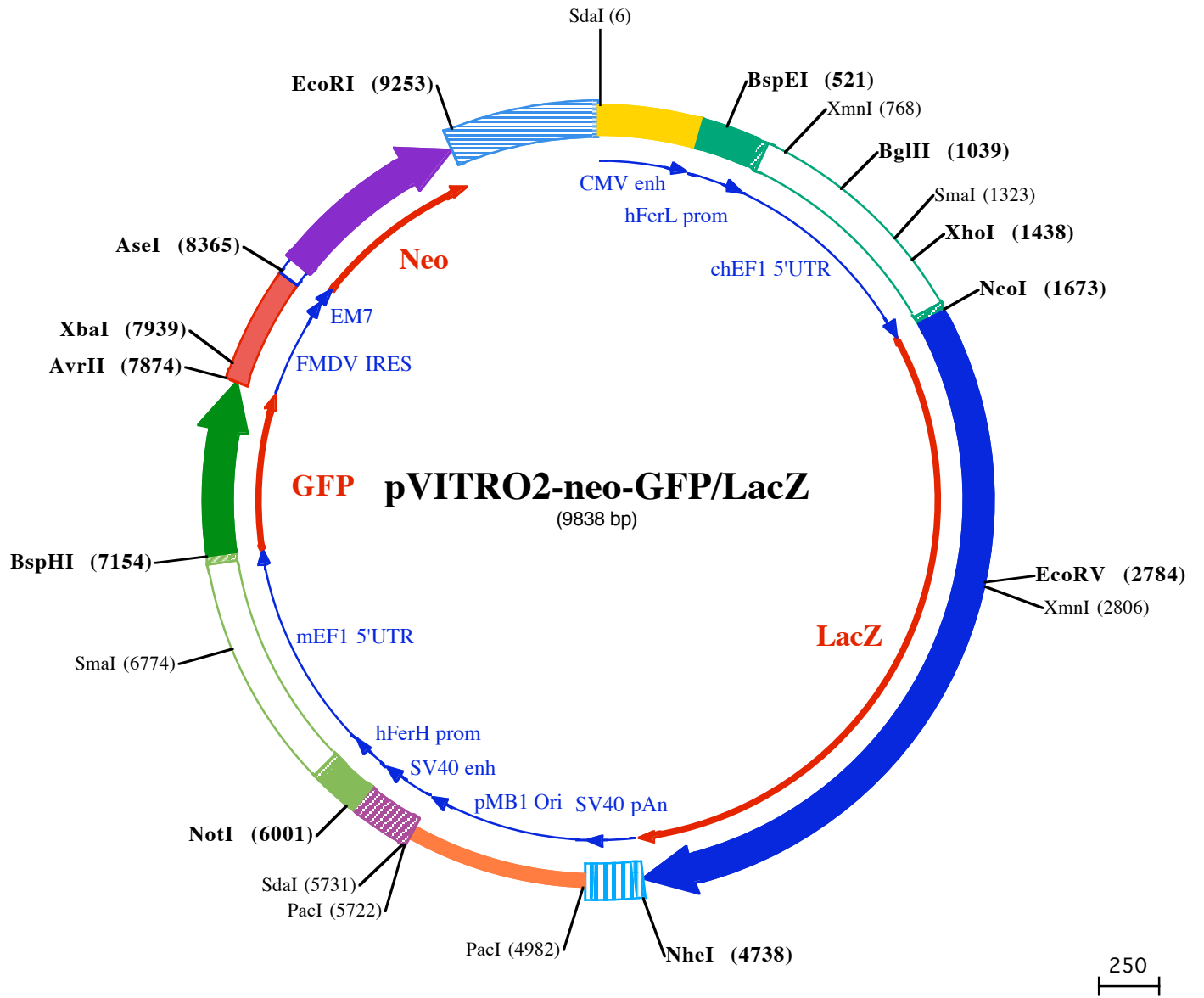
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3701 AACTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGACAACCTGTGGTAAACAGTGTAGGGTGGTTGAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACAT
676▶ IuLeuP roGl uLeuP roGl nP roGl uSer Al aGl yGl nLeuT rpLeuThr Val A rgVal Val Gl nP roAsnAl aThr Al aTrpSer Gl uAl aGl yHi s l l
3801 CTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCCTGCCTGCTGCCCTCATGCCATCCCTCACCTGACACATCTGAAATGGACTTC
709▶ eSer Al aTrpGl nGl nTrpArgLeuAl aGl uAsnLeuSer Val Thr LeuP roAl aAl aSer Hi sAl a l l eP roHi sLeuThr Thr Ser Gl uMeT AspPhe
3901 TGCATTGAGCTGGGCAACAGAGATGGCAGTTCAACAGCAGCTGCTGGCTCTCTCAGATGTGGATTGGAGCAAGAAAGCAGCTCCCTCACCTCTCA
743▶ Cys l l eGl uLeuGl yAsnLysArgT rpGl nPheAsnArgGl nSer Gl yPheLeuSer Gl nMeT rp l l eGl yAspLysLysGl nLeuLeuThr P roLeuA
4001 GGGACCAATTCACAGGGCTCCTCTGGACAATGACATTTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGTGGAGAGGTGGAAGGCTGCTGG
776▶ r gAspGl nPheThr ArgAl aP roLeuAspAsnAsp l l eGl yVal Ser Gl uAl aThr Arg l l eAspP roAsnAl aTrpVal Gl uArgTrpLysAl aAl aGl
4101 ACACTACCAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACACCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGCACCAAGGCAAG
809▶ yHi s TyrGl nAl aGl uAl aAl aLeuLeuGl nCysThr Al aAspThr LeuAl aAspAl aVal l eul l eThr Thr Al aHi sAl aTrpGl nHi sGl nGl yLys
4201 ACCCTGTTTCATCAGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTTGCCCTGACACACCTCACCTGC
843▶ Thr LeuPhe l l eSer ArgLysThr TyrArg l l eAspGl ySer Gl yGl nMeT al l l eThr Val AspVal Gl uVal Al aSer AspThr P roHi sP roAl aA
4301 GGATTGGCTGAACGTCAACTGGCACAGGTGGCTGAGAGGGTGAACCTGGCTGGGCTTAGGCCCTCAGGAGAATACCCTGACAGGCTGACAGCTGCCCTG
876▶ r gl l eGl yLeuAsnCysGl nLeuAl aGl nVal Al aGl uArgVal AsnTrpLeuGl yLeuGl yP roGl nGl uAsnTyrP roAspArgLeuThr Al aAl aCy
4401 CTTTGACAGGTGGACCTGCCTCTGACATGTACACCCCTATGTGTTCCCTCTGAGAAATGGCCTGAGGTGTGGCACCAGGAGCTGAACTATGGT
909▶ sPheAspArgTrpAspLeuP roLeuSer A sPMeT yTrpThr P roTyrVal PheP roSer Gl uAsnGl yLeuArgCysGl yThr ArgGl uLeuAsnTyrGl y
4501 CCTCACAGTGGAGGGAGACTTCCAGTTCAACATCTCCAGTACTCTCAGCAACAGCTCATGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGG
943▶ P roHi sGl nTrpArgGl yAspPheGl nPheAsn l l eSer ArgTyrSer Gl nGl nGl nLeuMeTGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uG
4601 GAACCTGGCTAACATTGATGGCTCCACATGGCATTGGAGGAGATGACTTTGGTCTCTTCTGTCTGTGAGTTCAGTTATCTGCTGGCAGGTA
976▶ l yThr TrpLeuAsn l l eAspGl yPheHi sMeTGl y l l eGl yGl yAspAspSer TrpSer P roSer Val Ser Al aGl uPheGl nLeuSer Al aGl yA rgTy

NheI (4738)

4701 CCACATCAGCTGGTGTGGTCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCAACTAGAATGCAG
1009▶ r Hi s TyrGl nLeuVal TrpCysGl nLys ●●●
4801 TGAAAAAATGCTTATTATTGTGAAATTTGTGATGCTATTGCTTTATTGTAACCATTATAAGTGAATAAACAGTTAACACAAACAATTGCATTCATT

PacI (4982)

4901 TTATGTTTCAGTTCAGGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATGTTAATTAAGTACAGCCATGACCAAA
5001 ATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTACAGCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCT
5101 TGCAACAAAAAACCCACCGCTACCAGCGGTGTTTTGTTTCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGAT
5201 ACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTG
5301 GCTGCTGCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGTTACCAGGATAAAGCCAGCGGTGGGCTGAACGGGGGTTCTGTGCA
5401 CACAGCCAGCTGGAGCGAACGACCTACACCGAAGTACCTACAGCGTGTAGCTATGAGAAAGCCACGCTTCCCGAAGGGAGAAAGCGGCAGC
5501 GTATCCGGTAAGCGGCGAGGTCGGAACAGGAGAGCGCAGGAGGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGCTGGGTTTCCGCACTC
5601 TGACTTGAGCGTGCATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGGCCCTTTTTACGGTTCCTGGCCTTTTGTCTGGC

PacI (5722) SdaI (5731)

5701 CTTTTGCTCACATGTTCTTAATTAACCTGACGGCCGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGG
5801 AATGTGTGTCAGTTAGGTTGTGAAAGTCCCGAGGCTCCCGAGCAGGAGAAGTATGAAAGCATGCATCTCAATTAGTCAGCAACAGGTTGGAAAGT

NotI (6001)

5901 CCCAGGCTCCCGAGCAGGAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCGCAGAGCGCGGAGGGCTCCAG
6001 CGGCCGCCCTCCCGACAGCAGGGCGGGTCCCGCCACCGAAGGAGCGGGCTCGGGCGGGCGGCTGATTGGCCGGGCGGGCTGACGCCG
6101 ACGGCGGTATAAGAGACCACAAGCGACCCGAGGGCCAGAGCTTCTTCCCGAAGCTTCCGTCAGAACGCAGGtgagggggcgggtggtgcttccgctggg
6201 ccgcccagctggaggctctgctccgagcgggcccggcccgcgtgctgctggcgggatttagctgagcattcccgttccgagttgcccggcggcgggga
6301 ggcagagtgagggcctagcggcaaccctgagctcgcctcgtgctccgcttagggcctagcgtggtgctccgcccgcgcgctgctactccggccg
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6501 ggagcccggagaggtcatggttggggaggaaatggaggacaggagtgccggcgggcccgccttccgagcacatgtccgacgcacctggatggg
6601 gcgagggcctggggTTTTTCCGAAGCAACAGGCTGGGTTAGCgtgcccagcaccggcagcatctggcttggcggcggcggcgt

SmaI (6774)

6701 tgccctgectccctaactagggtgaggccatcccgtccggcaccagttgctgctggaagatggccgctcccgggcccgttgaaggagctcaaat
6801 ggaggacgcccagcccgggtggagcggcgggtgagtcaccacacaaaggaagaggcctggctccctaccggctgctgcttctgtaccctcggtc
6901 ctatcggccgcaatagtcacctcgggcttttgagcagggctagtcgcccggggggggagggatgtaatggcgttggagttgttccacatttgggtggg
7001 agactagtcaggccagcctggcgtggaagtcatTTTTGAAATTTGTCCTTgagttttagcggagcattctcgggcttcttagcgttcaagggt

BspHI (7154)

7101 atcttttaaaccttttttagTGTGTGAAACACCCTAATCAAAGCAATCATGAGCAAGGGAGAAGAACTCTTACTGGTGTGTCCCAATTCTG
7201 GTTGAGCTGGATGGTGTGTAATGGCCACAATTTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTGAAAGTCTATTGTGA
16▶ Val Gl uLeuAspGl yAspVal AsnGl yHi sLysPheSer Val Ser Gl yGl uGl yAspAl aThr TyrGl yLysLeuThr LeuLysPhe l l eCysT
7301 CAACAGGAAAGCTGCGAGTGCCTTGGCAACTCTGGTACCCTGACTTATGGTGTTCATGTTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGA
49▶ hr Thr Gl yLysLeuP roVal P roTrpP roThr LeuVal Thr Thr LeuThr TyrGl yVal Gl nCysPheSer ArgTyrP roAspHi sMeT LysGl nHi sAA
7401 CTTCTTTAAATCTGCAATGCCAGAAGTTATGTTCCAGGAGGACAATCTCTTTAAGGATGATGGAATATAAGACAAGGAGGAGGAGGAAATGAA
82▶ pPhePheLysSer Al aMeT P roGl uGl yTyrVal Gl nGl uArgThr l l ePhePheLysAspAspGl yAsnTyrLysThr ArgAl aGl uVal LysPheGl u
7501 GGTGATACACTGTTTAAACAGAATTGAGCTGAAAGGCATTGATTTTAAAGGAGATGAAACATTTCTGGTGCAGAGCTGGAGTACAACATAAATCTCACA
116▶ Gl yAspThr LeuVal AsnArg l l eGl uLeuLysGl y l l eAspPheLysGl uAspGl yAsn l l eLeuGl yHi sLysLeuGl uTyrAsnTyrAsnSer Hi sA

7601 ATGTTTACATTATGGCAGATAAGCAGAAGTGAATTAAGGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTGGCAGACCATTA
149▶ snVal TyrI l eMe tAl aAspLysGl nLysAsnGl yI l eLysVal l AsnPheLysI l eArgH i sAsnI l eGl uAspGl ySer Val l Gl nLeuAl aAspHi sTy
7701 CCAGCAGAACACCCCTATTGGTGTATGGCCAGTTCCTCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGA
182▶ rGl nGl nAsnThr Pro l l eGl yAspGl yProVal l LeuLeuP roAspAsnHi sTyrLeuArgThr Gl nSerAl aLeuSer LysAspP roAsnGl uLysArg
AvrII (7874)
7801 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTAATCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCAATGACAC
216▶ AspHi sMe tVal l LeuLeuGl uPheVal l Thr Al aAl aGl yI l eThr LeuGl yMe tAspGl uLeuTyrLys●●●
XbaI (7939)
7901 AAAACGTGCAACTTAACTCCGCCTGGTCTTTCAGGCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTAG
8001 TAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAATCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGT
8101 CATGTGTGCAACCCAGCACGGGCACTTTACTGCGAAACCCACTTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTT
8201 CAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAGCGCTCGGTTTAAAAGCTTCTATGCCTGAATAGGTG
AseI (8365)
8301 ACCGGAGGTCGGCACCTTTCCTTTCGCAATTAAGTACCTGACCTATGAATACAACCTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAA
CGACTCACTATAGGAGGCCACCATGATTGAACAAGATGGATTGCACGCGAGTTCTCCGGCCGCTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACA
▶ Met l l eGl uGl nAspGl yLeuHi sAl aGl ySer ProAl aAl aTrpVal l Gl uArgLeuPheGl yTyrAspTrpAl aGl
8501 ACAGACAATCGGCTGCTCTGATGCCGCCGTTCGGCTGTACGCGAGGGCGCCGGTCTTTTGTCAAGACCGACCTGTCCGGTCCCTGAATGAA
26▶ nGl nThr l l eGl yCysSerAspAl aAl aVal l PheArgLeuSer Al aGl nGl yArgP roVal l LeuPheVal l LysThrAspLeuSer Gl yAl aLeuAsnGl u
8601 CTGCAAGACGAGGCAGCGCGCTATCGTGGCTGGCCAGCAGCGGGCTTCCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGC
60▶ LeuGl nAspGl uAl aAl aArgLeuSer TrpLeuAl aThr Thr Gl yVal l P roCysAl aAl aVal l LeuAspVal l Val l Thr Gl uAl aGl yA rgAspTrpLeuL
8701 TATTGGCGAAGTGC CGGGCAGGATCTCCTGTCTCACCTTCTCCTGCGGAGAAAGTATCCATCATGGCTGATGCAATGCGCGGCTGCATACGCT
93▶ euLeuGl yGl uVal l P roGl yGl nAspLeuLeuSer Ser Hi sLeuAl aP roAl aGl uLysVal l Ser l l eMe tAl aAspAl aMe tArgArgLeuHi sThr Le
8801 TGATCCGGCTACCTGCCATTCCGACCAACGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTGCATCAGGATGATCTGGAC
126▶ uAspP roAl aThr CysP roPheAspHi sGl nAl aLysHi sArg l l eGl uArgAl aArgThr ArgMe tGl uAl aGl yLeuVal l AspGl nAspAspLeuAsp
8901 GAAGAGCATCAGGGCTCGCGCCAGCCGAACGTTCGCCAGGCTCAAGCGAGCATGCCGACGGCGAGGATCTCGCTGACACATGGCGATGCCTGCT
160▶ Gl uGl uHi sGl nGl yLeuAl aP roAl aGl uLeuPheAl aArgLeuLysAl aSer Me tP roAspGl yGl uAspLeuVal l Val l Thr Hi sGl yAspAl aCysL
9001 TGCCGAATATCATGGTGGAAAATGGCCGCTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCG
193▶ euP roAsn l l eMe tVal l Gl uAsnGl yArgPheSer Gl yPhe l l eAspCysGl yA rgLeuGl yVal l Al aAspArgTyrGl nAsp l l eAl aLeuAl aThr Ar
9101 TGATATTGCTGAAGAGCTTGGCGCGAATGGGCTGACCGCTTCTCTGCTGCTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCTTCTATGCCTT
226▶ gAsp l l eAl aGl uGl uLeuGl yGl uT rpAl aAspArgPheLeuVal l LeuTyrGl y l l eAl aAl aP roAspSer Gl nArg l l eAl aPheTyrArgLeu
EcoRI (9253)
9201 CTTGACGAGTCTTCTGAGCGGACTCTGGGTTGAAATGACCGACCAAGCAATTCGATAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAG
260▶ LeuAspGl uPhePhe●●●
9301 TGGTGAAGAACGGTCTCAGAAGTGGTTCATTTGTTCAATTGGCCATTTAAGTTTATAGTAGTAAAAGACTGGTTAATGATAAACAATGCATCGTAAAAACCTTCAGA
9401 AGGAAAGGAGAATGTTTGTGGACCATTGGTTCCTTTTGGCGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGAAACAA
9501 CTTGACCAAAAATTTGTACAGAATTTTGAGACCCATTAAGTAAATGAGAAACCTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAG
9601 ACATCTAATCTGGTTTACGAATCTGGAACCTCTTGAAGTGAATCTTGGTAACTTCTGGTGGAGAATAGGTTGTTTTCCCCACATAA
9701 TTGGAAGGGGAAGAAATATCATTTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATCGACATGTTGCTGATTCCTGCTCACTAAAAC
9801 AGGCCAAAACCTGAGTCTTGGTTGCATAGAAAGCTG