

pVITRO2-blasti-GFP/LacZ

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

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

For research use only

Version 20H18-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-blasti-GFP/LacZ provided as lyophilized 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

pVITRO is a family of plasmids developed mainly for *in vitro* studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

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

pVITRO2-blasti-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (BspH I/Avr II for GFP and Nco I/Nhe I for LacZ) that allow for convenient cloning of ORF's.

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 water. 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.

PLASMID FEATURES

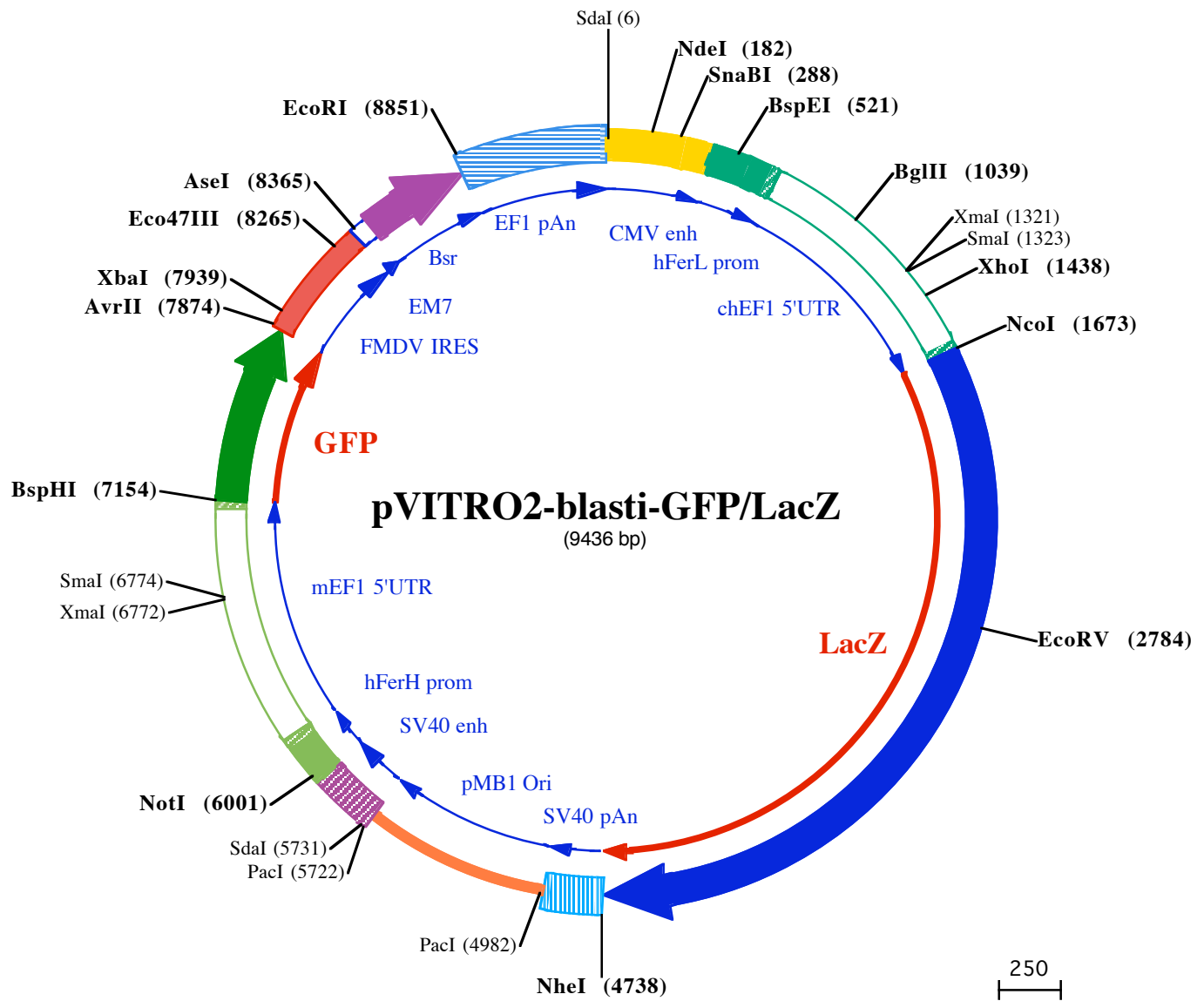
- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron 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 range². The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.
- **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*.
- **Blasti:** Resistance to blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the hFerH/mEF1α 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 RS. & Munro HN. 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.* 10:4248-58.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com





SdaI (6)
1 CCTGCAGGCGTTACATAA...
NdeI (182)
101 CGCCAATAGGGACTTTC...
SnaBI (288)
201 TATTGACGTCAATGAC...
301 GCTATTACCATGATG...
401 GGAGTTTGTGTTGACT...
BspEI (521)
501 GGGCTGAGACTCCTAT...
601 TCCGAGGGCCGCGCAC...
701 taagtgcgctgtgtggt...
801 ttgatcccgagcttcgg...
901 ctggggccgcccgcgt...
BglIII (1039)
1001 cttttttctggcgagat...
1101 agcgacacatgctcgg...
1201 gccgctgtatcgcccgc...
XmaI (1321)
SmaI (1323)
1301 tcaaatggaggacgccc...
XhoI (1438)
1401 cggagtaccggcgcgct...
1501 ccacactgagggtggag...
NcoI (1673)
1601 tcaagcctcagacagt...
1701 AGACTGGGAGAACCCT...
1801 AGCCAGCAGCTCAGGT...
1901 CTGACACTGTTGGTGC...
2001 GCCCACTGAGAACCCT...
2101 TCTGCTTCCACCTCT...
2201 AGAACAGGCTGGCTG...
2301 GCTGCACAAGCCCA...
2401 GGAGAAGCTCAGAGAC...
2501 AGAGAGGAGGCTATG...
2601 GCTGCACACTGCTGA...
EcoRV (2784)
2701 CCTCTGCTCATCAGGG...
2801 AGAACAACTTCAATG...
2901 CATTGACACATGGCAT...
3001 AGGAACCAACCCTCT...
3101 GCAGACTGTGCAGTAT...
3201 TGTGCCAAGTGGAGCA...
3301 GGCTTTGCAAGTACT...
3401 ATGGCAACCCCTGGT...
3501 CCCTGCCCTCAGAGG...
3601 GCAAATGAGCTCCTG...
643 AspAsnGI uLeuLeuHi sTrpMetValAlaLeuAspGI yLysProLeuAlaSerGI yGI uValProLeuAspValAlaProGI nGI yLysGI nLeuIleG

3701 AACTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGACAACCTGTGGCTAACAGTGGGGTGGTTACGCCAATGCAACAGCTTGGTCTGAGGCAGGCCACAT
676▶ I uLeuP roGl uLeuP roGl nP roGl uSer Al aGl yGl nLeuTr pLeuThr Val l ArgVal l Val Gl nP roAsnAl aThr Al aTr pSer Gl uAl aGl yHi s l l
3801 CTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCCTCTGTGACCCTGCCTGCCTCATGCCATCCCTCACCCTGACCAATCGAAATGGACTTC
709▶ eSer Al aTr pGl nGl nTr pArgLeuAl aGl uAsnLeuSer Val Thr LeuP roAl aAl aSer Hi sAl a l l eP roHi sLeuThr Thr Ser Gl uMe tAspPhe
3901 TGCATTGAGCTGGGCAACAAGATGGCAGTTCACAGGCAGTCTGGCTTCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCTCTCA
743▶ Cys l l eGl uLeuGl yAsnLysArgTr pGl nPheAsnArgGl nSer Gl yPheLeuSer Gl nMe tTr p l l eGl yAspLysLysGl nLeuLeuThr P roLeuA
4001 GGGACCAATTCACAGGGCTCCTCTGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGTGGAGAGGTGGAAGGCTGCTGG
776▶ r gAspGl nPheThr ArgAl aP roLeuAspAsnAsp l l eGl yVal Ser Gl uAl aThr Arg l l eAspP roAsnAl aTr pVal Gl uArgTr pLysAl aAl aGl
4101 ACACATACCAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACACCCTGGCTGATGTCTTCTGATCACCACAGCCATGCTTGGCAGCACCAGGCAAG
809▶ yHi s TyrGl nAl aGl uAl aAl aLeuLeuGl nCysThr Al aAspThr LeuAl aAspAl aVal l Leu l l eThr Thr Al aHi sAl aTr pGl nHi sGl nGl yLys
4201 ACCCTGTTTCATCAGCAGAAAGACCTACAGGATGTAGTGGCTCTGGACAGATGGCAATCACAGTGGATGGAGGTTGCCTCTGACACACCTCACCCTGC
843▶ Thr LeuPhe l l eSer ArgLysThr TyrArg l l eAspGl ySer Gl yGl nMe tAl a l l eThr Val AspVal Gl uVal Al aSer AspThr P roHi sP roAl aA
4301 GGATTGGCTGAACGTCAACTGGCAGGTTGGTGGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAGAATCACCCTGACAGGCTGACAGTGCCTG
876▶ r gl l eGl yLeuAsnCysGl nLeuAl aGl nVal Al aGl uArgVal l AsnTr pLeuGl yLeuGl yP roGl nGl uAsnTyrP roAspArgLeuThr Al aAl aC y
4401 CTTTGACAGTGGGACCTGCCTCTGTCTGACATGTACACCCTTATGTGTTCCCTTCTGAGAATGGCTGAGGTGGCACCAGGGAGCTGAACATATGGT
909▶ sPheAspArgTr pAspLeuP roLeuSer AspMe tTyrThr P roTyrVal l PheP roSer Gl uAsnGl yLeuArgCysGl yThr ArgGl uLeuAsnTyrGl y
4501 CCTCACCAGTGGAGGGGAGACTTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGGAAACTCTCACAGGACCTGCTCCATGACAGGAGG
943▶ P roHi sGl nTr pArgGl yAspPheGl nPheAsn l l eSer ArgTyrSer Gl nGl nGl nLeuMe tGl uThr Ser Hi sLeuLeuHi sAl aGl uGl uG
4601 GAACCTGGCTGAACATTGATGGCTTCCACATGGCATTGGAGGAGATGACTCTTGGTCTCCTTGTGTCTGTGAGTTCAGATTATCTGCTGGCAGGTA
976▶ l yThr Tr pLeuAsn l l eAspGl yPheHi sMe tGl y l l eGl yGl yAspAspSer Tr pSer P roSer Val Ser Al aGl uPheGl nLeuSer Al aGl yArgTy

NheI (4738)

4701 CCACTATCAGCTGGTGTGGTCCAGAAGTAAACCTGAGCTAGCTGGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAATAAGAATGCAG
1009▶ r Hi sTyrGl nLeuVal l Tr pCysGl nLys ●●●
4801 TGAATAAATGCTTTATTTGTGAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAACAACAACAAATGCATTCAAT

PaeI (4982)

4901 TTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAAATGTTAATTAAGTACGCATGACCAAA
5001 ATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTACAGCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGCGTAATCTGCTGCT
5101 TGCAAAACAAAAAACACCCTACCAGCGGTGTTTTGTTTGGCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGCTTACAGAGCGCAGAT
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5301 GCTGCTCCAGTGGCGATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAAAGCGCAGCGGTGGGCTGAACGGGGGGTTCGTGCA
5401 CACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAAGCTATGAGAAAAGCGCCAGCTTCCCGAAGGAGAAAGCGGACAG
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5601 TGACTTGAGCGTCGATTTTTGTGATGCTGCTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACCGCGCTTTTTACGGTTCCTGGCTTTTGTGCGC

PaeI (5722) SdaI (5731)

5701 CTTTTGCTCACATGTTCTTAATTAACCTGCAGGGCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACAGCTGTGG
5801 AATGTGTGTCAGTTAGGTTGGAAAGTCCCAAGCTCCCAAGCAGGAGAGATGCAAAAGCATGCATCTCAATTAGTCAGCAACAGGTGTGGAAAGT

NotI (6001)

5901 CCCCAGGCTCCCAGCAGGAGAGATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGGAGGGCCCTCCAG
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6101 ACGCGCTATAAGAGACCACAAGCGACCCGAGGGCCAGAGCTTCTCGCCGAAGCTTCCGCTCAGAACGCAAGtgaggggggggtgtggcttccgcggg
6201 ccgcccagctggaggtcctgctcagagcgggcccggcccgcgtgctgctggcggggattagctgagcattccgcttccgagttgcccggcgcgcgga
6301 ggcagagtgaggcctagcggcaacccgtagcctcctcgtgctcggcttaggcctagcgtggtgctccgcccgcgcgcgctgctactccggcgcg
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6601 gcgaggcctggggttttccgaagcaacaggctggggttagcgtgcccagggccatggtggcccagaccggcagcatctggcttggcgccgcgcgt

XmaI (6772)

SmaI (6774)

6701 tgccttgcctccctaactagggtagggccatccgctccggcaccagttgctgctgctggaagatggccgctcccgggcccctgttcaaggagctcaaaa
6801 ggaggacgcggcagcccgtggagcgggctgagtcaccacacaaaggaagaggcctggtccctcaccggctgctgcttctctgtagcccggtgctc
6901 ctatcgccgcaatagtcacctcggccttttagcagcggctagtcgcccgggggagggatgtaatggcgttggagttgttccacatttgggtggg
7001 agactagtcaggccagcctggcgtggaagtcatttttggaaattgtccccttagttttagcggagctaatctcgggcttcttagcggttcaaaaggt

BspHI (7154)

7101 atcttttaaaccttttttagGTGTTGTGAAAACCCGCTAATCAAAGCAATCATGAGCAAGGGAGAAGAACTCTTACTGGTGTGTCCAACTTCTG
1▶ Me tSer LysGl yGl uGl uLeuPheThr Gl yVal l Val P ro l l eLeu
7201 GTTGAGCTGGATGGTGTGAATGGCCACAATCTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTATGGAAGCTGACTCTGAAGTTCATTGTGA
16▶ Val l Gl uLeuAspGl yAspVal l AsnGl yHi sLysPheSer Val Ser Gl yGl uGl yGl uGl yAspAl aThr TyrGl yLysLeuThr LeuLysPhe l l eCysT
7301 CAACAGGAAAGCTGCCAGTGCCTTGGCCAACTCTGGTGAACACCTGACTTATGGTGTCAATGTTTCAGCAGGTACCTGACCACATGAAGCAGCATGA
49▶ hr Thr Gl yLysLeuP roVal l P roTr pP roThr LeuVal l Thr Thr LeuThr TyrGl yVal l Gl nCysPheSer ArgTyrP roAspHi sMe tLysGl nHi sAs
7401 CTTCTTAAATCTGCAATGCCAAGGTTATGTTCCAGGAGGACAATCTCTTAAAGGATGATGGAATTTATAAGACAAGGGCAGAAGTGAAGTTTGA
82▶ pPhePheLysSer Al aMe tP roGl uGl yTyrVal l Gl nGl uArgThr l l ePhePheLysAspAspGl yAsnTyrLysThr ArgAl aGl uVal l LysPheGl u
7501 GGTGATACACTGGTAAACAGAAATGAGCTGAAAGGATTGATTTTAAAGAAAGTGAACACTTGGGTCAACAGCTGGAGTACAACATATAATCTCACA
116▶ Gl yAspThr LeuVal l AsnArg l l eGl uLeuLysGl y l l eAspPheLysGl uAspGl yAsn l l eLeuGl yHi sLysLeuGl uTyrAsnTyrAsnSer Hi sA

7601 ATGTTTACATTATGGCAGAT[▶]AAGCAGAAGAA[▶]TGGAATTAAGGTTAAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACCTGGCAGACCATTA
149▶snVal TyrI leMetAl aAspLysGl nLysAsnGl yI leLysVal AsnPheLysI leArgHisAsnI leGl uAspGl ySer Val Gl nLeuAl aAspHisTy
7701 CCAGCAGAACCACCCCTATTGGTGATGCCAGTTCCTCCAGATAATCACTATCTCCGCACCTCAATCTGCTCTCCAAAGACCC[▶]TAATGAGAAAAAG
182▶rGl nGl nAsnThr Prol leGl yAspGl yProVal LeuLeuProAspAsnHis TyrLeuArgThr Gl nSerAl aLeuSer LysAspProAsnGl uLysArg
AvrII (7874)
7801 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTA[▶]CTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCAATGACAC
216▶AspHis sMetVal LeuLeuGl uPheVal ThrAl aAl aGl yI leThr LeuGl yMetAspGl uLeuTyrLys●●●
XbaI (7939)
7901 AAAACGTGCAACTTGA[▶]AACCTCCGCTGGTCTTCCAGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAG
TAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGA[▶]ACTCCTCTTGGTAACAAGGCCACGGGGCCAAAAGCCACGCCACACGGGGCCCGT
CATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTACATTGAAACTGGTACCACACACTGGTGACAGGCTAAGGATGCCCTT
Eco47III (8265)
8201 CAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTG
AseI (8365)
8301 ACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCC[▶]TATGAATACA[▶]ACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATA
CGACTCACTATAGGAGGGCCACCATGAAGAC[▶]TTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGA
▶MetLysThr PheAsnI leSer Gl nGl nAspLeuGl uLeuVal Gl uValAl aThr Gl uLysI leThr MetLeuTyrGl
8501 GGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGT
26▶uAspAsnLysHisHisVal Gl yAl aAl aI leArgThr LysThr Gl yGl uI leI leSerAl aVal HisI leGl uAl aTyrI leGl yArgVal Thr Val Cys
8601 GCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGGCTGTGAGGCACCCCTACTCTGATGAGGTGGACAGAT
60▶Al aGl uAl aI leAl aI leGl ySerAl aVal SerAsnGl yGl nLysAspPheAspThr I leValAl aVal ArgHisProTyrSerAspGl uVal AspArgS
8701 CCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAA
93▶er I leArgVal Val SerProCysGl yMetCysArgGl uLeuI leSerAspTyrAl aProAspCysPheVal LeuI leGl uMetAsnGl yLysLeuVal Ly
EcoRI (8851)
8801 AACCACATTGAGGA[▶]ACTCATCCCCCTCAAGTACACCAGGA[▶]ACTAAACCTGAATTTCGCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCAGTG
126▶sThr Thr I leGl uGl uLeuI leProLeuLysTyrThr ArgAsn●●●
8901 GTGGAAGAACGGTCTCAGA[▶]ACTGTTTGTTC[▶]CAATTGGCCATTTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAAG
GAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTAAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAACAAC
TGACAAAAATTTGTACAGAATTTTGAGACCCATTA[▶]AAAAAGTTAAATGAGAAACCTGTGTGTTCCCTTTGGTCAACACCGGAGACATTTAGGTGAAAGAC
ATCTAATCTGTTTTACGAATCTGGA[▶]AACTTCTTGAAATGTAATTTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACAATAAT
GGAAGGGGAAGGAATATCATT[▶]TAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAATGCAGCATGTTGCTGATTGCCTGCTACTAAAAACG
GCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG