

pVITRO1-neo-GFP/LacZ

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

Catalog code: pvitro1-ngfplacz

<https://www.invivogen.com/pvitro1-gfplacz>

For research use only

Version 19L17-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO1-neo-GFP/LacZ provided as lyophilized DNA

Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA is stable for 3 months at -20°C.
- Resuspended DNA is stable for at least one year at -20°C.

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. pVITRO plasmids allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels.

pVITRO1-GFP/LacZ contains the reporter genes GFP and LacZ and can be used as a control vector.

pVITRO1-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*NcoI*/*AvrII* for GFP and *BspHI*/*NheI* for LacZ) that allow for convenient cloning of ORF's which can be selected from InvivoGen's extensive pORF and pBLAST lists

PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-mcs plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart¹, both promoters display a strong activity that yield similar levels of expression. EF-1α promoters are expressed at high levels in all cell cycles and lower levels during G0 phase. EF-1α promoters are also non-tissue specific; they are highly expressed in all cell types.
- **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 several-fold more active than the SV40 enhancer³
- **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.⁴
- **pMB1 Ori** is 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 expression⁵.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Neo:** The neo gene from Tn5 encodes an aminoglycoside 3'-phosphotransferase (3' APH II) that confers resistance to the antibiotics kanamycin in bacteria 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 rat EF-1α 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.
- **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).

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system *Gene* 91(2):217-23. 2. Dean DA. *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* 41(2):521-30. 4. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 9(10): 4248-58. 5. Ramesh N. *et al.*, 1996. High-iter bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.

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 DH5α.

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 G418	Competent <i>E. coli</i> cells Selection antibiotic	gt116-11 ant-gn-1

TECHNICAL SUPPORT

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

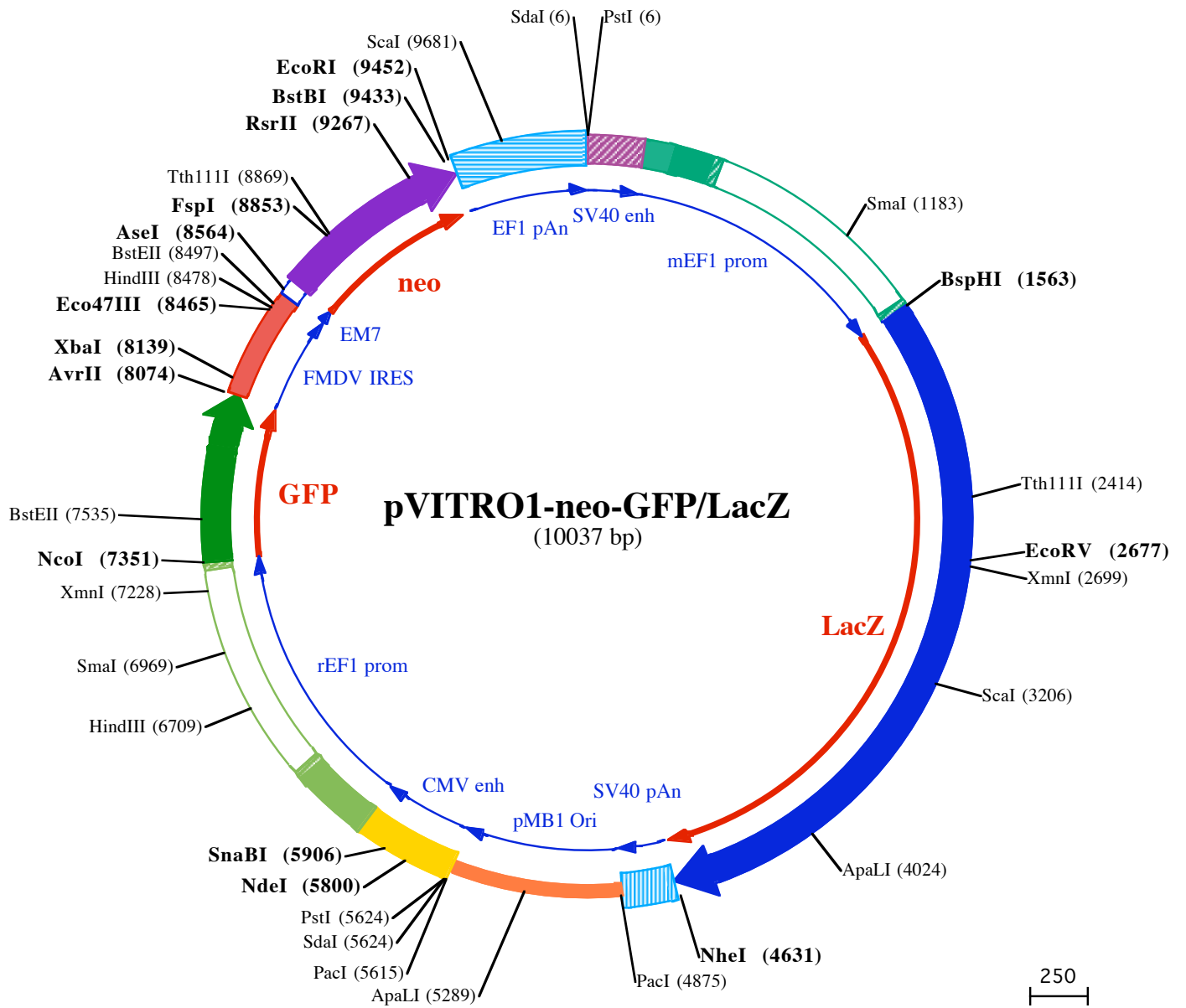
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PstI (6)
SdaI (6)
1 CCTGCAGGGCCGTAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGCGGAAAGAACAGCTGTGAATGTGTGTAGTTAGGGTGTGGAA
101 AGTCCCCAGGCTCCCCAGCAGGAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGAGAA
201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTGGAGCCGAGAGTAATTCATAAAAAGGAGGGATCGCTTCGCAAGGGGAGAG
301 CCCAGGGACCGTCCCTAAATCTCACAGACCCAAATCCCTGTAGCCGCCCCACGACAGCGCGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA
401 GCACACAAGCTCATAGACCTTGGTCGTGGGGGGGAGGACCGGGGAGCTGGCGGGGGCAAACCTGGGAAAGCGGTGTCTGTGTGCTGCCCTCCGCTTCTCC
501 CGAGGGTGGGGAGAACGGTATATAAGTGGCGAGTGCCTTGGACGTTCTTTTTGCAACGGGTTTGGCTCAGAACGCAAGtgaggggggggtgtggc
601 ttccgggggcccgagctggaggtcctgctccgagcggggccggcccgctgtcgtcggcggggatttagctcgagcattcccgtcttcgagttgctgggg
701 ggcgcgggaggcagagtgagggcctagcggcaacccccgtagcctgcctcgtgctcggcttgaggcctagcgtggtgtccgcgcgcgcgcgcgctgcta
801 ctccggccgactctggctcttttttttttttttggttgtgtgttcctgctgccttcgattgccgttcagcaataggggctaacaaggagggggtcggggct
901 tgctcgcgggagcccggagaggtatggttggggaggaatggagggacaggagtggcggctggggccgcccgccttcggagcacatgtccgacgccc
1001 ctggatggggcgagcctggggtttttccgaagcaaccaggctggggttagcgtgccgagccatgtggcccagaccggcacgatctggcttggcg

SmaI (1183)
1101 gcgccgcttgccctgcctcctaactagggtagggcctcccgtccggcaccagttgctgctggaagatggccgctccggccctgttgcagga
1201 gctcaaatggaggacgcggcagcccggtagggcggggcgggtgagtcacccacacaaaggaagaggcctggccctcaccggctgctcctcctgtagc
1301 cccggtgctctatcggccgcaatagtcacctcgggcttttgagcacgctagtcgcccggggggggggatgtaatggcggtggagtttgttcacattt
1401 ggtgggtggagactagtcaggccagcctggcgtggaagtcattttggatttggcccttgagtttgagcggagtaattctcgggcttcttagcgg

BspHI (1563)
1501 ttcaaggatctctttaaacccttttttagGTGTTGTGAAACCACCGTAATCAAGCAATCATGAGCCTGGCTGTTGTGCTGCAAAAGGAGAGACTGG
1601 GAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCTCCCTTTTGCCTCTTGGAGAACTCTGAGGAAGCCAGGACAGACAGGCCCCAGCCAGC
13> GluAsnProGlyVal Thr GluLeuAsnArgLeuAlaAlaHisProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGluN
1701 AGTCAGGCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCTCCCTGAACTGTGCCTGAGTCTGGCTGGAGTGTGACCTCCAGAGGCTGACAC
46> InLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAsnTh
1801 TGTTGTGGTGGCCAGCAACTGGCAGATGCATGGCTATGATGCCCATCTACACCAATGTCACCTACCCATCACTGTGAACCCCTTTTGTGCCACT
79> ValValValProSerAsnTrpGluMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProPheValProThr
1901 GAGAACCCTGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGCCAGCAGCAGGATCATCTTTGATGGAGTCAACTGCTC
113> GluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGluGluGlyNThrArgIleIlePheAspGlyValAsnSerAlaP
2001 TCCACCTCTGGTGAATGGCAGGTGGGTTGGCTATGGCCAAGCAGCAGGCTGCCCTCTGAGTTTACCTCTGCTTCTCAGAGCTGGAGAGAACAG
146> HeHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGluAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnAr
2101 GCTGGCTGTCATGGTGGCTGATGGCAGCTACCTGATGGAGCAGCAAGCAAGCTGGAGGATGTGGAGGATGTCTGGCATCTTGGCATCTTGGCAGC
179> GLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGluAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHis
2201 AAGCCACCACCCAGATTTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGGAGAAC
213> LysProThrThrGluNileSerAspPheHisValAlaThrArgPheAsnAspPheSerArgAlaValLeuPheAlaGluAlaGluNMetCysGlyGluL
2301 TCAGAGACTACTGAGAGTACAGTGAAGCTGAGCCTGATGTTGGATTGAGAGAGCTCAGGATGAGATGGCCCTGCTGAGCTCAATGGACAGCCTGCTG
246> euArgAspTyrLeuArgValThrValSerLeuTrpGluGlyGluThrGluValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlu

Thi111I (2414)
2401 AGGCTATGCTGACAGATCACCTGAGGCTCAATGTGGAGAACCCEAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGGCTGTTGGAGCTGCAC
279> yGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHis
2501 ACTGCTGATGGCAGCCTGATGAGCTGAGCCTGTGATGTTGGATTGAGAGAGCTCAGGATGAGATGGCCCTGCTGAGCTCAATGGACAGCCTGCTG
313> ThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuL

EcoRV (2677) XmnI (2699)
2601 TCATCAGGGAGTCAACAGGCATGACACCACCTCTGCATGGACAAGTGTGGATGAACAGACAATGGTGAAGATATCTGCTAATGAAGCAGAACAA
346> euIleArgGlyValAsnArgHisGlyHisHisProLeuHisGlyGluNValMetAspGluGluNThrMetValGluNAspIleLeuLeuMetLysGluNAsnAs
2701 CTTCAATGCTGCTCAGGTGCTCTCACTACCCCAACACCCTCTCTGGTACCCCTGTGTGACAGGATGGCCTGATGTTGTTGATGAAGCCAAACATTGAG
379> nPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGlu
2801 ACACATGGCATGGTCCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCTGCCATGTCTGAGAGAGTGACCAGGATGGTGCAGAGAGACAGGAACC
413> ThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGluNArgAspArgAsnH
2901 ACCCTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAACTGATGCTCTACAGGTGGATCAAGTCTTGGACCCAGCAGACC
446> isProSerValIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgPr
3001 TGTGCAATGAAGGAGGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCC
479> oValGluNThrGluGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGluNProPheProAlaValPro
3101 AAGTGGAGCATCAAGAAGTGGCTCTCTGCTGGAGAGACCAGCCTCTGATCTGTGTAATGACATGCAATGGCAACTCTCTGGGAGGCTTTG
513> LysTrpSerIleLysLysTrpLeuSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheA

Scal (3206)
3201 CCAAGTACTGGCAAGCCTTCCAGACAGTACCCAGGCTGCAAGGAGGATTTGTGTGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAA
546> IaLysTyrTrpGluNAlaPheArgGluNTrpProArgLeuGluGlyGlyPheValTrpAspTrpValAspGluNserLeuIleLysTyrAspGluAsnGlyAs
3301 CCCCTGGTCTGCTATGAGGAGACTTTGGTACACCCCAATGAGCCAGTCTGCTGATGAGTGGCTGGTCTTTGGACAGGACCCCTCACCTCCG
579> nProTrpSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGluNpheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAla
3401 CTCACAGAGGCCAAGCACCAGCAACAGTCTCCAGTTCAGCTGTCTGGACAGACCATGAGGTGACATCTGAGTACCTTCCAGGCACTGACAATG
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3501 AGCTCTGCACTGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTCTGGTGAAGTGGCTTGGATGGCCCTCAAGGAAAGCAGCTGATTGAACCTGCC
646> IuLeuLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGluGlyLysGluNleuIleGluLeuPr
3601 TGAGCTGCCTCAGCAGAGTCTGCTGCAACTGTGGCTAACAGTGGGTTGAGGTTGAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACATCTCTGCA
679> oGluLeuProGluNProGluuSerAlaGlyGluNLeuTrpLeuThrValArgValValGluNProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAla
3701 TGGCAGCAGTGGAGGCTGGCTGAGAACCCTCTGTGACCTGCCTGCTGCTCATGCCATCCCTCACCTGACAACATCTGAATGGACTTCTGCAATTG
713> TrpGluNgluNTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleG
3801 AGCTGGCAACAGAGATGGCAGTTCACAGGCAAGTCTGGCTCTGCTGATGAGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCAGGAGCA
746> IuLeuGlyAsnLysArgTrpGluNpheAsnArgGluNserGlyPheLeuSerGluNMetTrpIleGlyAspLysLysGluNleuLeuThrProLeuArgAspGlu
3901 ATTCACAGGCTCCTCTGGACAATGACATTTGGATGTCTGAGGCCACAGGATGACCCAAATGCTTGGTGGAGGTTGGAAGGCTGCTGGACACTAC
779> nPheThrArgAlaProLeuAspAsnAspIleGlyValSerGluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisThr

ApaLI (4024)
4001 CAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACACCTGGCTGATGCTGTTCTGATCACACAGCCCATGCTTGGCAGCACCAAGGCAAGACCCTGT
813▶ Gl nAl aGl uAl aAl aLeuLeuGl nCysThrAl aAspThrLeuAl aAspAl aVal LeuI leThr ThrAl aHi sAl aTrpGl nHi sGl nGl yLysThrLeuP
4101 TCATCAGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGTTGCCTCTGACACACCTCACCTGCAAGGATTGG
846▶ heI leSerArgLysThr TyrArgI leAspGl ySer Gl yGl nMeTAl al leThr ValIAspVal Gl uValAl aSerAspThr ProHi sProAl aArgI leGl
4201 CCTGAACGTCACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAACTACCTGACAGGCTGACAGCTGCCTGCTTTGAC
879▶ yLeuAsnCysGl nLeuAl aGl nValAl aGl uArgValAsnTrpLeuGl yLeuGl yProGl nGl uAsnTyrP roAspArgLeuThrAl aAl aCysPheAsp
4301 AGGTGGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTTCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACATGGTCCACC
913▶ ArgTrpAspLeuP roLeuSerAspMeTyrThr ProTyrValI PheP roSer Gl uAsnGl yLeuArgCysGl yThrArgGl uLeuAsnTyrGl yProHi sG
4401 AGTGGAGGGGAGACTTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGACAGAGGAGGAACTGG
946▶ InTrpArgGl yAspPheGl nPheAsnI leSerArgTyrSer Gl nGl nLeuMeTGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uGl yThr Tr
4501 GCTGAACATTGATGGCTCCACATGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCAGTTATCTGCTGCCAGTACCACAT
979▶ pLeuAsnI leAspGl yPheHi sMeTGl yI leGl yGl yAspAspSer TrpSer ProSer Val SerAl aGl uPheGl nLeuSerAl aGl yArgTyrHi sTyr

NheI (4631)
4601 CAGCTGGTGTGGTCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGCAAACCACTAGAAATGACAGTAAAAA
1013▶ Gl nLeuValI TrpCysGl nLys•••
4701 AATGCTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAACCATTATAAGCTGCAATAAACAAGTTAACAAACAATTGCATTCATTTTATGTT

PacI (4875)
4801 TCAGGTTCAAGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGTATGGAATGTTAATTAACATAGCCATGACCAAAATCCCTT
4901 AACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTCAAA
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5101 ACTGTTCTTAGTGATGCCGTAGTTAGGCCACCCTCAAGAAGCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG

ApaLI (5289)
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PstI (5624)
PacI (5615) SdaI (5624)
5601 TCACATGTTCTTAATTAACCTGCAGGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGA

NdeI (5800)
5701 CGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGGTATCA
5801 TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTAC

SnaBI (5906)
5901 ATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCC
6001 ACCCAATTGACGTCAATGGGAGTTGTTTTGACTAGTGGAGCGGAGATAATTCATAAAAAGGAGGATCGCCTTCGAAGGGGAGAGCCAGGGACCG
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6601 gccactccggccgactatgctgtttttgtccttgcctgctgcctcgattgcttccagcagcatgggctaacaaggagggtgtgggctcactcttaagg

HindIII (6709)
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SmaI (6969)
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XmnI (7228)
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NotI (7351)
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7401 GTTGAGCTGGATGGTGTGAATGGCCAAAATCTCTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTTGAAGTTCATTTGTA
17▶ Val Gl uLeuAspGl yAspValAsnGl yHi sLysPheSer Val Ser Gl yGl uGl yGl uAspAl aThr TyrGl yLysLeuThr LeuLysPheI leCysT
7501 CAACAGGAAAGCTGCCAGTGCCTTGGCCAACTCTGGTGAACACCCTGACTTATGGTGTTCATGTTTCAGCAGGTACCTGACCACATGAAGCAGCATGA
50▶ hr Thr Gl yLysLeuP roValI P roTrpP roThr LeuVal Thr ThrLeuThr TyrGl yValI Gl nCysPheSer ArgTyrP roAspHi sMeTLysGl nHi sAs

7601 CTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTCCAGGAGAGGACAATCTTCTTTAAGGATGATGGAATATAAGACAAGGGCAGAAGTGAAGTTTGAA
83▶ pPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGlu
7701 GGTGATACACTGGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGAAACATCTGGGTACCAAGCTGGAGTACAATAATCTCACA
117▶ GlyAspThrLeuValAsnArgIleGluLeuLysGlyIleAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisA
7801 ATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTGGCAGACCATTA
150▶ snValTyrIleMetAlaAspLysGlnLysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTy
7901 CCAGCAGAACACCCTATTGGTGTGGCCAGTTCTCCTCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAA
183▶ rGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArg
AvrII (8074)
8001 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTAATCTGGAATGGATGAGCTGTACAAGTAAACCTAGAGCAGGTTTCCCAATGACAC
217▶ AspHisMetValLeuLeuGluPheValThrAlaAlaGlyIleThrLeuGlyMetAspGluLeuTyrLys●●●
XbaI (8139)
8101 AAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCAGGCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTAG
8201 TAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACCTCCTCTGGTAACAAGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGT
8301 CATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTT
Eco47III (8465) HindIII (8478) BstEII (8497)
8401 CAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTG
AseI (8564)
8501 ACCGGAGGTCGGCACCTTTCCTTTGCAATTAAGTACCTGACCTATGAATACACTGACTGTTTGAACAATTAATCATCGGCATAGTATATCGGCATAGTATAATC
8601 GACTCACTATAGGAGGGCCACCATGATTGAACAAGATGGATTGCACGCAAGTTCCTCCGGCCCTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAA
1▶ MetIleGluGlnAspGlyLeuHisAlaGlySerProAlaAlaTrpValGluArgLeuPheGlyTyrAspTrpAlaGln
8701 CAGACAATCGGCTGCTGTATGCCCGTGTCCGGCTGCAGCGCAGGGCCCGGTTCTTTTGTCAAGCCAGCTGTCGGTGCCTGAATGAAC
27▶ GlnThrIleGlyCysSerAspAlaAlaValPheArgLeuSerAlaGlnGlyArgProValLeuPheValLysThrAspLeuSerGlyAlaLeuAsnGluL
FspI (8853) Tth11I (8869)
8801 TGCAAGCAGGCGCAGCGGGCTATCGTGGCTGCCACGCGGGCTTCTTGGCAGCTGTGCTGACGTTGCTACTGAAGCGGGAAGGACTGGCTGCT
60▶ euGlnAspGluAlaAlaArgLeuSerTrpLeuAlaThrThrGlyValProCysAlaAlaValLeuAspValValThrGluAlaGlyArgAspTrpLeuLe
8901 ATTTGGCGAAGTGCCTGGGCGAGGATCCTGTCTCATCTCACCTTGCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATCGCGCGCTGCATACGCTT
93▶ uLeuGlyGluValProGlyGlnAspLeuLeuSerSerHisLeuAlaProAlaGlyLysValSerIleMetAlaAspAlaMetArgArgLeuHisThrLeu
9001 GATCCGGCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAAGCCGGTCTTGTCTGATCAGGATGATCTGGAGC
127▶ AspProAlaThrCysProPheAspHisGlnAlaLysHisArgIleGluArgAlaArgThrArgMetGluAlaGlyLeuValAspGlnAspAspLeuAspG
9101 AAGAGCATCAGGGCTCGCCGACGCCAAGTGTCCGAGGCTCAAGCGCAGCATGCCCGACGGCGAGGATCTCGTCTGACACATGGCGATGCCTGCTT
160▶ IuGluHisGlnGlyLeuAlaProAlaGluLeuPheAlaArgLeuLysAlaSerMetProAspGlyGluAspLeuValValThrHisGlyAspAlaCysLe
RsrII (9267)
9201 GCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGT
193▶ uProAsnIleMetValGluAsnGlyArgPheSerGlyPheIleAspCysGlyArgLeuGlyValAlaAspArgTyrGlnAspIleAlaLeuAlaThrArg
9301 GATATTGCTGAAGACTTGGCGCGAATGGGCTGACCGCTTCTCGTCTTACGGTATCGCCGCTCCCGATTCCGACGGCATCGCTTCTATCGCTT
227▶ AspIleAlaGluGluLeuGlyGlyTrpAlaAspArgPheLeuValLeuTyrGlyIleAlaAlaProAspSerGlnArgIleAlaPheTyrArgLeuL
BstBI (9433) EcoRI (9452)
9401 TTGACGAGTCTTCTGAGCGGGACTCTGGGTTGCAATGACCGACCAAGCGAATTGCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCAGT
260▶ euAspGluPhePhe●●●
9501 GGTGGAAGAACGGTCTCAGAAGTGTGTTTCAATTGGCCATTTAAGTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAA
ScaI (9681)
9601 GGAAAGAGAATGTTTTGTGGACCACCTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGAAACAAC
9701 TTGACCAAAAATTTGTACAGAAATTTGAGACCATAAAAAAGTTAAATGAGAAACCTGTGTTCCTTTGGTCAACCCGAGACATTTAGGTGAAAGA
9801 CATCTAATTCGGTTTACGAATCTGGAACCTCTTGAATGTAATCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAAT
9901 TGGAAGGGAAGGAATATCATTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAATGCAGCATGTTGCTGATTGCCTGCTCAAAAACA
10001 GGCCAAAACCTGAGTCCTTGGTTGCATAGAAAAGCTG