

pVITRO1-GFP/LacZ

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

Catalog code: pvitro1-gfplacz

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

For research use only

Version 18J10-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-GFP/LacZ provided as lyophilized DNA
- 1 ml Hygromycin B Gold at 100 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 Hygromycin B Gold 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 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 (*Nco* I/*Avr* II for GFP and *Bsp*HI/*Nhe* I for LacZ) that allow for convenient cloning of ORFs.

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*.
- **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 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. & Albwine 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 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.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

TECHNICAL SUPPORT

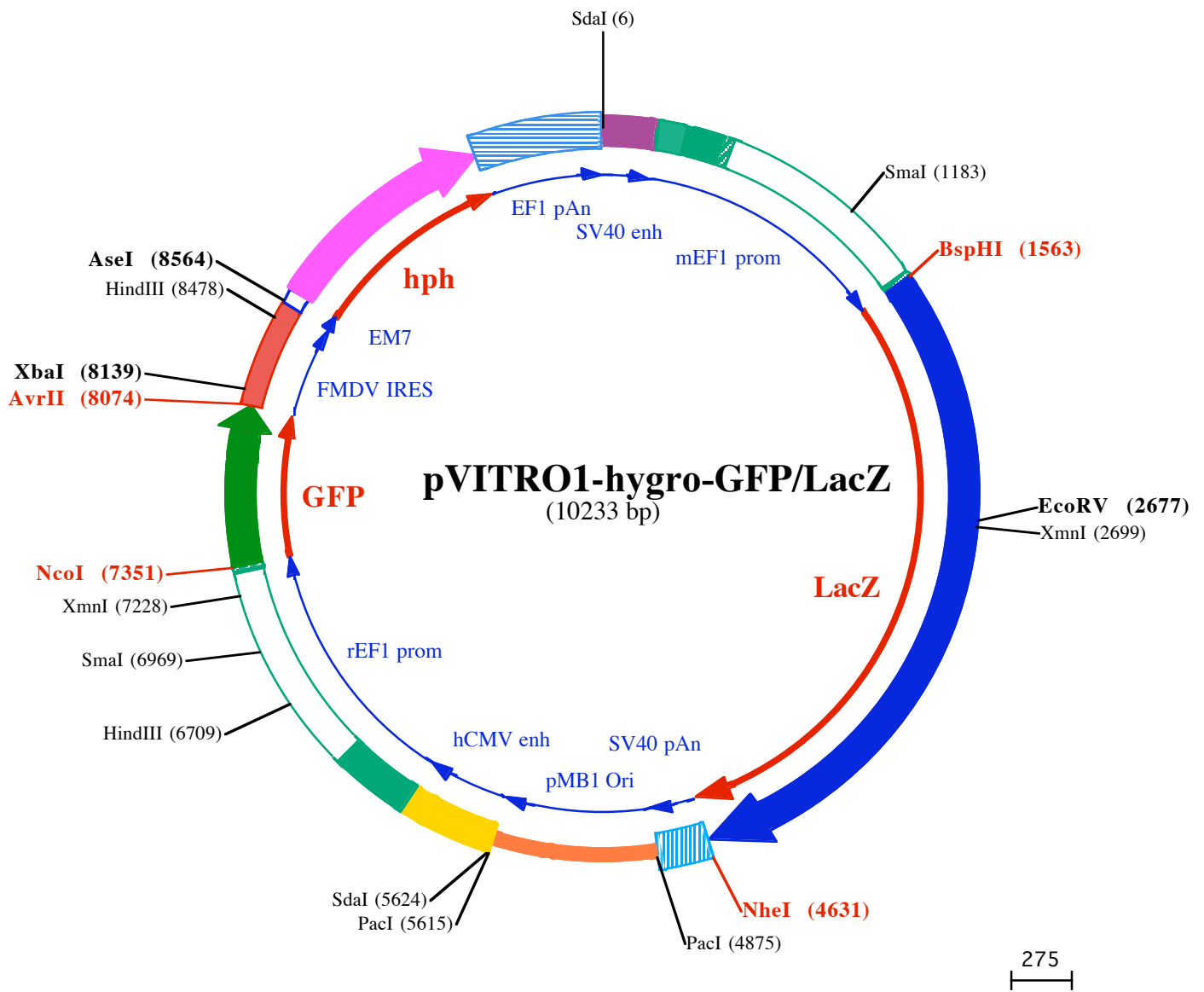
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SdaI (6)
1 CCTGCAGGGCCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGCGGAAAGAACCAAGCTGTGGAATGTGTGCAGTTAGGGTGTGGAA
101 AGTCCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAAGGTTGGAAAAGTCCCAGGCTCCCAGCAGGCAGAAG
201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG
301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGCAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA
401 GCACACAAGCTCATAGACCTGGTCTGTGGGGGGAGGACCGGGGAGCTGGCGGGGGCAAACCTGGGAAAGCGGTGTCTGTGTCTGGCTCCGCCCTCTTCC
501 CGAGGGTGGGGGAGAACGGTATATAAGTGGCGCAGTCGCTTGGACGTTCTTTTTCGCAACGGGTTTGCCGTGAGAACGAGGTGAGGGGCGGGTGTGGC
601 TTCCGGGGCCGCCGAGCTGGAGGCTCTGCTCCGAGCGGGCCGGCCCCGCTGTCTGTGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGC
701 GCGCGGGGAGGCAGAGTGCAGGCGCTAGCGGCAACCCGCTAGCTCGCTCGTGTCCGGCTTAGGCTAGCGTGGTGTCCGCGCCCGCCGCTGTGTA
801 CTCCGGCCGACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCTTCCCTGCTGCCTTCGATTGCCGTTAGCAATAGGGGCTAACAAAGGGAGGGTGCGGGGCT
901 TGCTCGCCCGGAGCCGGAGAGGTATGTTGGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCCGCTTCGGAGCACATGTCCGACGCCAC
1001 CTGGATGGGGCAGGCGCTGGGGTTTTTCCGAAGCAACCAAGGCTGGGGTTAGCTGCGGAGGCCATGTGGCCCCAGACCCGCGACGATCTGGCTTGGCG

SmaI (1183)
1101 GCGCCGCTTGCCTTCCCTAACTAGGGTGAGGCCATCCCGTCCGGCACCAGTTGCGTGGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGA
1201 GCTCAAATGGAGGACGCGGAGCCCGTGGAGCGGGGGTGGAGTACCACACAAAGGAAGAGGGCCTGGTCCCTCACCGGCTGCTTCTCTGTGAC
1301 CCCGTTGCTATCGGCCGAATAGTCACCTCGGCTTTTGGACCGGCTAGTCGCGGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTT
1401 GGTGGTGGAGACTAGTCAGGCCAGCCTGGCGTGAAGTCATTTTTGGAATTTGCCCCTTGGATTTTGGAGGAGTAAATCTCGGGCTTCTAGCGG

BspHI (1563)
1501 TTCAAAGGTATCTTTTAAACCCCTTTTTAGGTGTTGTGAAAACCACCCTAATTCAAAGCAATCATGAGCCTGGCTGTGTGCTGCAAAGGAGAGACTGG
1601 GAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCCTCCCTTTGGAGGAACCTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCCAGC
13> Gl uAsnProGlyVal Thr Gl nLeuAsnArgLeuAl aAl aHi sProProPheAl aSer TrpArgAsnSer Gl uGl uAl aArgThrAspArgProSer Gl nG
1701 AGCTCAGGCTCTCAATGGAGAGTGGAGGTTGGCTGGTCCCTGCCCTGAAGCTGTGCGCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACAC
46> InLeuArgSer LeuAsnGlyGl uTrpArgPheAl aTrpPheProAl aProGl uAl aVal ProGl uSer TrpLeuGl uCysAspLeuProGl uAl aAspTh
1801 TGTGTGGTGGCCAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTCACTACCCCATCACTGTGAACCCCTTTTGTGCCACT
79> rVal Val Val ProSerAsnTrpGlnMetHisGlyTyrAspAl aProI l eTyrThrAsnVal Thr TyrProI l eThr Val AsnProProPheVal ProThr
1901 GAGAACCCTGGAGTGCACAGCTGACCTCAATGTTGTGAGAGCCAGGCTGGCAAGAAAGCCAGACAGGATCATCTTTGATGGAGTCAACTGCTGCCT
113> Gl uAsnProThr GlyCysTyrSer LeuThr PheAsnVal AspGl uSer TrpLeuGl nGl uGl nThr ArgI l eI l ePheAspGl yVal AsnSerAl aP
2001 TCCACCTCTGGTGAATGGCAGGTGGTGGCTATGGCCAAAGACAGCAGGCTGCCCTCTGAGTTGACCTCTCTGCCTTCTCAGAGCTGGAGAGAACAG
146> heHisLeuTrpCysAsnGlyArgTrpVal GlyTyrGly nAspSerArgLeuProSer Gl uPheAspLeuSerAl aPheLeuArgAl aGlyGly uAsnAr
2101 ACTGGCTGTCTGGTCTCAGTGGTCTGATGGCAGCTACTGGCAAGCAAGACATGTGGAGGATGCTGGATCTTCAGGATGTGAGCCTGCTGCAC
179> gLeuAl aVal MetVal LeuArgTrpSerAspGl ySer TyrLeuGl uAspGl nAspMetTrpArgMetSer Gl yI l ePheArgAspVal SerLeuLeuHis
2201 AAGCCACCCAGCAGATTCTGACTTCCATGTTGCCACAGGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAAC
213> LysProThr Thr Gl nI l eSerAspPheHisValAl aThrArgPheAsnAspPheSerArgAl aVal LeuGl uAl aGl uAl Gl nMetCysGlyGly uL
2301 TCAGAGACTACTGAGTACAGTGCAGTGCAGTCTGGCAAGTGGAGCCAGGCTGGCCCTTGGCACAGCCCTTTGGCACAGGAGATGATGAGAGAGG
246> euArgAspTyrLeuArgVal Thr Val SerLeuTrpGlnGlyGly uThr Gl nValAl aSer Gl yThrAl aProPheGlyGlyGly uI l eI l eAspGl uArgGl
2401 AGGCTATGCTGACAGAGTCACTGAGGCTCAATGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAACCTTACAGGGCTGTGTGGAGCTGCAC
279> yGlyTyrAl aAspArgVal ThrLeuArgLeuAsnVal Gl uAsnProLysLeuTrpSerAl aGl uI l eProAsnLeuTyrArgAl aVal Val Gl uLeuHis
2501 ACTGCTGATGGCACCCCTGATTGAAGCTGAAGCCTGTGATGTGGATTTCAGAGAAGTCAGGATGAGAAATGGCCTGCTGCTCAATGGCAAGCCTCTGC
313> ThrAl aAspGl yThrLeuI l eGl uAl aGl uAl aCysAspVal GlyPheArgGl uValArgI l eGl uAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuL

EcoRV (2677) XmnI (2699)
2601 TCATCAGGGGAGTCAACAGGCATGAGCACCACCCTCTGCATGGACAAGTGGATGAACAGACAATGGTCAAGATATCTGCTAATGAAGCAGAACA
346> eul l eArgGlyVal AsnArgHisGly uHis sHi sProLeuHis sGlyGly nVal MetAspGl uGl nThr MetVal Gl nAspI l eLeuLeuMetLysGl nAsnAs
2701 CTTCAATGCTGTGAGGCTCTCACTACCCCAACCCTCTCTGGTACACCCTGTGTGACAGGTATGGCTGTATGTTGTTGATGAAGCCAACATTGAG
379> nPheAsnAl aValArgCysSerHis sTyrProAsnHis sProLeuTrpTyrThr LeuCysAspArgTyrGlyLeuTyrVal Val AspGl uAl aAsnI l eGl u
2801 ACATATGGATGGTCCCATGAACAGGCTCACAGATGACCCAGGTTGGCTGCCATGTCTGAGAGAGTGACCAGGATGGTGCAGAGAGACAGGAACC
413> ThrHis sGlyMetVal ProMetAsnArgLeuThrAspAspProArgTrpLeuProAl aMetSer Gl uArgVal ThrArgMetVal Gl nArgAspArgAsnH
2901 ACCCTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAAACATGATGCTCTCTACAGTGGATCAAGTCTGTTGACCCAGCAGACC
446> i sProSerVal I l eI l eTrpSerLeuGl yAsnGlySer Gl yHis sGlyAl aAsnHis sAspAl aLeuTyrArgTrpI l eLysSerVal AspProSerArgP
3001 TGTGAGTATGAAGGAGTGGAGCAGACACCAGCCACAGACATCATCTGCCCATGATGCCAGGGTTGATGAGGACCAGCCCTTCCCTGTGTGCC
479> oVal Gl nTyrGly uGlyGlyAl aAspThr ThrAl aThrAspI l eI l eCysProMetTyrAl aArgVal AspGl nProPheProAl aVal Pro
3101 AAGTGGAGCATCAAGAAGTGGCTCTCTGCTGGAGAGACCAGCTCTGATCTGTGTAATGCACATGCAATGGCAACTCTCTGGGAGGCTTTG
513> LysTrpSer I l eLysLysTrpLeuSerLeuProGlyGly uThrArgProLeuI l eLeuCysGlyTyrAl aHis sAl aMetGlyAsnSerLeuGlyGlyPheA
3201 CCAAGTACTGGCAAGCCTTACAGAGTACCCAGGCTGCAAGGAGGATTTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAA
546> l aLysTyrTrpGly nAl aPheArgGl nTyrProArgLeuGlyGlyPheVal TrpAspTrpVal AspGl nSerLeuI l eLysTyrAspGl uAsnGlyAs
3301 CCCCTGGTCTGCCTATGGAGGAGACTTTGGTACACCCCAATGACAGGCACTTCTGCATGAATGGCTGGTCTTTGCAGACAGGACCCCTCACCTGCC
579> nProTrpSerAl aTyrGlyGly AspPheGlyAspThrProAsnAspArgGl nPheCysMetAsnGlyLeuVal PheAl aAspArgThrProHis sProAl a
3401 CTCACAGAGGCCAAGCACCAGCAACAGTCTTCCAGTTCAGGCTGTCTGGACAGACCAATTGAGGTGACATCTGAGTACCTTTCAGGCACTGTGACAATG
613> LeuThrGl uAl aLysHis sGlnGl nGlnPhePheGlnPheArgLeuSer Gl yGlnThr I l eGl uVal ThrSer Gl uTyrLeuPheArgHis sSerAspAsnG
3501 AGCTCTGCACTGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGGTGGCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAAGTCC
646> l uLeuLeuHis sTrpMetValAl aLeuAspGlyLysProLeuAl aSer Gl yGly uVal ProLeuAspValAl aProGlnGlyLysGlnLeuI l eGl uLeuP
3601 TGAGTGCCTCAGCAGAGTCTGCTGGCAACTGTGGCTAACAGTGGGTTGAGGCTTCCAGCCCAATGCAACAGCTTGGTCTGAGGACGCCACATCTCTGCA
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3701 TGGCAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCTGCCTGCTCCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTG
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3801 AGCTGGGCAACAAGAGATGGCAGTTCACAGGCAGTCTGGCTTCTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGGACCA
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4101 TCATCAGCAGAAAGACCTACAGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTGGCTCTGACACACCTCACCTGCAAGGATTGG
846▶ helIleSerArgLysThrTyrArgIleAspGlySerGlyGlnMetAlaIleThrValAspValGluValAlaSerAspThrProHisSProAlaArgIleG
4201 CCTGAACGTCAACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAGAATACCTGACAGGCTGACAGCTGCCTGCTTTGAC
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4301 AAGTGGGACCTGCTGTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCTGAGGTGGCACCAGGGAGCTGAACATATGGTCCCTCAC
913▶ ArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisS
4401 AGTGGAGGGGAGACTTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGGAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTG
946▶ InTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyThrTr
4501 GCTGAACATTGATGGCTCCACATGGCATTGGAGGAGATGACTTTGGTCTCCTCTGTCTGCTGAGTCCAGTTATCTGCTGGCAGGTACCATTAT
979▶ pLeuAsnIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGlyArgTyrHisTyr

NheI (4631)

4601 CAGCTGGTGTGGTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAA
1013▶ GlnLeuValTrpCysGlnLys●●●

4701 AATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTATTTTATGTT

PacI (4875)

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5501 AGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACCGCGCTTTTTACGGTCTCTGGCCTTTTCTGGCCTTTTCTG

PacI (5615) SdaI (5624)

5601 TCACATGTTCTTAATTAACCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGA
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HindIII (6709)

6701 agcccatgaagcttacgttggataggaatggaagggcaggaggggagcactggggcccgcctcggagcacatgtccgacgccactggatggggcg
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SmaI (6969)

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XmnI (7228)

7201 ctagtcaggccagcctggcgtggaagctatttggaaatttggcctttgagtttgagcaggagctaatctcaagcctcttagcggttcaaggattt

NcoI (7351)

7301 ttctaaaccgtttcagGTGTTGTGAAAGCCACCCTAATTC**AAAGCAAc**ATGGTTTCTAAGGGAGAAGAACTTTACTGGTGTGTCCCAATTCTG
1 MetVal Ser LysGlyGluLeuPheThrGlyValValProIleLeu

7401 GTTGAGCTGGATGTTGATGTGAATGGCCACAAATCTCTGTGTCTGGTGAAGGTGAAGGAGATGCACTTATGAAAGCTGACTCTGAAGTTCATTTGTA
17 ValGluLeuAspGlyAspValAsnGlyHisLysPheSerValSerGlyGluGlyGluGlyAspAlaThrTyrGlyLysLeuThrLeuLysPheIleCysT

7501 CAACAGGAAAGCTGCCAGTGCCTTGGCCAACCTCTGGTGACCACCCTGACTTATGGTGTTC**CAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGA**
50 hrThrGlyLysLeuProValProTrpProThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAs

7601 CTTCTTTAAATCTGCAATGCCAGAAGTTATGTTCCAGGAGAGGACAATCTTCTTTAAGGATGATGAAATATAAGACAAGGGCAGAAAGTGAAGTTTGAA
83 pPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGlu

7701 GGTGATACACTGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAAGGAAGATGAAACATTCTGGGTCAACAGCTGGAGTACAACATAATTCTCACA
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7801 ATGTTTACATTATGCAGATAAGCAGAAGAATGGAATTAAGGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACCTGGCAGACCATTA
150 snValTyrIleMetAlaAspLysGlnLysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTy

7901 CCAGCAAGAACCCCTATTTGGTGTATGGCCAGTTCTCCAGATAATCTCCGCACTCAATCTCTGTC**CAAGACCC**TAATGAGAAAAGA
183 rGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArg

AvrII (8074)

8001 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTA**CTCTGGAAATGGATGAGCTGTACAAGTAA**CCCTAGGACGAGGTTTCCCAATGACAC
217 AspHisMetValLeuLeuGluPheValThrAlaAlaGlyIleThrLeuGlyMetAspGlyLeuTyrLys•••

XbaI (8139)

8101 AAAACGTGCAACTTGAAACTCCGCTGGTCTTCCAGGTCTAGAGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAG

8201 TAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCTCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCTG

8301 CATGTGTGCAACCCAGCAGCGGCAGCTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACTGGTGACAGGCTAAGGATGCCCTT

HindIII (8478)

8401 CAGGTACCCCGAGGTAACACGCGACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAGCGCTCGGTTTAAAAGCTTCTATGCCTGAATAGGTG

AseI (8564)

8501 ACCGAGGTCGGCACCTTCTCTTGAATTACTGACCCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATAC

8601 GA**CTCACTATAGGAGGCCACC**ATGAAGAACTGAACTGACAGCACTCTGTTGAGAAGTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATG
1 MetLysLysProGluLeuThrAlaThrSerValGluLysPheLeuIleGluLysPheAspSerValSerAspLeuMet

8701 CAGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTCTTTTGGTGTGGAGGAAGAGGTTATGTTCTGAGGGTCAATTTCTGTGCTGATGGTTTTACAAAAG
27 GlnLeuSerGluGlyGluSerArgAlaPheSerPheAspValGlyGlyArgGlyTyrValLeuArgValAsnSerCysAlaAspGlyPheTyrLysA

8801 ACAGATATGTTTACAGACACTTTGCCTCTGCTGCTGCCAATTCAGAAGTTCTGGACATTGGAGAATTTCTGAATCTCTCACTACTGCATCAGCAG
60 spArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProIleProGluValLeuAspIleGlyGluPheSerGluSerLeuThrTyrCysIleSerAr

8901 AAGAGCACAAGGAGTCACTCCAGGATCTCCCTGAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGAT
93 gArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGluLeuProAlaValLeuGlnProValAlaGluAlaMetAspAlaIleAlaAlaAlaAsp

9001 CTGAGCCAAACCTCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCACTACACCCTGGAGGGATTTCAATTTGTGCCATTGCTGATCTCATGTCT
127 LeuSerGlnThrSerGlyPheGlyProPheGlyProGlnGlyIleGlyGlnTyrThrThrTrpArgAspPheIleCysAlaIleAlaAspProHisValIT

9101 ATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCAAGCACTGGATGAACTCATGCTGTGGGCAGAAGATTGCTCTGAAGTCAGACA
160 yRHisTrpGlnThrValMetAspAspThrValSerAlaSerValAlaGlnAlaLeuAspGluLeuMetLeuTrpAlaGluAspCysProGluValArgHis

9201 CCTGGTCCATGCTGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTTGGAGATCTCAA
193 sLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAspAsnGlyArgIleThrAlaValIleAspTrpSerGluAlaMetPheGlyAspSerGln

9301 TATGAGGTTGCCAATTTTTTTTGGAGACCTTGGCTGGCTGCATGGAACAACAACAAGATATTTTGAAGAAAGACACCAGAACTGGCTGGTTCC
227 TyrGluValAlaAsnIlePhePheTrpArgProTrpLeuAlaCysMetGluGlnGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerP

9401 CCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCACTGTATCAATCTCTGGTTGATGAAACTTTGATGATGCTGCTTGGGCACAAGGAAGATG
260 roArgLeuArgAlaTyrMetLeuArgIleGlyLeuAspGlnLeuTyrGlnSerLeuValAspGlyAsnPheAspAlaAlaTrpAlaGlnGlyArgCys

9501 TGATGCCATTGTGAGGTCTGGTGTGGAATGTTGGAAGAACAATTTGCAAGAAGGCTGCTGCTGTTGGACTGATGGATGTTTGAAGTCTGGCT
293 sAspAlaIleValArgSerGlyAlaGlyThrValGlyArgThrGlnIleAlaArgArgSerAlaAlaValTrpThrAspGlyCysValGluValLeuAla

9601 GACTCTGGAACAGGAGACCTCCACAAGACCCAGACCAAGGAATGATAATTAGCTAGATTATCCCTAATACCTGCCACCCCACTTAAATCAGTGGTG
327 AspSerGlyAsnArgArgProSerThrArgProArgAlaLysGlu•••

9701 GAAGAACGGTCTCAGAACTGTTTCAATTTGGCCATTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAAACTTCAGAAGGAA

9801 AGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGAAAACAACCTGA

9901 CCAAAAATTTGTCACAGAATTTTGGAGCCATTAATAAAGTTAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGTGAAAGACATC

10001 TAATCTGGTTTTACGAATCTGGAACCTTCTTGAAAATGTAATCTTGTAGTTAACACTCTGGGTGGAGAATAGGGTGTTTTTCCCCACATAATTGGA

10101 AGGGAAAGGAATATCATTTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCC

10201 AAAACTGAGTCTTGGGTTGCATAGAAAGCTG