

pVITRO1-neo-GFP/LacZ

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

Catalog # pvitro1-ngfplacz

For research use only

Version # 05L08-SV

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-neo-GFP/LacZ provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Kan

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Kan pouches at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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

pVITRO1-neo-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*H I/*Nhe* I for LacZ) that allow for convenient cloning of ORFs 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).

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.

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

E. coli Fast-Media® Kan is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Kan is a TB (liquid) or LB (solid) based medium with kanamycin, and contains stabilizers.

E. coli Fast-Media® Kan can be ordered separately (catalog code # fas-kn-1, fas-kn-s).

Method:

- 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:

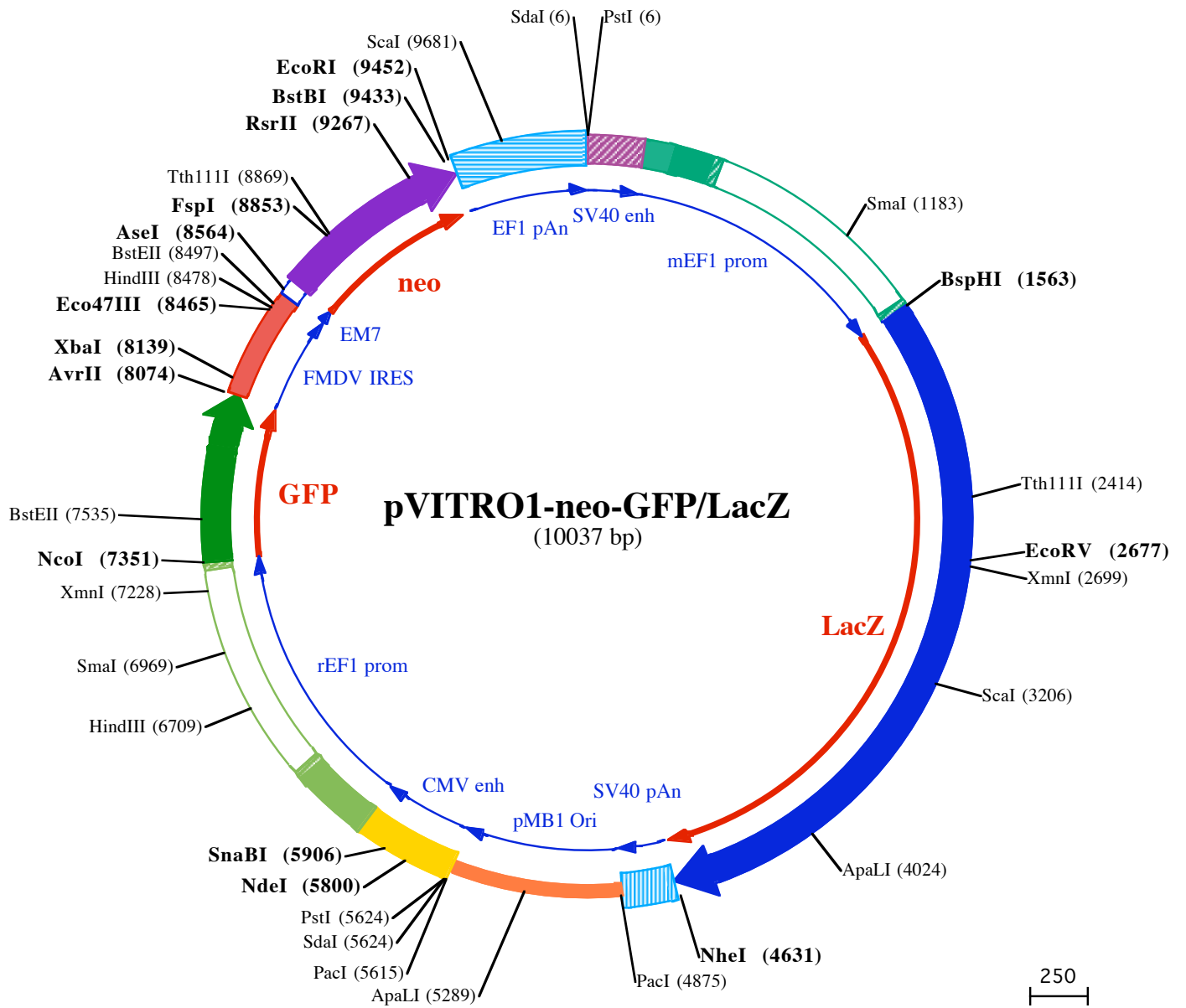
1. Kim DW. *et al.* 1990. Gene 91(2):217-23.
2. Dean DA. *et al.* 1999. Exp. Cell. Res. 253:713-22
3. Boshart M. *et al.* 1985. Cell 141(2):521-30
4. Carswell S., and Alwine JC. 1989. Mol. Cell Biol. 10: 4248-4258.
5. Ramesh N *et al.* 1996. Nucleic Acids Res. 24(14):2697-700.

TECHNICAL SUPPORT

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ApaI (4024)
4001 CAGGCTGAGGCTGCCCTGCTCCAGTGCACAGACACCTGGCTGATGCTGTTCTGATCACACAGCCCATGCTTGGCAGCACCAAGGCAAGACCCTGT
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 CCTGAACGTCAACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAACTACCTGACAGGCTGACAGCTGCCTGCTTTGAC
879▶ yLeuAsnCysGl nLeuAl aGl nValAl aGl uArgValAsnTrpLeuGl yLeuGl yProGl nGl uAsnTyrP roAspArgLeuThrAl aAl aCysPheAsp
4301 AGGTGGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACATGGTCCACC
913▶ ArgTrpAspLeuP roLeuSerAspMeTyrThr ProTyrValI PheP roSer Gl uAsnGl yLeuArgCysGl yThr ArgGl uLeuAsnTyrGl yProHi sG
4401 AGTGGAGGGGAGACTTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGACAGAGGAGGAACTCG
946▶ I nTrpArgGl yAspPheGl nPheAsnI leSer ArgTyrSer Gl nGl nLeuMeTGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uGl yThr Tr
4501 GCTGAACATTGATGGCTCCACATGGCATTGGAGGAGATGACTCTTGGTCTCTTCTGTGTCTGCTGAGTTCAGTTATCTGCTGCCAGGTACCCTAT
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 AATGCTTATTTGTGAATTTGTGATGCTATTGCTTTATTTGTAAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTT

PacI (4875)
4801 TCAGGTTCAAGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGTATGGAATGTTAATTAACATAGCCATGACCAAAATCCCTT
4901 AACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTCAAA
5001 AAAAAAACCCCGCTACCAGCGGTGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGTAACTGGCTTACGACAGCGCAGATACCAAT
5101 ACTGTTCTTAGTGATGCCGTAGTTAGGCCACCCTCAAGAAGCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG

ApaI (5289)
5201 CCAGTGGCGATAAGTCGTGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCC
5301 CAGCTTGAGCGAACGACCTACACCGAAGTACAGCTACAGCGTGTAGCTATGAGAAGCGCCACGCTTCCGAAGGAGAAGGCGGACAGGTATCCG
5401 GTAAGCGGAGGGTGGAAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACCGCTGGTATCTTTATAGCTCTGCGGTTTCGCCACCTCTGACTTG
5501 AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGGAGCCTATGAAAAACGCCAGCAACCGGCCCTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGC

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

NdeI (5800)
5701 CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGGTATCA
5801 TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTAC

SnaBI (5906)
5901 ATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCC
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6101 TCCCTAAATCTCACAGACCAATCCCTGTAGCCGCCACGACAGCGGAGGATGCGCCAGGGCTGAGCGGGGTAGATCAGAGCACACAAGCT
6201 CACAGTCCCCGGCGGTGGGGGAGGGGCGCGCTGAGCGGGGCCAGGGAGCTGCGCGGGGCAAACTGGGAAAGTGGTGTCTGTGCTGGCTCCGCCCTC
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6401 tggcttccgagggccccggagctggagccctgctctgagggggggctgatatgagtgctgctcgcaggggttagctgtgagcattccacttccga
6501 gtggcgggctgctgggggtgagagtgagagcctagcggcaacccctagcctcgcctcgtgctcggcttgaggcctagcgtggtgctcgcgcgcgct
6601 gccactccggccgactatgctgtttttgtccttgcctcgtgctcctcagcagcagtgaggtaaacaaaggagggtgtgggctcactcttaagg

HindIII (6709)
6701 agcccatgaagcttacggttaggaatggaagggcaggaggggagctggggcccccgccttcggagcacatgtccgacgccacctggatggggcg
6801 aggcctgtggctttccgaagcaatcggcgctgagtttagcctacctggccatgtggccctagcactgggcacggctggcctggcggtccgcttccc

SmaI (6969)
6901 ttgctcccaacaagggtagggcgtcccggcaccagttgcttgcgcggaagatggcgctccggggccctgttgcaaggagctcaaaatggag
7001 gacgcggcagcccggtagggcgggtagtaccacacaaaggaagagggccttgcccctcgcggcgctgttctctgtgaccccggtgtatc
7101 ggccgcatagtcacctcggcctctcttagcaccgctcgtcgcggcgggggagggatctaaggcgttgagttgttccatcttgggtgggagga

XmnI (7228)
7201 ctagtacggccagcctggcgctggaagtcattcttggaaattgccctttgagttggagcaggctaattctcaagcctcttagcgttcaaaaggatt

NotI (7351)
7301 ttctaaccggctttccagGTGTTGTGAAAGCACCGCTAATTCAAAGCAAcATGGTTTCTAAGGGAGAAGAACTCTTACTGGTGTGCCAAATTCTG
7401 GTTGAGCTGGATGGTGTGAATGGCCACAAATCTCTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTTGAAGTTCATTTGTA
17▶ Val Gl uLeuAspGl yAspValAsnGl yHi sLysPheSer Val Ser Gl yGl uGl yGl uAspAl aThr TyrGl yLysLeuThr LeuLysPheI leCysT
7501 CAACAGGAAAGCTGCCAGTGCCTTGGCCAACTCTGGTGAACACCCTGACTTATGGTGTTCATGTTTTCAGCAGGTACCTGACCACATGAAGCAGCATGA
50▶ hr Thr Gl yLysLeuP roValI ProTrpP roThr LeuVal Thr ThrLeuThr TyrGl yValI Gl nCysPheSer ArgTyrP roAspHi sMeTLysGl nHi sAs

7601 CTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAATATAAGACAAGGGCAGAAGTGAAGTTTGAA
83▶ pPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGlu
7701 GGTGATACACTGGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGAAACATCTGGGTACAAAGCTGGAGTACAATAATCTCACA
117▶ GlyAspThrLeuValAsnArgIleGluLeuLysGlyIleAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisA
7801 ATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTGGCAGACCATTA
150▶ snValTyrIleMetAlaAspLysGlnLysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTy
7901 CCAGCAGAACACCCTATTGGTGTGGCCAGTTCTCCTCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTTCCAAAGACCCTAATGAGAAAAA
183▶ rGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArg
AvrII (8074)
8001 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTAATCTGGAATGGATGAGCTGTACAAGTAAACCTAGAGCAGGTTTCCCAATGACAC
217▶ AspHisMetValLeuLeuGluPheValThrAlaAlaGlyIleThrLeuGlyMetAspGluLeuTyrLys●●●
XbaI (8139)
8101 AAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCAGGCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTAG
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8301 CATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCCACTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTT
Eco47III (8465) HindIII (8478) BstEII (8497)
8401 CAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTG
AseI (8564)
8501 ACCGGAGGTCGGCACCTTTCCTTTGCAATTAAGTACCTGACCTATGAATACACTGACTGTTTGAACAATTAATCATCGGCATAGTATAATAC
8601 GACTCACTATAGGAGGGCCACCATGATTGAACAAGATGGATTGCACGCAAGTTCCTCCGGCCCTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAA
1▶ MetIleGluGlnAspGlyLeuHisAlaGlySerProAlaAlaTrpValGluArgLeuPheGlyTyrAspTrpAlaGln
8701 CAGACAATCGGCTGCTGTATGCCCGTGTCCGGCTGTGAGCGCAGGGCCGCCGGTCTTTTGTCAAGCCAGCTGTCCGGTGCCTGAATGAAC
27▶ GlnThrIleGlyCysSerAspAlaAlaValPheArgLeuSerAlaGlnGlyArgProValLeuPheValLysThrAspLeuSerGlyAlaLeuAsnGluL
FspI (8853) Tth11I (8869)
8801 TGCAAGCAGGCGAGCCGGCTATCGTGGCTGCCACGCGGGCTTCTTGGCAGCTGTGCTGACGTTGCTACTGAAGCGGGAAGGACTGGCTGCT
60▶ euGlnAspGluAlaAlaArgLeuSerTrpLeuAlaThrThrGlyValProCysAlaAlaValLeuAspValValThrGluAlaGlyArgAspTrpLeuLe
8901 ATTTGGCGAAGTGCCTGGGCGAGGATCCTGTCTCATCTCACCTTGCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGCGGCTGCATACGCTT
93▶ uLeuGlyGluValProGlyGlnAspLeuLeuSerSerHisLeuAlaProAlaGlyLysValSerIleMetAlaAspAlaMetArgArgLeuHisThrLeu
9001 GATCCGGCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAAGCCGGTCTTGTCTGATCAGGATGATCTGGAGC
127▶ AspProAlaThrCysProPheAspHisGlnAlaLysHisArgIleGluArgAlaArgThrArgMetGluAlaGlyLeuValAspGlnAspAspLeuAspG
9101 AAGAGCATCAGGGCTCGCCGACCCGAAGTGTCCGAGGCTCAAGCGAGCATGCCCGACGGCGAGGATCTCGTCTGACACATGGCGATGCCTGCTT
160▶ IuGluHisGlnGlyLeuAlaProAlaGluLeuPheAlaArgLeuLysAlaSerMetProAspGlyGluAspLeuValValThrHisGlyAspAlaCysLe
RsrII (9267)
9201 GCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGT
193▶ uProAsnIleMetValGluAsnGlyArgPheSerGlyPheIleAspCysGlyArgLeuGlyValAlaAspArgTyrGlnAspIleAlaLeuAlaThrArg
9301 GATATTGCTGAAGACTTGGCGCGAATGGGCTGACCGCTTCTCTGCTTACGGTATCGCCGCTCCCGATTCCGACGGCATCGCTTCTATCGCTT
227▶ AspIleAlaGluGluLeuGlyGlyTrpAlaAspArgPheLeuValLeuTyrGlyIleAlaAlaProAspSerGlnArgIleAlaPheTyrArgLeuL
BstBI (9433) EcoRI (9452)
9401 TTGACGAGTCTTCTGAGCGGGACTCTGGGTTGCAATGACCGACCAAGCGAATTGCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCAGT
260▶ euAspGluPhePhe●●●
9501 GGTGGAAGAACGGTCTCAGAAGTGTGTTTCAATTGGCCATTTAAGTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAA
ScaI (9681)
9601 GGAAAGAGAATGTTTTGTGACCACCTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGAAACAAC
9701 TTGACCAAAAATTTGTACAGAAATTTGAGACCATAAAAAAGTTAAATGAGAACTGTGTGTTCTTTGGTCAACCCGAGACATTTAGGTGAAAGA
9801 CATCTAATTCGGTTTACGAATCTGAAACTTCTGAAAATGTAATCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAAT
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10001 GGCCAAAACCTGAGTCCTTGGTTGCATAGAAAAGCTG