

# pVIVO2-GFP/LacZ

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

Catalog code: pvivo2-gfplacz

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

For research use only

Version 19J02-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pVIVO2-GFP/LacZ is provided as lyophilized DNA.
- 1 ml of 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

pVIVO2 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector.

- **pVIVO2-GFP/LacZ** contains the reporter genes GFP and LacZ and can be used as a control vector.

- **pVIVO2-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 ORFs which can be selected from InvivoGen's extensive list of genes. For more information, visit: <https://www.invivogen.com/genes>.

## 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 protein<sup>1</sup>. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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 plasmids<sup>2</sup>.
- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), 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 enhancer<sup>3</sup>.

- **LacZ-ΔCpG 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. In order to reduce the immunogenicity of this bacterial gene the 298 CpG motifs from the wildtype have been removed by chemically synthesizing the gene.

- **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.*<sup>4</sup>

- **pMB1 Ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

- **Hygro-ΔCpG** is a new allele of the *hph* gene conferring resistance to hygromycin B. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Hygro-ΔCpG* gene allows the selection of *E. coli* clones transformed with a pVIVO plasmid.

*Note: Stable transfection of mammalian clones cannot be performed due to the absence of a eukaryotic promoter upstream of the Hygro-ΔCpG gene.*

- **Term:** The *E. coli rps O* terminator allows efficient transcription termination of the *Hygro-ΔCpG* gene.

- **LGFP** is a new allele of the green fluorescent protein. The gene has been chemically synthesized to remove the CpGs to ensure long-lasting expression.

- **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.

## 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 DH5a.

### 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.

## References

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58.
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.* 10: 4248-4258.

## TECHNICAL SUPPORT

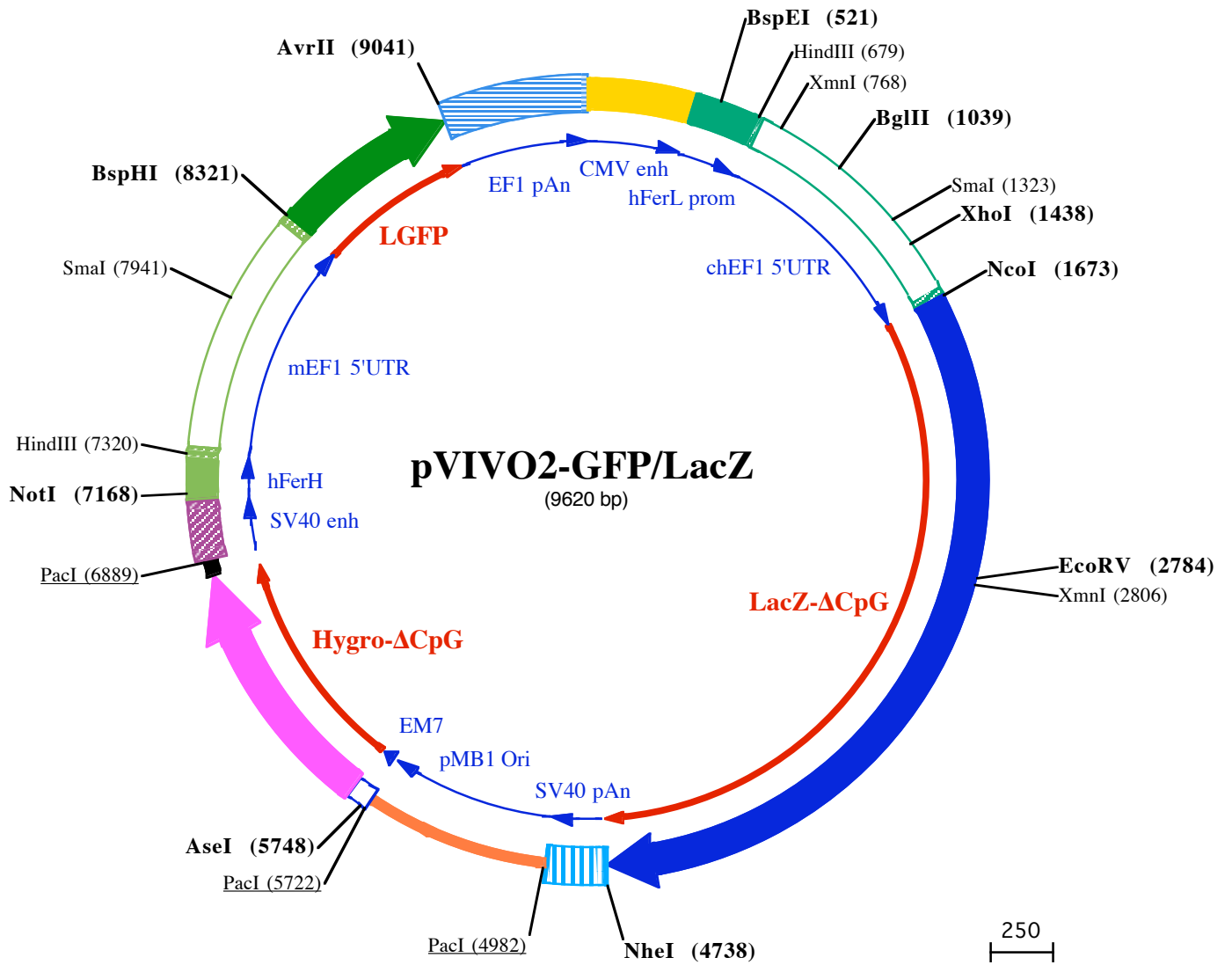
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1 CCTGCAGCGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA  
101 CGCCAATAGGGACTTTCATTGACGTCAATGGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC  
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGGTGGATAGCGGTTTGACTIONCACGGGATTCCAAAGTCCACCCATTGACGTCAATG  
401 GGAGTTTGTGTTGACTAGTCAGGGCCCAACCCCCCAAGCCCATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCTTCTTTGGGCGGG  
501 GGGCTGAGACTCCTATGTCTCCGATTGGTCAGGCACGGCTTCGGCCCGCTCTGCCACCGCAGATTGGCCGCTAGCCCTCCCGAGCGCCCTGCC  
601 TCCGAGGGCCGCGCACCATAAAGAAGCCGCTTAGCCACGTCCCTCGCAGTTTCGGCGGTCCCGCGGTCTGTCTCAAGCTTGGCCCAAGACACAGg  
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1801 9) gAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgPro  
1901 43) SerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAla  
2001 76) IAspThrValValValProSerAsnTrpGlnMethisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProPheVal  
2101 109) IProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGlyGlnThrArgIleIlePheAspGlyValAsn  
2201 143) SerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyG  
2301 176) IAsnArgLeuAlaValMethValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMethTrpArgMethSerGlyIlePheArgAspValSerLe  
2401 209) uLeuHisLysProThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMethCys  
2501 243) GlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspG  
2601 276) IuArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValG  
2701 309) uLeuHisThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValAlaArgIleGluAsnGlyLeuLeuLeuAsnGlyLys  
2801 343) ProLeuLeuIleArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlyValMethAspGluGlnThrMethValGlnAspIleLeuLeuMethLysG  
2901 376) IAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAs  
3001 409) nIleGluThrHisGlyMethValProMethAsnArgLeuThrAspAspProArgTrpLeuProAlaMethSerGluArgValThrArgMethValGlnArgAsp  
3101 443) ArgAsnHisProSerValIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProS  
3201 476) erArgProValGlnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMethTyrAlaArgValAspGluAspGlnProPheProAl  
3301 509) aValProLysTrpSerIleLysLysTrpLeuSerLeuProGlyGlyGlyThrArgProLeuIleLeuCysGlyTyrAlaHisAlaMethGlyAsnSerLeuGly  
3401 543) GlyPheAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAla

3401 ATGGCAACCCCTGGTCTGCCTATGGAGGAGACTTTGGTGACACCCCAATGACAGGCAGTTCGCATGAATGGCCTGGTCTTTGCAGACAGGACCCCTCA  
576 ▶ snGl yAsnProTrpSerAl aTyrGl yGl yAspPheGl yAspThr ProAsnAspArgGl nPheCysMe tAsnGl yLeuVal lPheAl aAspArgThr ProHi  
3501 CCCTGCCTCACAGGGCAAGCACCAGCAACAGTTCTCCAGTTCAGGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTTCAGGCACTCT  
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643 ▶ AspAsnGl uLeuLeuHi sTrpMe tVal lAl aLeuAspGl yLysProLeuAl aSer Gl yGl uVal lProLeuAspVal lAl aProGl nGl yLysGl nLeu l l eG  
3701 AACTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGACAACCTGTGGTAACAGTGGAGGTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACAT  
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3801 CTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCTGCCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTC  
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743 ▶ Cys l l eGl uLeuGl yAsnLysArgTrpGl nPheAsnArgGl nSer Gl yPheLeuSer Gl uMe tTrp l l eGl yAspLysLysGl nLeuLeuThr ProLeuA  
4001 GGGACCAATTACCAGGGCTCCTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGGTGGAGAGGTGGAAGGCTGCTGG  
776 ▶ rGAspGl nPheThr ArgAl aProLeuAspAsnAsp l l eGl yVal lSer Gl uAl aThr Arg l l eAspProAsnAl aTrpVal lGl uArgTrpLysAl aAl aGl  
4101 ACCTACCAGGCTGAGGCTGCCTCCAGTGCACAGCAGACCCCTGGCTGATGCTGTTCTGATCACCACAGCCCATGCTTGGCAGCACCAGGCAAG  
809 ▶ yHi sTrpGl nAl aGl uAl aLeuLeuGl nCysThr Al aAspThr LeuAl aAspThr LeuAl aVal Leu l l eThr Thr Al aTrpGl nHi sAl aGl nGl yLys  
4201 ACCCTGTTTCATCAGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTGGCTCTGACACACCTCACCTGCAA  
843 ▶ Thr LeuPhe l l eSer ArgLysThr TyrArg l l eAspGl ySer Gl yGl nMe tAl a l l eThr Val lAspVal lGl uVal lAl aSer AspThr ProHi sProAl aA  
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876 ▶ rGl l eGl yLeuAsnCysGl nLeuAl aGl nVal lAl aGl uArgVal lAsnTrpLeuGl yLeuGl yProGl nGl uAsnTyrPAspArgLeuThr Al aGl uCy  
4401 CTTTGACAGGTGGGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTCCCTTCTGAGAATGGCTGAGGTGTGGCACCAGGGAGCTGAACATGGT  
909 ▶ sPheAspArgTrpAspLeuProLeuSerAspMe tTyrThr ProTyrVal lPheProSer Gl uAsnGl yLeuArgCysGl yThr ArgGl uLeuAsnTyrGl y  
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943 ▶ ProHi sGl nTrpArgGl yAspPheGl nPheAsn l l eSer ArgTyrSer Gl nGl nGl nLeuMe tGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uG  
4601 GAACCTGGCTGAACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCTTCTGTGTCTGAGTTCAGTTATCTGCTGGCAGGTA  
976 ▶ l yThr TrpLeuAsn l l eAspGl yPheHi sMe tGl y l l eGl yGl yAspAspSer TrpSer ProSer Val lSerAl aGl uPheGl nLeuSerAl aGl yArgTy

**NheI (4738)**

4701 CCACTATCAGCTGGTGTGGTGCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAC TAGAATGCAG  
1009 ▶ rHi sTyrGl nLeuVal lTrpCysGl nLys ●●●  
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**PacI (4982)**

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**PacI (5722)**

**AseI (5748)**

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1 ▶ MetLysLysProGl uLeuThrAl aThr Ser Val lGl uLysPheLeu l l eGl uLysPheAspSer Val lSer AspLeuMe tGl nLeuSer Gl uGl yG  
5901 AAGAAAGCAGAGCCTTTCTTTTGATGTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATTCTTGTGCTGATGGTTTTTACAAGACAGATATGTTACAG  
32 ▶ l uGl uSer ArgAl aPheSer PheAspVal lGl yGl yArgGl yTyrVal lLeuArgVal lAsnSer CysAl aAspGl yPheTyrLysAspArgTyrVal lTyrAr  
6001 ACACCTTGCCTCTGCTGCTCCAACTCCAGAACTTGGCAATCTGGACTTCTGAACTCTCACCTACTCAGCAGAAGAGCAAGGATGTC  
65 ▶ gHi sPheAl aSerAl aAl aLeuPro l l eProGl uVal lLeuAsp l l eGl yGl uPheSer Gl uSer LeuThr TyrCys l l eSer ArgArgAl aGl nGl yVal l  
6101 ACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTG  
99 ▶ Thr LeuGl nAspLeuProGl uThr Gl uLeuProAl aVal lLeuGl nProVal lAl aGl uAl aMe tAspAl a l l eAl aAl aAl aAspLeuSer Gl nThr Ser G  
6201 GATTTGGCTCTTTGGTCCCAAGGCATTGGTCAAGTACACCTTGGAGGGATTTCTTTGGCCATTGCTGATCCATGCTATCACTGGCAGACTGT  
132 ▶ l yPheGl yProPheGl yProGl nGl y l l eGl yGl nTyrThr Thr TrpArgAspPhe l l eCysAl a l l eAl aAspProHi sVal lTyrHi sTrpGl nThr Va  
6301 GATGGATGACACAGTTCTGCTTCTGTTGCTCAGGCCTGGATGAACCTCATGCTGTGGGCAGAAGATTGCTCCTGAAGTCAAGACCTGGTCCATGCTGAT  
165 ▶ l Me tAspAspThr Val lSerAl aSer Val lAl aGl nAl aLeuAspGl uLeuMe tLeuTrpAl aGl uAspCysProGl uVal lArgHi sLeuVal lHi sAl aAsp  
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199 ▶ PheGl ySerAsnAsnVal lLeuThrAspAsnGl yArg l l eThrAl aVal l l eAspTrpSer Gl uAl aMe tPheGl yAspSer Gl nTyrGl uVal lAl aAsn l  
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232 ▶ l ePhePheTrpArgProTrpLeuAl aCysMe tGl uGl nGl nThr ArgTyrPheGl uArgArgHi sProGl uLeuAl aGl ySer ProArgLeuArgAl aTy  
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265 ▶ r Me tLeuArg l l eGl yLeuAspGl nLeuTyrGl nSer LeuVal lAspGl yAsnPheAspAspAl aAl aTrpAl aGl nGl yArgCysAspAl a l l eVal lArg  
6701 TCTGGTCTGGAACCTGTTGGAAGAATCAAATGCAAGAAGTCTGCTGCTGTTGGACTGATGGATGTGTTGAAGTCTGGCTGACTCTGGAACAGGA  
299 ▶ Ser Gl yAl aGl yThr Val lGl yArgThr Gl n l l eAl aArgArgSerAl aAl aVal lTrpThrAspGl yCysVal lGl uVal lLeuAl aAspSer Gl yAsnArgA

**PacI (6889)**

6801 GACCCTCCACAAGACCAGAGCCAAAGGAATGAATATTAGCTAGGAGTTTCAGAAAAGGGGCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAGG  
332 ▶ rGProSer ThrArgProArgAl aLysGl u ●●●  
6901 GCCTGAATAAACCCTGAAAGAGGAACCTGGTATAGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCA  
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NotI (7168)

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HindIII (7320)

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SmaI (7941)

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BspHI (8321)

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MetSer LysGlyGluLeuPheThr GlyVal Val Prol LeuVal GluLeuAspGlyAspValAsnGlyHisLys  
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27 PheSer Val Ser GlyGluGlyGluAspAlaThr TyrGlyLysLeuThr LeuLysPhe IleCysThr Thr GlyLysLeuProl ProlTrpProlThrL  
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8601 TCAGGAGAGGACAATCTTCTTAAAGGATGATGAAATTATAAGACAAGGGCAGAAGTGAAGTTGAAGGTGATACACTGGTTAACAGAATTGAGCTGAAA  
93 I GluGluArgThr IlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluVal LysPheGluGlyAspThr LeuValAsnArgIleGluLeuLys  
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127 GlyIleAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleMetAlaAspLysGluLysAsnG  
8801 GAATTAAGTAAATTCAGATTAGACACAACATTGAGGATGGATCTGTCCAACCTGGCAGACCATTACCAGCAGAACACCCCTATTGGTGTGGCCAGT  
160 IyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGluLeuAlaAspHisTyrGluGluAsnThrProlIleGlyAspGlyProVa  
8901 TCTCTCCAGATAATCACTATCTCCGCACTCAATCTGCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCTCTGGAGTTTGTGACAGCA  
193 ILeuLeuProlAspAsnHisTyrLeuArgThrGluSerAlaLeuSerLysAspProlAsnGluLysArgAspHisMetValLeuLeuGluPheValThrAla

AvrII (9041)

9001 GCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGAAGAACGGTCTC  
227 AlaGlyIleThrLeuGlyMetAspGluLeuTyrLys  
9101 AGAACTGTTTGTTCATTCAGCCATTTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTT  
9201 GTGGACCACTTTGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGGAAACAACCTTGACAAAAATTTGTC  
9301 ACAGAATTTTGGAGCCATTAAAAAGTTAAATGAGAAACCTGTGTTCCTTTGGTCAACACCGAGACATTTAGTGAAAGACATCTAATTTCTGGTTTT  
9401 ACGAATCTGAAACTTCTTGAATGTAATCTTGTAGTTAACACTTCTGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAGGAATA  
9501 TCATTTAAAGCTATGGGAGGTTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTAAAACAGGCCAAAACTGAGTCC  
9601 TTGGTTGCATAGAAAGCTG