

# pSELECT-neo-LacZ

A LacZ-expression plasmid selectable with Kanamycin/G418

Catalog code: psetn-lacZ

<https://www.invivogen.com/pselect-neo>

For research use only

Version 19L13-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pSELECT-neo-LacZ plasmid provided as lyophilized DNA

### Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 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

pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human β-globin polyA terminates the transcription of the selectable marker.

pSELECT-LacZ plasmids can be used as control vectors or for cloning of an open reading frame, as the LacZ gene is flanked by two unique restriction sites: Nco I at the 5' end that encompasses the Start codon and Nhe I at the 3' end.

## PLASMID FEATURES

### First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat<sup>2</sup>. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **LacZ**: 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<sup>3</sup>.
- **ori**: a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

### Second expression cassette

- **CMV enh/prom**: The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Neo**: The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. The neo gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>4</sup>.

1. Kim D.W. *et al.*, 1990. Use of human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene*. 91(2):217-23. 2. Takebe, Y. *et al.*, 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol.* 1: 466-472. 3. 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: 4248-4258. 4. Yu J. & Russell J.E., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. *Mol Cell Biol*, 21(17):5879-88.

## 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 H<sub>2</sub>O. 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

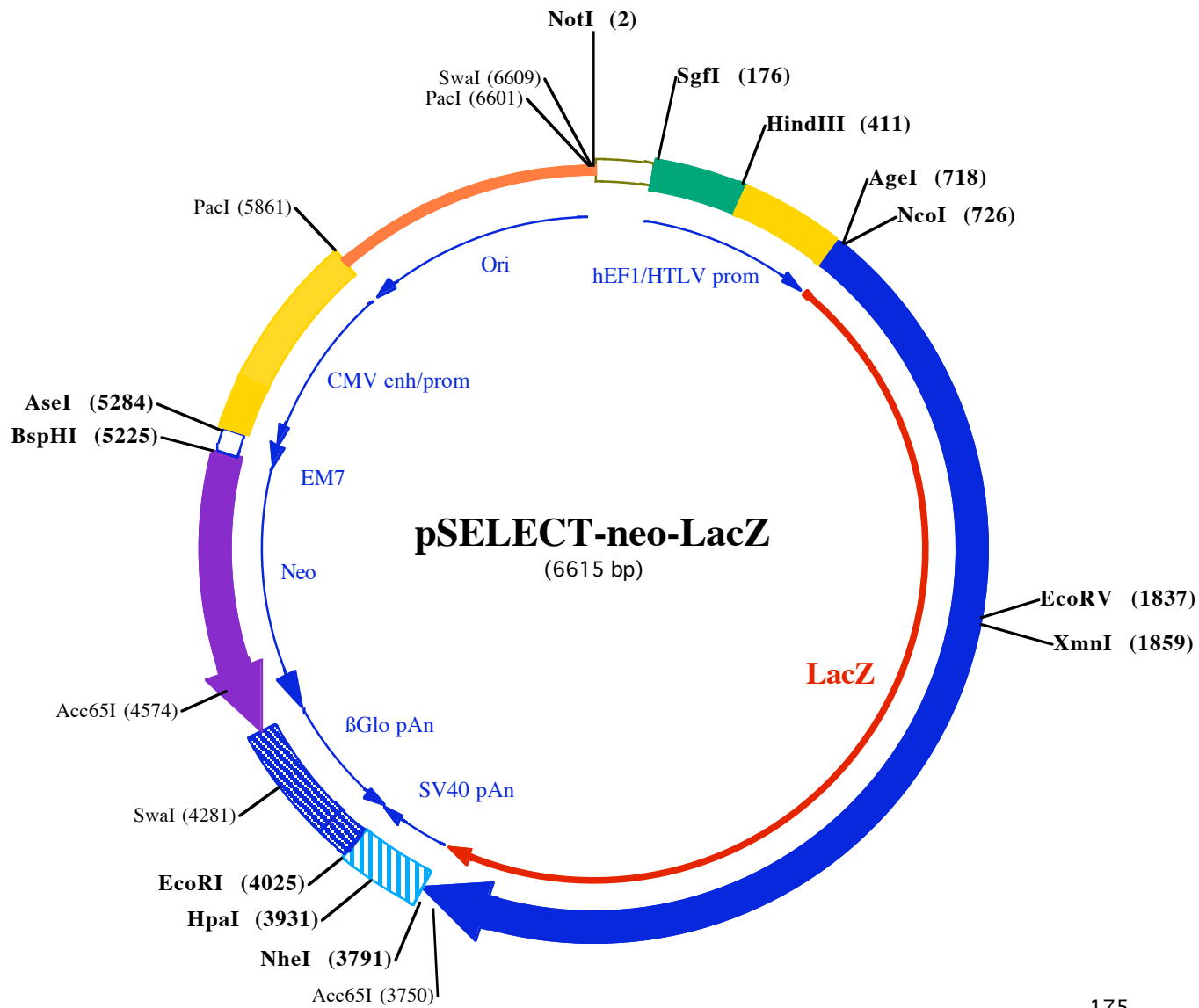
InvivoGen USA (International): +1 (858) 457-5873

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InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

 **InvivoGen**  
www.invivogen.com



**NotI (2)**

1 GCGGCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA  
 101 AAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCATCGCTCCGGTGCCTCGAGTGGGA  
 201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGATG  
 301 TCGTGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCGGTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAG

**SgfI (176)**

**HindIII (411)**

401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCCACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCT  
 501 CCGCGCTGTGGTGCCTCCTGAACGCGTCCGCGCTAGGTAAGTTAAAGCTCAGGTGCAGACCGGGCTTTGTCCGGCGCTCCCTTGGAGCTACCTA  
 601 GACTCAGCCGGCTCCACGCTTGGCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

**NcoI (726)**

**AgeI (718)**

701 GCGCGCTACCTGAGATCAccggtcacCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCC  
 1▶MetAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaH  
 801 ACCCTCCCTTTGCCTCTGGAGGAACTCGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGCCTGGTT  
 25▶iSProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPh  
 901 CCCTGCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTGTGGTCCCACTGGCAGATGCATGGCTATGAT  
 58▶eProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAsp  
 1001 GCCCCATCTACACCAATGTCACTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCTGGCTGCTACAGCTGCCTCAATGTTG  
 92▶AlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAla  
 1101 ATGAGAGCTGGCTGCAAGAAGGCCAGACAGGATCATCTTTGATGGAGTCAACTCTGCTTCCACCTCTGGTGAATGGCAGGTGGGTGGCTATGGCCA  
 125▶spGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGly  
 1201 AGACAGCAGGCTGCCCTGTAGTTGACCTCTCTGCTTCCCTCAGAGCTGGAGAGAACCAGGCTGGCTGTATGGTGCAGGTGGTCTGATGGCAGCTAC  
 158▶nAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGlyuAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyr  
 1301 CTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAAGGATGTGAGCCTGCTGCACAGCCACCACCCAGATTCTGACTTCCATGTTGCCACCA  
 192▶LeuGluAspGluAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAspPheHisValAlaThrA  
 1401 GTTTCAATGATGACTTCAGCAGAGCTGTCTGGAGCTGAGGTGCAGTGTGGAGAACTCAGAGACTACCTGAGAGTCAACAGTGGCTCTGGCAAGG  
 225▶rGheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGly  
 1501 TGAGACCAGGTGGCTCTGGCAGACGCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCCACCTGAGGCTCAATGGGAG  
 258▶yGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGlyIleIleAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGlu  
 1601 AACCCCAAGCTGTGCTCTGATGAGTCCCAACCTCAGAGGCTGTTGTGGAGTGCACACTGCTGATGGCAGATGATTAAGCTGAAGCTGTGATG  
 292▶AsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspV  
 1701 TTGGATTGAGAGAAGTCAAGGATGAGAAATGGCTGCTGCTCAATGGCAAGCCTCTGCTCATAGGGGAGTCAACAGGCATGAGCACCACCTCTGCA  
 325▶alGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuAsnGlyLysProLeuLeuIleArgGlyValAsnArgHisGlyuHisHisProLeuHis

**EcoRV (1837)**

**XmnI (1859)**

1801 TGGACAAGTGATGGATGAACAGACAATGGTGAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTGTCAGGTGCTCTACTACCCCAACCACCT  
 358▶sGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisPro  
 1901 CTCTGGTACACCTGTGTGACAGGATGGCCTGTATGTTGTTGATGAAGCCAACATTGAGACACATGGCATGGTGCCTGAACAGGCTCACAGATGACC  
 392▶LeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluuThrHisGlyMetValProMetAsnArgLeuTrpAspAsp  
 2001 CCAGGTGGCTGCTGCTGACATGTCTGAGAGAGTGACCAGGATGGTGCAGAGAGACAGGAACCACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGG  
 425▶roArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpSerLeuGlyAsnGluSerGly  
 2101 ACATGGAGCCAAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGAGTATGAAGGAGGTGGAGCAGACCCACAGCCACA  
 458▶yHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValGlnTyrGluGlyGlyAlaAspThrThrAlaThr  
 2201 GACATCALTGCCCCATGTCAGGTTGATGCCAGGTTGATGAGGACAGCCCTTCCCTGTGTCGCAAGTGGAGCATCAAGAAGTGGCTCTCTGCTGGAGAGA  
 492▶AspIleIleCysProMetTyrAlaArgValAspGluAspGluProPheProAlaValProLysTrpSerIleLysLysTrpLeuSerLeuProGlyGlyuT  
 2301 CCAGACCTCTGATCTGTGTAATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGGCAAGCCTTCCAGACAGTACCCAGGCTGCA  
 525▶hrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgLeuTyrProArgLeuGly  
 2401 AGGAGGATTTGTGGGAGTGGGTGGGCAATCTCTCATCAAGATGATGAGAAATGGCAACCCTGGTCTGCATGGAGAGACTTGGTGGACACCCCC  
 558▶nGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGlyAspPheGlyAspThrPro  
 2501 AATGACAGGCAGTCTGCATGAATGGCTGGTCTTTGACAGACAGACCCCTCACCTGCCCTCACAGAGGCCAAGCACCAGCAACAGTCTCTCCAGTTCA  
 592▶AsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGlnPhePheGlnPheA  
 2601 GGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTCAGGCACTTGACAATGAGCTCTGlyMetValProMetAsnArgLeuTrpAspAsp  
 625▶rGLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeuLeuHisTrpMetValAlaLeuAspGlyLysProLe  
 2701 GGCTTCTGGTGGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACCTGAGCTGCCTCAGCCAGAGTCTGCTGGCAACTGTGGCTA  
 658▶uAlaSerGlyGlyuProLeuAspValAlaProGlnGlyGlnLeuIleGluLeuProGluLeuProGluProGluuSerAlaGlyGlnLeuTrpLeu  
 2801 ACAGTGAAGGTGGTTGAGCCCAATGCAACAGCTTGTGCTGAGGACAGCCACATCTGCTGAGCAGCAGTGGAGGCTGGCTGAGAAGCTCTCTGTGACC  
 692▶ThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrL  
 2901 TGCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTGGGCAACAAGAGATTGGCAGTTCACAGGCAGTCTGG  
 725▶euProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleGluLeuGlyAsnLysArgTrpGlnPheAsnArgGlnSerGly  
 3001 CTTCTGTCTCAGATGTGATGGAGCAAGAGCAGCTCTCACCCCTCtPheAsnAlaAlaTCCAGGCTCCTCTGAGCAATGACATTTGGATGTCT  
 758▶yPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspAsnAspIleGlyValSer  
 3101 GAGGCCACCAGGATTGACCCAAATGCTTGGGTGGAGAGTGAAGGCTGCTGGACACTACCAGGCTGAGGCTGCCTGCTCCAGTGCACAGCAGACCCC  
 792▶GluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisTyrGlnAlaGluAlaAlaLeuLeuGlnCysThrAlaAspThrL  
 3201 TGGCTGATGTTCTTGTGATCACCACAGCCATGCTTGGCAGCACAAGCAAGCAGCCTGTTCATCAGCAGAAAGACTACAGGATGATGGCTCTGGACA  
 825▶euAlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheIleSerArgLysThrTyrArgIleAspGlySerGlyGly  
 3301 GATGGCAATCACAGTGGATGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCTGAACTGTCAACTGGCACAGGTGGCTGAGAGGTGAAC  
 858▶nMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgIleGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsn  
 3401 TGGCTGGCTTAGCCCTCAGGAGAATACCCTGACAGCTGACAGCTGCTGCTTGGACAGGTGGACCTGCCTGTGATGTACACCCCTATG  
 892▶TrpLeuGlyLeuGlnProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrV  
 3501 TGTTCCTTCTGAGAATGGCTGAGGTGGCACCAGGAGCTGAACATGGTCTCACAGTGGAGGGAGACTTCCAGTCAACATCTCCAGGTACTC  
 925▶alPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSer

3601 TCAGCAACAGCTCATGGAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTGGCTGAACATTGATGGCTCCACATGGGCATTGGAGGAGAT  
958▶r Gl nGl nGl nLeuMe tGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uGl yThr TrpLeuAsnI l eAspGl yPheHi sMetGl yI l eGl yGl yAsp  
3701 GACTCTTGGTCTCCTTCTGTGTCTGTGAGTTCCAGTTATCTGTGGCAGGTACCACATCAGCTGGTGGTGCCAGAAGTAAACCTGAGCTAGCTAGCTGGC  
992▶AspSer TrpSer P roSer Val Ser Al aGl uPheGl nLeuSer Al aGl yArgTyrHi sTyrGl nLeuVal TrpCysGl nLys●●●  
3801 CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT

HpaI (3931)

3901 TGTAACCATTATAAGCTGCAATAAACAGTTAACAAACAACAAATTGCATTCAATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAG

EcoRI (4025)

4001 TAAAACCTCTACAAATGTGGTATGGAATTTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA

4101 TAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCCTCACCTTCTTTTATGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTT

SwaI (4281)

4201 GAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGA

4301 AAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAAACAAAGGAACCTTTAATA

4401 GAAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTTAGAGAAGCTCATCAAGAAGTCTGTAGAAGGCAATTTCTCTGGGAGTCAGGGGCTGCAATGCCATAG

264▶PhePheGl uAspLeuLeuArgTyrPheAl a l eArgGl nSer AspP roAl aAl a l eGl yTyrL

Acc65I (4574)

4501 AGCACTAGGAACCTGTCTGCCACTCTCCCTAGCTCTTCTGTATGTCCCTGGTGTAGGGCAATGTCCTGGTACTGTCCAGCCACTCCCAGCCTGC

242▶euVal LeuPheArgAspAl aTrpGl uGl yGl yLeuGl uGl uAl a l eAspArgThrAl aLeuAl a l eAspGl nTyrArgAspAl aVal l Gl yLeuArgGl

4601 CACAGTCTATGAAGCCAGAGAACCTTCCATTTTCAACCATGATGTTGGGAAGGCAGGCATCCCCATGAGTCACCACTAGGTCCTACCCTCTGGCATGGA

209▶yCysAsp l ePheGl ySer PheArgGl yAsnGl uVal l Me t l eAsnP roLeuCysAl aAspGl yHi sThr Val l Val LeuAspGl uGl yAspP roMetSer

4701 TGCCTTGAGCCTGGCAAATAGTTCAGCAGGGGCCAGGCCCTGGTGTCTTCCATCCAAGTCATCTTGGTCCACCAGGCCAGCTCCATCCTGGTTCTGGCC

176▶Al aLysLeuArgAl aPheLeuGl uAl aP roAl aLeuGl yGl nHi sGl uGl uAspLeuAspAspGl nAspVal l LeuGl yAl aGl uMe tArgThrArgAl aA

4801 CTCTCTATCCTGTGCTTGGCCTGGTGGTCAAAGGGGCGAGTGGCTGGTCAAAGGGTGTGGAGTCTTCTCATGGCATCAGCCATGATTGACACTTCTCAG

142▶r gGl u l eArgHi sLysAl aGl nHi sAspPheP roCysThr Al aP roAspLeuThrHi sLeuArgArgMe tAl aAspAl aMe t l eSer Val l LysGl uAl

4901 CTGGAGCTAGGTGAGAGGAAAGGAGTCTGCCAGGCACCTCACCTAGTAGGAGCCAGTCCCTCCAGCTTCTGTGACCACATCAAGGACAGCTGCACA

109▶aP roAl aLeuHi sSer Ser LeuLeuAspGl nGl yP roVal l Gl uGl yLeuLeuLeuTrpAspArgGl yAl aGl uThr Val l Val AspLeuVal l Al aAl aCys

5001 GGGACCCAGTTGTTGCCAACAGGAGTCTGGCAGCCTCATCTGGAGCTCATTGAGAGCCCACTGAGGTCTGTCTTTACAAAAAGGACTGGCCTG

76▶P roVal l Gl yThr ThrAl aLeuTrpSer LeuArgAl aAl aGl uAspGl nLeuGl uAsnLeuAl aGl ySer LeuAspThr LysVal l PheLeuVal l P roArgG

5101 CTTTGGGCTGAAAGTCTGAAACTGTGCATCAGACCAACCAATGGTCTGCTGTGCCAGTCATAGCCAAACAGTCTCTCAACCCAGGCAGCTGGAGAAC

42▶l yGl nAl aSer LeuArgPheVal l Al aAl aAspSer CysGl y l eThr Gl nGl nAl aTrpAspTyrGl yPheLeuArgGl uVal l TrpAl aAl aP roSer Gl

BspHI (5225)

AseI (5284)

5201 CTGCATGTAGGCCATCTTGTTCATCATGATGGCCCTCTATAGTGAGTCTGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGC

9▶yAl aHi sLeuGl yAspGl nGl u l eMe t

5301 GTGGATGGCGCTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTGGCGTCAATGGGGC

5401 GGAGTTGTTACGACATTTTGAAAGTCCCGTTGATTTACTAGTCAAAAACAACTCCCAATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCA

5501 AACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGTAGGAAAGTCCATA

5601 AGGTCATGTAAGTGGGCATAATGCCAGGCGGGCCATTACCGTCAATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTAAGTCCCAAGT

5701 GGCAGTTTACCGTAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGCGGGGG

PacI (5861)

5801 TCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACCGCTGCGAGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAA

5901 CCGTAAAAAGGCCGCTTGTGGCGTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA

6001 GGACTATAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTT

6101 CGGGAAGCGTGGCGTTTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAGCTGGGCTGTGTGCACGAACCCCGTTCA

6201 GCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC

6301 AGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGC

6401 CAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAACAAACCCGCTGGTAGCGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAG

PacI (6601)

6501 AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAA

SwaI (6609)

6601 TTAACATTTAAATCA