

pSELECT-zeo-LacZ

A LacZ Reporter Gene System Selectable with Zeocin™

Catalog code: psetz-lacz

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-zeo-LacZ plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

Product is shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.

Store Zeocin™ at 4 °C or at -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

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 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.

pSELECT-LacZ can serve as a gene reporter system for the study of eukaryotic gene expression and regulation. The *E. coli lacZ* gene encoding β-galactosidase is the classical histochemical reporter gene. β-Galactosidase catalyzes the hydrolysis of X-Gal producing a blue precipitate that can be easily visualized under a microscope.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1a (EF-1a) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². The EF-1a promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1a core promoter to enhance stability of RNA.

• **LacZΔCpG gene:** a humanized and CpG-free allele of the LacZ gene. This CpG-free gene is ten times more active than the wild-type gene in mammalian cells. It can be used for *in vitro* or *in vivo* applications.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

• **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*.

• **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

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 other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
ChemiComp GT116	gt116-11
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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

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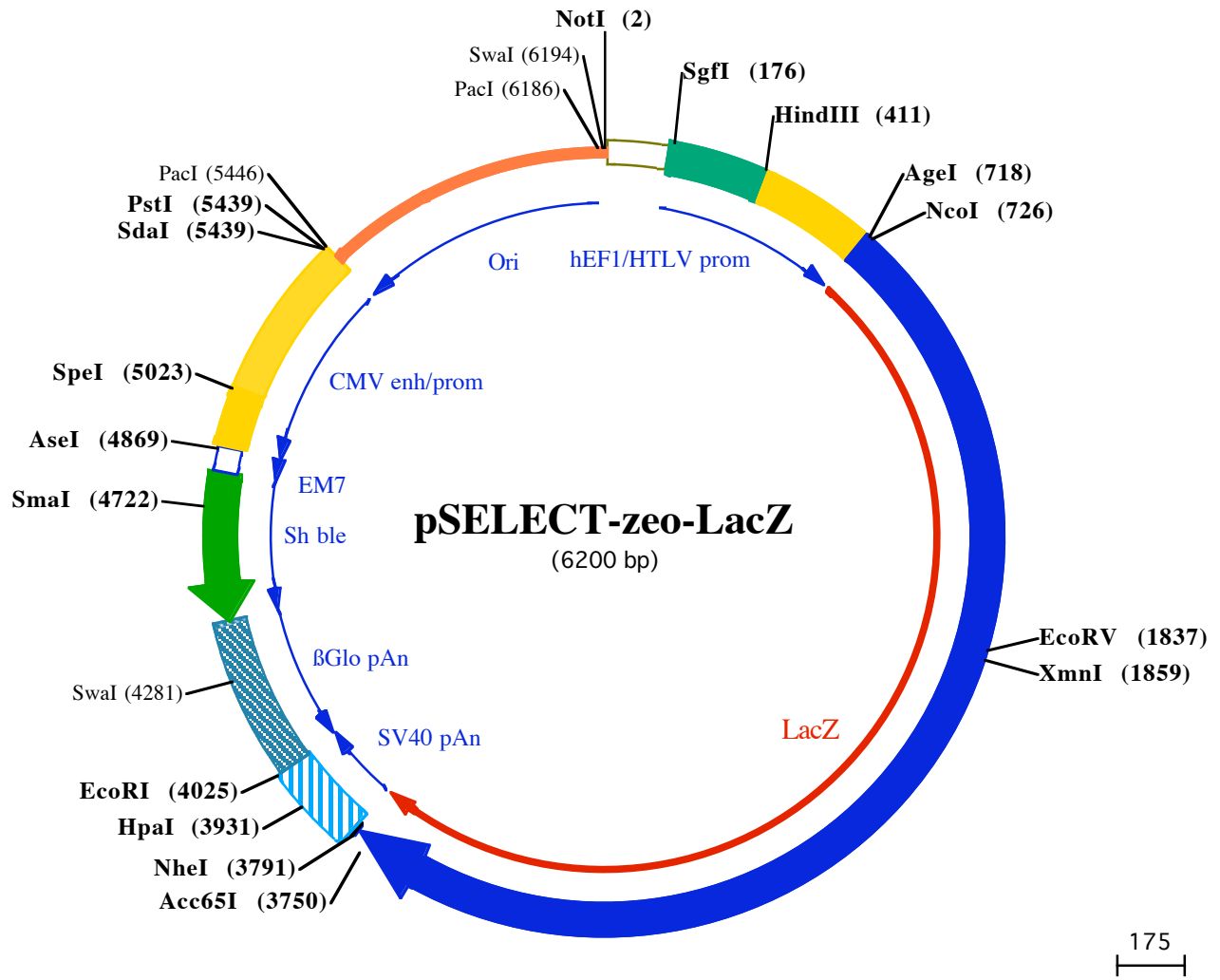
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NotI (2)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAAACGAAACA
SgfI (176)
101 AAACAACTAGCAAATAGGCTGTCCCAAGTGAAGTGCAGGTCGAGAACATTTCTCTATCGAAGGATCTCGCATCGCTCCGGTGCCCGTCAGTGGGCA
201 GAGCGCATCTGCCACAGTCCCGAGAAGTTGGGGGAGGGTGGCAATTGAACGGGTGCCTAGAGAAGTGGCGGGGTAACATGGAAAGTGATG
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGAGAACCCTATATAAGTCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAG
HindIII (411)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCCAGCGCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCTT
501 CCCGCTGTGGTGCTCCTGAACTGCGTCCGCGCTAGGTAAAGTTAAAGCTCAGTTCGAGACCGGGCCTTTGTCGGCGCTCCCTTGGAGCCTACCTA
601 GACTCAGCGGCTCCACGCTTTGCTGACCTGCTTCTCAACTCTACGTTCTTGTTCGTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC
NcoI (726)
AgeI (718)
701 GCGCCCTACTGAGATCAccggtcacCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCC
1MetAspProVal Val LeuGlnArgArgAspTrpGluAsnProGluVal ThrGlnLeuAsnArgLeuAlaAlaH
801 ACCCTCCCTTTGCCCTCTGGAGAACTCTGAGGAAGCCAGACAGACAGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGGCTGGTT
25PisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGluGluTrpArgPheAlaTrpPh
901 CCCTGCCCTGAAGCTGTGCTGAGTCTTGGCTGGAGTGTACCTCCAGAGGCTGACACTGTTGTGGTCCCGCAACTGGCAGATGCATGGCTATGAT
58PeProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTrpGlnMetHisGluGluYTrpAsp
1001 GCCCCATCTACCAATGTACCTACCCATCACTGTGAACCCCTTTGTGCCACTGAGAACCCTGGCTGTACAGCCTGACCTTCAATGTTG
92AlaProIleThrThrAsnValThrTyrProIleThrValAsnProPheValProThrGluAsnProThrGluCysTyrSerLeuThrPheAsnValAla
1101 ATGAGAGCTGGCTGCAAGAAGCCAGCAGGATCATCTTTGATGGAGTCACTTCGCTCCACCTCTGGTGAATGGCAGGTTGGCTATGGCCAA
125spGluSerTrpLeuGlnGluGluGlnThrArgIleIlePheAspGluValAsnSerAlaPheHisLeuTrpCysAsnGluYArgTrpValGluYTrpGluGlu
1201 AGACAGCAGGCTGCCCTCTGAGTTGACCTCTCTGCTTCTCAGAGCTGGAGAGAAGCAGGCTGGCTGTGATGGTCTCAGTGGTCTGATGGCAGCTAC
158nAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGluGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGluYSerTrp
1301 CTGGAAGCAAGACATGTGGAGGATGTCTGGCATCTTCAGGATGTGAGCTGCTGCACAAGCCACCACCCAGATTTCTGACTTCCATGTTGCCACCA
192LeuGluAspGluAspMetTrpArgMetSerGluIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAspPheHisValAlaThrA
1401 GGTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGTGCAGATGTGTGGAGAAGTGCAGACTACCTGAGAGTGCAGAGTGGCCTGGCAGG
225rPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGluYLeuArgAspTyrLeuArgValThrValSerLeuTrpGluGln
1501 TGAGACCCAGTGGCCTCTGGCAGACCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTACCCTGAGGCTCAATGGAG
258yGluThrGlnValAlaProGluYThrAlaProPheGluYglYglulIleAspGluArgGluYglYThrAlaAspArgAlaThrLeuArgLeuAsnValGlu
1601 AACCCCAAGCTGTGGTCTGCTGAGTCCCAACCTCTACAGGCTGTGTGGAGCTGCACACTGCTGATGGCACCCTGATTTGAAGCTGAAGCTGTGATG
292AsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGluYThrLeuIleGluAlaGluAlaCysAspV
1701 TTGATTACAGAAAGTACAGGATGAGAATGGCTGCTGCTCAATGGCAAGCCTGCTGCTATCAGGGAGTCAACAGGATGAGCACCCTCTGCA
325AlGluPheArgGluValArgIleGluAsnGluYLeuLeuLeuAsnGluYLysProLeuLeuIleArgGluYValAsnArgHisGluHisProLeuHis
EcoRV (1837) XmnI (1859)
1801 TGGACAAGTGTGATGAACAGACAATGGTGCAGATATCTGTCTAATGAAGCAGAACAACTTCAATGTCTCAGTGTCTCACTACCCCAACCACCT
358sGluYglYnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisPro
1901 CTCTGGTACACCCCTGTGTGACAGGTATGGCCTGTATGTTGTTGATGAAGCCAACTTGGAGACATGGCATGGTCCCATGAACAGGCTCACAGATGACC
392LeuTrpTyrThrLeuCysAspArgTyrGluYLeuTyrValValAspGluAlaAsnIleGluThrHisGluMetValProMetAsnArgLeuThrAspAsp
2001 CCAGTGGCTGTGCTGCCATGTCTGAGAGAGTGCACAGGATGCTGGAGGCTGCAGAGACAGGAACCCCTCTGTGATCATCTGGGCAATGAGTGTGG
425roArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpSerLeuGluYAsnGluSerGlu
2101 ACATGGAGCAACCATGATGCTCTACAGGTGGATCAAGTCTGTGACCCCAAGCAGACCTGTGCAGTATGAAGGAGTGGAGCAGACACACAGCCACA
458yHisGluYAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValGlnTyrGluGluYglYAlaThrAlaThr
2201 GACATCATCTGCCCATGTATGCCAGGTTGATGAGGACAGCCCTTCCCTGCTGTGCCAAGTGGAGCATCAAGAAGTGGCTCTCTCTGCTGGAGAGA
492AspIleIleCysProMetTyrAlaArgValAspGluAspGlnProPheProAlaValProLysTrpSerIleLysLysTrpLeuSerLeuProGluYglY
2301 CCAGACTGTGATCCTGTGAATATGCACATGCAATGGGCAACTCTGGGAGGCTTTCGCAAGTACTGGCAAGCCTTCAGACAGTCCAGCCAGCTCA
525hrArgProLeuIleLeuCysGluYThrAlaHisAlaMetGluYAsnSerLeuGluYglYpHeAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlu
2401 AGGAGGATTTGTGGGACTGGGTGGACCAATCTCTCAAGTATGATGAGAATGGCAACCCCTGGTCTGCCTATGGAGGAGACTTTGGTGCACACCCC
558nGluYglYpHeValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluYAsnGluYAsnProTrpSerAlaTyrGluYglYAspPheGluYAspTrpPro
2501 AATGACAGGCGATTCTGCATGAATGGCCTGGTCTTTCAGACAGGACCCCTCACCTGCCCTCACAGAGGCCAAGCACCAGCAACAGTCTTCCAGTTCA
592AsnAspArgGlnPheCysMetAsnGluYLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGluGlnGlnPhePheAlaPheA
2601 GGCTGTGGACAGCAATGAGTGCATCTGAGTACCTTTCAGCAGCTTGCACATGAGCTCCTGACTGGATGGCCCTGGATGGCAGCCTCTCTGTGACCC
625rGLeuSerGluYglYnThrIleGluValThrSerGluYThrLeuPheArgHisSerAspAsnGluYLeuLeuHisTyrMetValAlaLeuAspGluYLysProLe
2701 GGCTTCTGGTGGAGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGACAACCTGTGGCTA
658AlaSerGluYglYnValProLeuAspValAlaProGlnGluYLysGlnLeuIleGluLeuProGluYLeuProGluYSerAlaGluYThrLrpLeu
2801 ACAGTGAGGGTGGTTTCAGCCCAATGCAACAGCTTGGCTGTGGCAGCCAGCAGTCTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCC
692ThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGluYHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrL
2901 TGCCTGCTGCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTGGCAACAAGAGATGGCAGTTCAACAGGCAGTCTGG
725euProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleGluYLeuGluYAsnLysArgTrpGlnPheAsnArgGlnSerGlu
3001 CTTCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGGCAAACTTCCACAGGGCTCCTCTGGACAATGACATGGAGTGTCT
758yPheLeuSerGlnMetTrpIleGluYAspLysLysGlnLeuLeuLeuTrpProLeuArgAspGlnPheThrArgAlaProLeuAsnAspIleGluYAlaSer
3101 GAGGCCACAGGATTTGACCCAAATGCTGGGTGGAGAGGTTGGAAGGCTGTGGACTACCAAGCTGAGGCTGGCTGGCTGCAAGTCCAGTGCACAGCACCC
792GluAlaThrArgIleAspProAsnAlaTrpValGluYArgTrpLysAlaAlaGluYHisTyrGlnAlaGluAlaAlaLeuLeuGlnCysThrAlaAspThrL
3201 TGGCTGATGCTTCTGATCACCACAGCCATGCTTGGCAGCACCAGGCAAGCAGCCCTGTTTCATCAGCAGAAAGCCTACAGGATGATGGCTCTGGACA
825AlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGluGlnYLysThrLeuPheIleSerArgLysThrTyrArgIleAspGluYAspGluYglY
3301 GATGGCAATCAGTGGATGGAGGTTGCCCTGACACACCTCACCTGCAAGGATTGGCCTGAACGTCACTGGCAGAGTGGCTGAGAGGTTGAAAC
858nMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgIleGluYLeuAsnCysGluYLeuAlaGluYnValAlaGluYArgValAsn
3401 TGGCTGGCTTAGGCCCTCAGGAGAATCCTGACAGGCTGACAGCTGCCTTGGAGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAG
892TrpLeuGluYLeuGluYProGlnGluYAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrV
3501 TGTTCCTCTGAGAATGGCTGAGTGTGGCACCAGGAGCTGAACTATGGTCTCACCAGTGGAGGGGAGACTTCCAGTTCAACATCTCCAGGTACTC
925AlPheProSerGluYAsnGluYLeuArgCysGluYThrArgGluYLeuPheAsnIleSerHisGlnTrpArgGluYAspPheGluYAspPheGluYSer
3601 TCAGCAACAGCTCATGAAACCTCTCACAGCACCTGCTCCATGCAAGAGGAGGAACTGGCTGAACATTGATGGCTTCCACATGGCATTGGAGGAGAT
958rGlnGlnGlnLeuMetGluYThrSerHisArgHisLeuLeuHisAlaGluYglYThrTrpLeuAsnIleAspGluYpHeHisMetGluYIleGluYglYAsp
Acc65I (3750) NheI (3791)
3701 GACTCTTGGTCTCTCTGTGTCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCCTATCAGTGGTGGTGGCCAGAAGTAAACCTGAGCTAGCTGGC
992AspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGluYArgTyrHisTyrGluYLeuValTrpCysGlnLys -
3801 CAGACATGATAAGATACATTGATGAGTTGGACAAACCAACTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT

3901 **HpaI (3931)**
TGTAACCATTATAAGCTGCAATAAACAGTTAAACAACAACATTGCATTCTATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAG

4001 **EcoRI (4025)**
TAAAACTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA

4101 TAAGGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTT

4201 **SmaI (4281)**
GAAGTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATGTAATAATTCAGAAATAATTTAAATACATCATTGCAATGA

4301 AAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATA

4401 GAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTGCTGCCACAAAGTGCACGAGTTGCCGGCCGGTCCGCGAGGGCAACT

4501 CCCGCCCCACGGTGTCTGCGGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCCTCGGCGTACAGCTCGTC
127 rLeuGlyTrpProGlnGluIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAsp
104 uArgGlyTrpProGlnGluIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAsp

4601 CAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGCTCTGGACCGCGCTGATGAACAGGGTCACTGCTCCCGACACACCCGGCG
71 LeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaP

4701 **SmaI (4722)**
AAGTCGCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCAGAACCTCGACCCTCCGGCGACGTCGCGCGGTTGAGCACCCGGAACGGCACTGG
37 heAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerTh

4801 **AseI (4869)**
TCAACTGGCCATGATGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCC
4 rLeuLysAlaMet

4901 AGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCCTACCGCCATTTGCGTCAATGGGGGGAGTGTGTACGACA

5001 **SpeI (5023)**
TTTTGAAAGTCCCGTTGATTTACTAGTCAAACAACACTCCCATGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGC

5101 CCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGG

5201 CATAATGCCAGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGACGTTTACCGTAA

5301 ATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGCGGGGGTCTGTGGCGGTCAG

5401 **PstI (5439)**
SdaI (5439)
CCAGCGCGGCCATTACCCTGAAGTTATGTAACGCCGTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGC

5501 GTTGCTGGCGTTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC

5601 CAGGCGTTTCCCTGGAAGTCCCTCGTGGCTCTCTGTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTCGGGAAGCGTGGCGC

5701 TTTCTCATAGCTACGCTGTAGTATCTCAGTTCGGTGTAGTCTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGC

5801 CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTA

5901 GCGGTGTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA

6001 AAAGATTGGTAGCTTTGATCCGGCAAACAACCCGCTGGTAGCGGTGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCA

6101 **PacI (6186) SmaI (6194)**
AGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCTAGGCTAGTTAATTAACATTTAAATCA