

pSELECT-neo-LacZ

A LacZ-expression plasmid selectable with Kanamycin/G418

Catalog # psetn-lacZ

For research use only

Version # 09K25-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-neo-LacZ plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Kan (2 TB and 2 Agar)

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 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.

Store *E. coli* Fast-Media® Kan 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

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 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-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 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*.
- **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 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.

Selection of bacteria with *E. coli* Fast-Media®

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic.

Fast-Media® Kan can be ordered separately (#fas-kn-l (liquid), #fas-kn-s (solid)).

Method:

- 1- Pour the contents of a Fast-Media® 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:

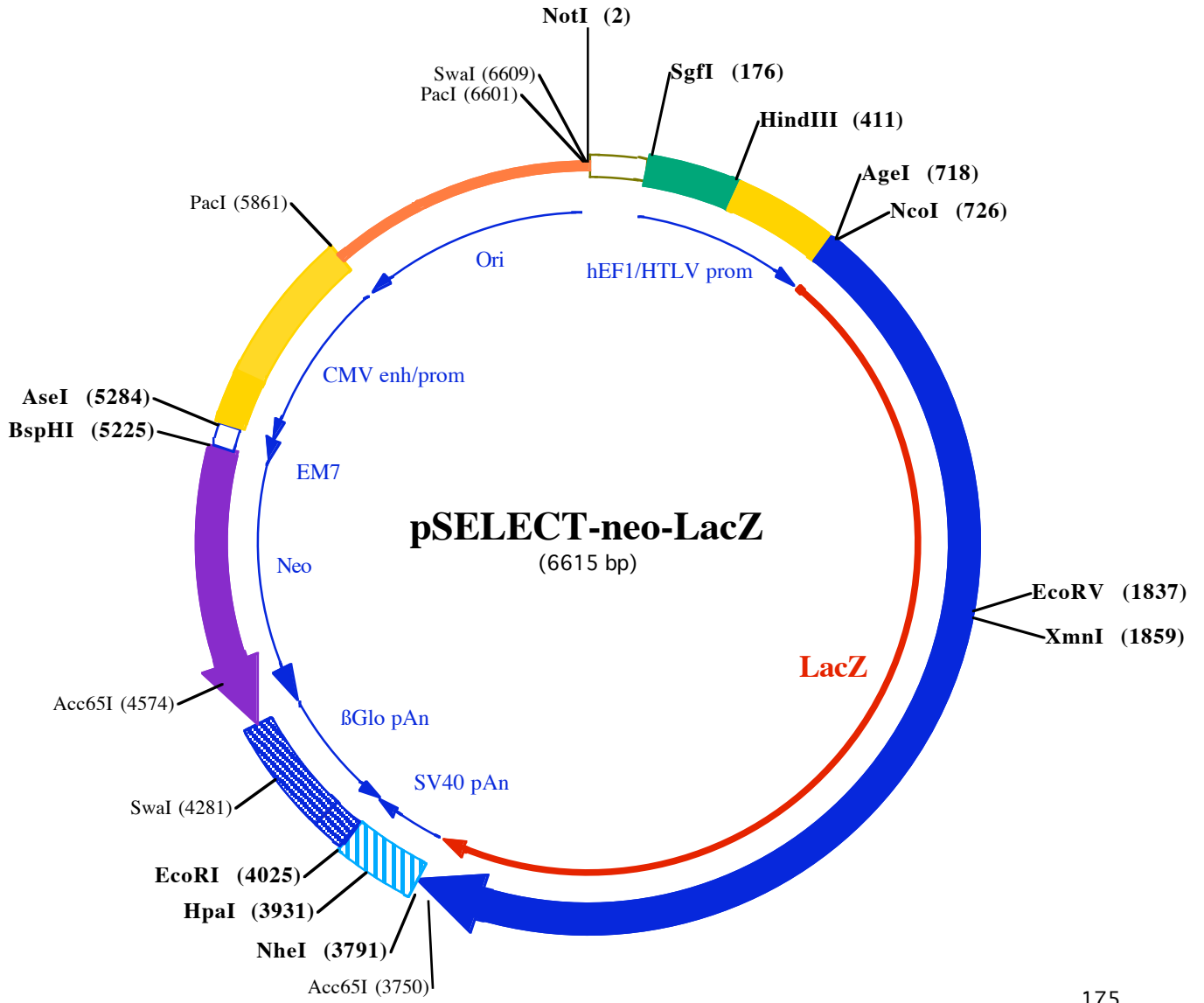
1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

TECHNICAL SUPPORT

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NotI (2)

1 GCGGCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA
 101 AAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCATCGCTCCGGTCCCGTCAAGTGGGCA
 201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGATG
 301 TCGTGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCGCGTGAACGTTCTTTTTTCGCAACGGGTTTCCGCCACG

SgfI (176)

HindIII (411)

401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCCACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCT
 501 CCGCGCTGTGGTGCCTCCTGAACGCGTCCGCGCTAGGTAAGTTAAAGCTCAGGTGCAGACCGGGCTTTGTCCGGCGCTCCCTTGGAGCTACCTA
 601 GACTCAGCCGGCTCCACGCTTGCCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

NcoI (726)

AgeI (718)

701 GCGCGCTACCTGAGATCAccggtcacCATGGACCTGTTGTCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCC
 1▶MetAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHis
 801 ACCCTCCCTTTGCCTCTGGAGGAACTCGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGCCTGGTT
 25▶iSProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPh
 901 CCCTGCCCTGAAGCTGTGCTGAGTCTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTGTGGTCCCAAGCACTGGCAGATGCATGGCTATGAT
 58▶eProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAsp
 1001 GCCCCATCTACACCAATGTCACTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCTGGCTGCTACAGCTGCCTCAATGTTG
 92▶AlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAla
 1101 ATGAGAGCTGGCTGCAAGAAGGCCAGACAGGATCATCTTTGATGGAGTCAACTCTGCTTCCACCTCTGGTGAATGGCAGGTGGGTGGCTATGGCCA
 125▶spGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGly
 1201 AGACAGCAGGCTGCCCTGTAGTTGACCTCTCTGCCCTCCTCAGAGCTGGAGAGAACCAGGCTGGCTGTATGGTGTCAAGTGGTCTGATGGCAGCTAC
 158▶nAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGlyuAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyr
 1301 CTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAAGGATGTGAGCCTGCTGCACAGCCACCACCCAGATTCTGACTTCCATGTTGCCACCA
 192▶LeuGluAspGluAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAspPheHisValAlaThrA
 1401 GTTTCAATGATGACTTCAGCAGAGCTGTCTGGAGCTGAGGTGCAGTGTGGAGAACTCAGAGACTACCTGAGAGTCAACAGTGGCTCTGGCAAGG
 225▶rGlyPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGlyLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGly
 1501 TGAGACCAGGTGGCTCTGGCAGACGCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCCACCTGAGGCTCAATGGGAG
 258▶yGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGlyIleIleAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGlu
 1601 AACCCCAAGCTGTGCTCTGATGATCCCAACCTCAGAGGCTGTTGTGGAGTGCACACTGCTGATGGCAGTATTGAAGCTGAAGCTGTGATG
 292▶AsnProLysLeuTrpSerAlaGlyIleProAsnLeuTyrArgAlaValValGluLeuHisSThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspV
 1701 TTGGATTGAGAGAAGTCAAGGATGAGAAATGGCTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGAGTCAACAGGCATGAGCACCACCTCTGCA
 325▶alGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuAsnGlyLysProLeuLeuIleArgGlyValAsnArgHisGlyuHisSHisProLeuHis

EcoRV (1837)

XmnI (1859)

1801 TGGACAAGTGGATGAACAGACAATGGTGAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTGCAGGTGCTCTACTACCCCAACCACCT
 358▶sGlyGlyValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisPro
 1901 CTCTGGTACACCTGTGTGACAGGATGGCTGTATGTTGTTGATGAAGCCAACATTGAGACACATGGCATGGTCCCAAGGCTCACAGATGACC
 392▶LeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluuThrHisGlyMetValProMetAsnArgLeuTrpAspAsp
 2001 CCAGGTGGCTGCTGCTGACATGTCTGAGAGAGTGACCAGGATGGTGCAGAGAGACAGGAACCACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGG
 425▶roArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpSerLeuGlyAsnGluSerGly
 2101 ACATGGAGCCAAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGAGTATGAAGGAGGTGGAGCAGACCCACAGCCACA
 458▶yHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValGlnTyrGluGlyGlyAlaAspThrThrAlaThr
 2201 GACATCAGTCCCCATGATGCCAGGTTGATGAGGACAGCCCTCCTGCTGTGCGCAAGTGGAGCATCAAGAAGTGGCTCTCTGCTGCTGGAGAGA
 492▶AspIleIleCysProMetTyrAlaArgValAspGluAspGluProPheProAlaValProLysTrpSerIleLysLysTrpLeuSerLeuProGlyGlyuT
 2301 CCAGACCTCTGATCTGTGTAATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGGCAAGCCTTCCAGACAGTACCCAGGCTGCA
 525▶hrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgLeuTyrProArgLeuGly
 2401 AGGAGGATTTGTGGGAGTGGGTGGGCAATCTCTCATCAAGATGATGAGAAATGGCAACCCTGGTCTGCATGGAGAGACTTTGGTGACACCCCC
 558▶nGlyGlyPheValTrpAspTrpValAspGluSerLeuIleLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGlyAspPheGlyAspThrPro
 2501 AATGACAGGCAGTCTGCATGAATGGCTGGTCTTTGACAGACAGACCCCTCACCCTGCCCTCAGAGGCGCAAGCACCAGCAACAGTCTCTCCAGTTCA
 592▶AsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlyGlnGlnPhePheGlnPheA
 2601 GGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTCAGGCACTGTGACAATGAGCTCTGCACTGGATGGTGGCCCTGGATGGCAAGCCTCT
 625▶rGlyLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeuLeuHisSrpMetValAlaLeuAspGlyLysProLe
 2701 GGCTTCTGGTGGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACCTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGCAACTGTGGCTA
 658▶uAlaSerGlyGlyuProLeuAspValAlaProGlnGlyGlnLeuIleGluLeuProGluLeuProGluNProGluSerAlaGlyGlnLeuTrpLeu
 2801 ACAGTGAAGGTGGTTGAGCCCAATGCAACAGCTTGTGCTGAGGACAGCCACATCTGCTGAGCAGCAGTGGAGGCTGGCTGAGAAGCTCTCTGTGACC
 692▶ThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrL
 2901 TGCTGCTGCCTCTCATGCCATCCCTCACCCTGACAACATCTGAAATGGACTTCTGCATTGAGCTGGGCAACAAGAGATTGGCAGTTCACAGGCAGTCTGG
 725▶euProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleGluLeuGlyAsnLysArgTrpGlnPheAsnArgGlnSerGly
 3001 CTTCTGTCTCAGATGTGATGGAGCAAGAGCAGCTCCTCACCCTCACPheCysAlaTTCACCAGGCTCCTCTGAGCAATGACATTTGGATGTCT
 758▶yPheLeuSerGluMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspAsnAspIleGlyValSer
 3101 GAGGCCACCAGGATTGACCCAAATGCTTGGGTGGAGAGTGAAGGCTGCTGGACACTACCAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACCCC
 792▶GluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisTyrGlnAlaGluAlaAlaLeuLeuGlnCysThrAlaAspThrL
 3201 TGGCTGATGTTCTTGTGATCACCACAGCCATGCTTGGCAGCACAAGCAAGCAGCCTGTTCATCAGCAGAAAGACTACAGGATGATGGCTCTGGACA
 825▶euAlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheIleSerArgLysThrTyrArgIleAspGlySerGlyGly
 3301 GATGGCAATCAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCCTGCAAGGATTGGCTGAACTGTCAACTGGCACAGGTGGCTGAGAGGTGAAC
 858▶nMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgIleGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsn
 3401 TGGCTGGCTTAGCCCTCAGGAGAATACCCTGACAGCTGACAGTCTGCTGCTTGCAGAGTGGGACCTGCCTGTGATGTACACCCCTATG
 892▶TrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrV
 3501 TGTTCCTTCTGAGAATGGCTGAGGTGGCACCAGGAGCTGAACATGGTCTCACCAGTGGAGGGAGACTTCCAGTCAACATCTCCAGGTACTC
 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 GACTCTTGGTCTCCTTCTGTGTCTGTGAGTTCAGTTATCTGTGGCAGGTACCACATCAGCTGGTGGTGCCAGAAGTAAACCTGAGCTAGCTGGC
992▶AspSer TrpSer P roSer Val Ser Al aGl uPheGl nLeuSer Al aGl yArgTyrHi sTyrGl nLeuVal TrpCysGl nLys●●●
3801 CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT

HpaI (3931)

3901 TGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACATTGCATTCTTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAG

EcoRI (4025)

4001 TAAAACCTCTACAAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA

4101 TAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTT

SwaI (4281)

4201 GAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGA

4301 AAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAAACAAAGGAACCTTTAATA

4401 GAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGAGAAGCTCATCAAGAAGTCTGTAGAAGGCAATTTCTCTGGGAGTCAGGGGCTGCAATGCCATAG

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 CACAGTCTATGAAGCCAGAGAACCTTCCATTTTCAACCATGATGTTGGGAAGGCAGGCATCCCCATGAGTCACCACTAGGTCCTACCATCTGGCATGGA

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 CTCTCTATCCTGTGCTTGGCCTGGTGGTCAAAGGGGCAGGTGGCTGGTCAAAGGGTGGAGTCTTCTCATGGCATCAGCCATGATTGACACTTCTCAG

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 Al aAl aCys

5001 GGGACCCAGTTGTTGCCAACCAGGAGTCTGGCAGCCTCATCTGGAGCTCATTGAGAGCCCACTGAGGTCTGTCTTTACAAAAAGGACTGGCCTG

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

5101 CTTGGGCTGAAAGTCTGAAACTGTCATCAGACCAACCAATGGTCTGCTGTGCCAGTCATAGCCAAACAGTCTCTCAACCCAGGCAGCTGGAGAAC

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

BspHI (5225)

AseI (5284)

5201 CTGCATGTAGGCCATCTTGTTCATCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGC

9▶yAl aHi sLeuGl yAspGl nGl u l eMe t

5301 GTGGATGGCGCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTGGCGTCAATGGGGC

5401 GGAGTTGTTACGACATTTTGAAAGTCCCGTTGATTTACTAGTCAAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCA

5501 AACCGCTATCCAGCCCATTTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGTAGGAAAGTCCATA

5601 AGGTCATGTAAGTGGCATAATGCCAGGCGGGCCATTTACCGTCAATGAGGGGCGTACTTGGCATATGATACACTTGTACTGCAAGTGGCAAGTGG

5701 GGCAGTTTACCGTAATACTCCACCCATTGACGTCATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGCAAGTCAATGGCGGGGG

PacI (5861)

5801 TCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACCGCTGCGAGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAA

5901 CCGTAAAAAGGCCGCTTGTGGCGTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA

6001 GGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTT

6101 CGGGAAGCGTGGCGTTTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAGCTGGGCTGTGTGCACGAACCCCGTTCA

6201 GCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC

6301 AGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGC

6401 CAGTTACCTTCGAAAAAGAGTTGGTAGCTTGTATCCGGCAACAAACCCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAG

PacI (6601)

6501 AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAA

SwaI (6609)

6601 TTAACATTTAAATCA