

pSELECT-blasti-LacZ

A LacZ-expression plasmid selectable with Blasticidin

Catalog # psetb-lacz

For research use only

Version # 04A08-MT

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-blasti-LacZ plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Blas (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 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® Blas 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*.
- **Bsr:** Resistance to Blasticidin S is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* 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® Blas can be ordered separately (#fas-bl-l (liquid), #fas-bl-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:

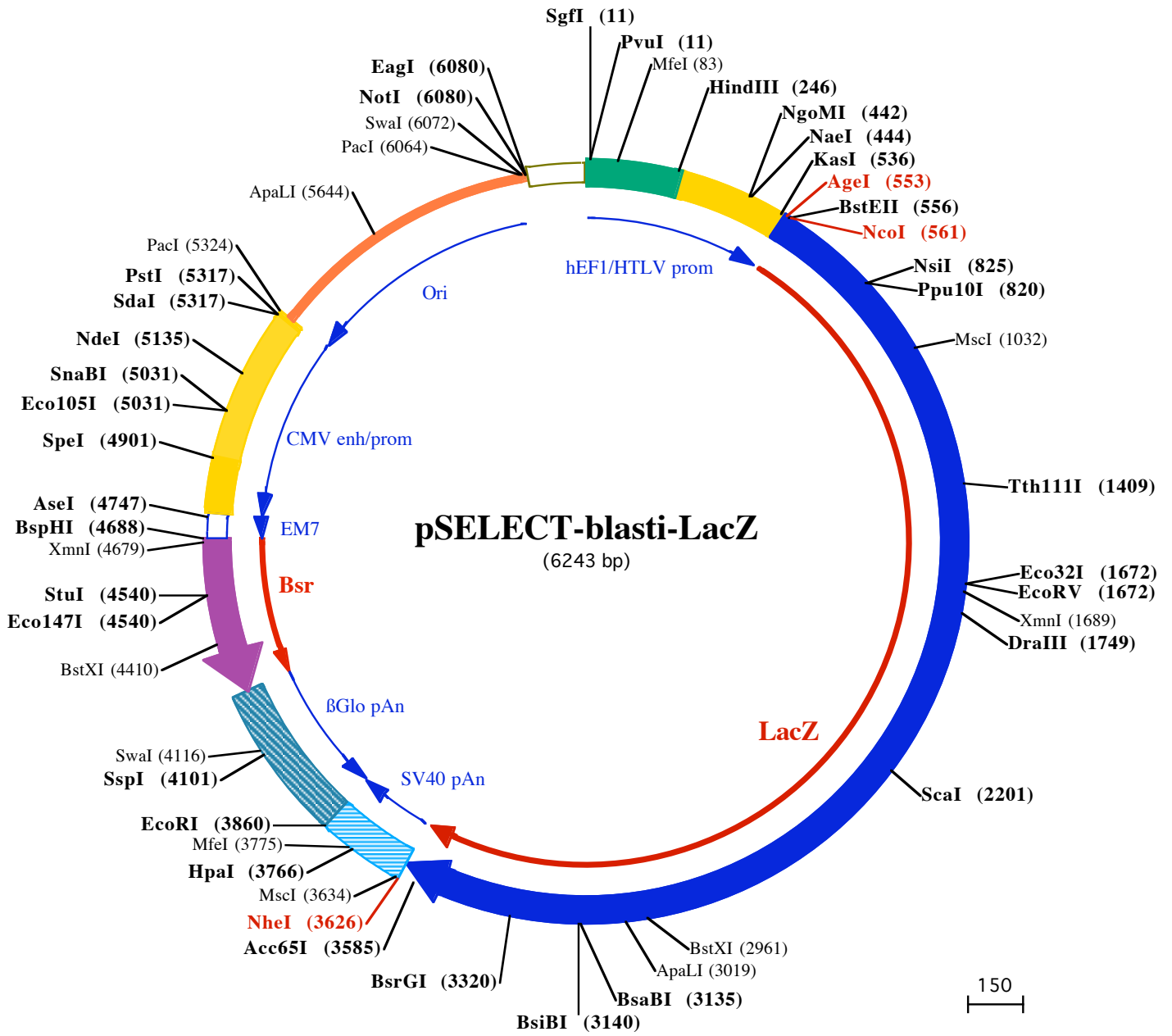
1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

TECHNICAL SUPPORT

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PvuI (11) SgfI (11) MfeI (83)

1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGCGAGAGCCACATGCCACAGTCCCGAGAAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA

101 GAGAAGTGGCGGGGTAACCTGGAAAGTGTATGCTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)

201 GTGAACGTTCTTTTCGCAACGGGTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACTGAGGCC

301 GCCATCCACGCGGGTTGAGTGGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGCTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMI (442) NaeI (444)

401 GGGCCTTTGTCGGCGCTCCCTTGGAGCTACCTAGACTCAGCGGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTCTGTT

NcoI (561) BstEII (556) KasI (536) AgeI (553)

501 TCTGTTCTGGCGGTTACAGATCCAAGCTGTGACCGGGCTACTCTGAGATCACCGGTACCCTGGACCCTGTTGTGCTGCAAAAGGAGAGCTGGGAGAA

601 CCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCTCCCTTTGGCTTTGGAGAACTCTGAGGAAGCCAGGACAGACAGCCAGCCAGCAGCTC

130 nProGlyVal Thr Gl nLeuAsnArgLeuAl aAl aHi sP roP roPheAl aSer TrpArgAsnSer Gl uGl uAl aArgThrAspArgP roSer Gl nGl nLeu

701 AGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTCCCTGCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTG

470 ArgSer LeuAsnGly uTrpArgPheAl aTrpPheProAl aProGly uAl aVal P roGly uSer TrpLeuGly uCysAspLeuP roGly uAl aAspThr Val V

Ppu10I (820) NsiI (825)

801 TGGTCCAGCACTGGCAGATGCATGGCTATGATGCCCATCTACACCAATGTCACCTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAA

800 aI Val P roSerAsnTrpGly nMetHisGly TyrAspAl aP roI eTyrThrAsnVal Thr TyrP roI eThr Val AsnP roP roPheVal P roThr Gl uAs

901 CCCACTGGCTGCTACAGCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAGGCCAGACAGGATCATCTTTGATGGAGTCAACTCTGCTTCCAC

1130 nP roThr Gl yCysTyrSer LeuThr PheAsnVal AspGly uSer TrpLeuGly nGl uGly uGl nThr ArgI l eI l ePheAspGly yVal AsnSer Al aPheHis

MscI (1032)

1001 CTCTGGTCAATGGCAGGTGGTGGCTATGGCCAAGCAGCAGGCTGCCCTGAGTTTGACCTCTCTGCTTCCCTCAGAGCTGGAGAGAAGCAGGCTGG

1470 LeuTrpCysAsnGly yArgTrpVal Gl yTyrGly uGl nAspSerArgLeuP roSer Gl uPheAspLeuSer Al aPheLeuArgAl aGly yGl uAsnArgLeuA

1101 CTGTCATGGTCTCAGTGGTCTGATGGCAGCTACCTGGAGACCAAGACATGTGGAGGATGCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCC

1800 l aVal MetVal LeuArgTrpSerAspGly ySer TyrLeuGly uAspGly nAspMetTrpArgMetSer Gl yI l ePheArgAspVal Ser LeuLeuHisLysP r

1201 CACCACCAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGCTGAGGTGCAGATGTGGAGAACCTCAGA

2130 oThr Thr Gl nI l eSerAspPheHisVal Al aThr ArgPheAsnAspAspPheSer ArgAl aVal LeuGly uAl aGly uVal Gl nMetCysGly yGl uLeuArg

1301 GACTACCTGAGAGTACAGTGGCCTCTGGCAAGTGGAGCCAGTGGCTCTGGCACAGCCCTTTGGAGAGAGATCATTGATGAGAGAGGAGGCT

2470 AspTyrLeuArgVal Thr Val Ser LeuT rpGly nGly uThr Gl nVal Al aSer Gl yThr Al aP roPheGly yGly uI l eI l eAspGly uArgGly yGly T

Tth111I (1409)

1401 ATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCACCAAGCTGTGGTCTGCTGAGATCCCAACCTCTACAGGGCTGTGTGGAGCTGCACACTGC

2800 yAl aAspArgVal Thr LeuArgLeuAsnVal Gl uAsnP roLysLeuTrpSer Al aGly uI l eP roAsnLeuTyrArgAl aVal Val Gl uLeuHis sThr Al

1501 TGTGGCAAGCTGATGAAGCTGAAGCTGTGATGTGGATTGGATTGAGAACTCAGAGGATTCAGGATTCAGAAATGGCTGCTGCTGCTGCTGCTGCTGCTCATC

3130 aAspGly yThr LeuI l eGly uAl aGly uAl aCysAspVal Gl yPheArgGly uVal ArgI l eGly uAsnGly yLeuLeuLeuLeuAsnGly yLysP roLeuLeuI l e

EcoRV (1672) Eco32I (1672) XmnI (1689)

1601 AGGGGAGTCAACAGGCATGAGCACCACCTCTGCATGGACAAGTGGATGAACAGACAATGGTGAAGATATCCCTGTAATGAAGCAGAACCACTTCA

3470 ArgGly yVal AsnArgHis sGly uHis sP roLeuHis sGly yGl nVal MetAspGly uGl nThr MetVal Gl nAspI l eLeuLeuMetLysGly nAsnAsnPheA

DraIII (1749)

1701 ATGCTGTCAAGTGTCTCACTACCCCAACCCCTCTCTGGTACACCTGTGTGACAGGTATGGCTGTATGTTGTGTGATGAAGCCAACATTGAGACACA

3800 snAl aVal ArgCysSer His sTyrP roAsnHis sP roLeuTrpTyrThr LeuCysAspArgTyrGly yLeuTyrVal Val AspGly uAl aAsnI l eGly uThrHi

1801 TGGCATGGTCCCATGAACAGGCTCACAGATGACCCAGGCTGGCTGCCATGCTGAGAGAGTGCAGCAGGATGACAGGATGGTGCAGACAGCAACCC

4130 sGly yMetVal P roMetAsnArgLeuThr AspAspP roArgTrpLeuP roAl aMetSer Gl uArgVal Thr ArgMetVal Gl nArgAspArgAsnHis sP ro

1901 TCTGTGATCATCTGGCTCTGGCAATGAGTCTGGACATGGAGCCAACATGATGCTCTCTACAGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGC

4470 Ser Val I l eI l eTrpSer LeuGly yAsnGly uSer Gl yHis sGly yAl aAsnHis sAspAl aLeuTyrArgTrpI l eLysSer Val AspP roSer ArgP roVal G

2001 AGTATGAAGGAGTGGAGCAGACACCACGCCACAGACATCATCTGCCCATGTATGCCAGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCCAAGT

4800 l nTyrGly uGly yGly yAl aAspThr Thr Al aThrAspI l eI l eCysP roMetTyrAl aArgVal AspGly uAspGly nP roPheP roAl aVal P roLysTr

ScaI (2201)

2101 GAGCATCAAGAAGTGGCTCTCTGCTGGAGAGACCAGACCTCTGATCCTGTGTAATGCACATGCAATGGGCAACTCTCTGGAGGCTTTGCCAAG

5130 pSer I l eLysLysTrpLeuSer LeuP roGly uThrArgP roLeuI l eLeuCysGly uTyrAl aHis sAl aMetGly yAsnSer LeuGly yGly yPheAl aLys

2201 TACTGGCAAGCTTCAAGCAGTACCCAGGCTCAAAGGAGGATTTGCTGGAGCTGGGTGGACCAATCTCTCATCAAGTATGATGAGAATGCAACCCCT

5470 TyrTrpGly nAl aPheArgGly nTyrP roArgLeuGly nGly yGly yPheVal TrpAspTrpVal AspGly nSer LeuI l eLysTyrAspGly uAsnGly yAsnP roT

2301 GGTCTGCATGAGGAGACTTTGGTGGACCCCAATGACAGGAGTCTGATGATGAGGCTGGCTTTTGCAGACAGGACCCCTCACCTGCCCTCAC

5800 rpSer Al aTyrGly yGly yAspPheGly yAspThr P roAsnAspArgGly nPheCysMetAsnGly yLeuVal PheAl aAspArgThr P roHis sP roAl aLeuTh

2401 AGAGGCCAAGCACCAGCAACAGTCTTCCAGTTCAGGCTGTCTGGACAGACATTGAGGTGACATCTGAGTACCTCTTACGGCCTCTGACAAAGAGCTC

6130 r Gl uAl aLysHis sGly nGly nPhePheGly nPheArgLeuSer Gl yGly nThr I l eGly uVal Thr Ser Gl uTyrLeuPheArgHis sSerAspGly uLeu

2501 CTGCACTGGATGGTGGCCCTGGATGGCAAGCTCTGGCTTCTGGTGGAGTGCCTCTGGATGGCCCTCAAGGAAGCAGCTGATTGAACCTGCTGAGC

6470 LeuHis sTrpMetVal Al aLeuAspGly yLysP roLeuAl aSer Gl yGly uVal P roLeuAspVal Al aP roGly nGly yLysGly nLeuI l eGly uLeuP roGly uL

2601 TGCCTCAGCCAGAGTCTGCTGGACAACCTGGCTAACAGTGAAGGTTGCTCAGCCCAATGCAACAGCTTGGCTGAGGAGCCACATCTCTGCATGGCA

6800 euP roGly nP roGly uSer Al aGly yGly nLeuTrpLeuThr Val ArgVal Val Gl nP roAsnAl aThr Al aTrpSer Gl uAl aGly yHis sI l eSer Al aTrpGly

2701 GCACTGGAGGCTGGTGAACCTCTCTGACCTGGCTGCTGCTGATGACCAACATCTGAATGGACTTCTGATGAGCTG

7130 nGly nTrpArgLeuAl aGly uAsnLeuSer Val Thr LeuP roAl aAl aSer His sAl aI l eP roHis sLeuThr Thr Ser Gl uMetAspPheCysI l eGly uLeu

2801 GGCAACAGAGATGGCAGTTCACAGGCAGCTGGCTTCTGCTCAGATGGATTGGAGACAAGAAGCAGCTCTCACCCCTCTCAGGACCAATTC

7470 Gl yAsnLysArgTrpGly nPheAsnArgGly nSer Gl yPheLeuSer Gl nMetTrpI l eGly yAspLysLysGly nLeuLeuThr P roLeuArgAspGly nPheT

BstXI (2961)

2901 CCAGGCTCCTTGGACAATGACATTTGAGTGTCTGAGGCCACAGGATGACCAATGCTTGGTGGAGAGGTGGAAGCTGCTGGACACTACAGGC

7800 hr ArgAl aP roLeuAspAsnAspI l eGly yVal Ser Gl uAl aThr ArgI l eAspP roAsnAl aTrpVal Gl uArgTrpLysAl aAl aGly yHis sTyrGly nAl

ApaLI (3019)

3001 TGAGGCTGCCCTGCTCAGTGCACAGACACCCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGCACAAGCAAGACCTGTTTCATC

8130 aGly uAl aAl aLeuLeuGly nCysThr Al aAspThr LeuAl aAspAl aVal LeuI l eThr Thr Al aHis sAl aTrpGly nHis sGly nGly yLysThr LeuPheI l e

BsiBI (3140) BsaBI (3135)

3101 AGCAGAAAGACCTACAGATTGATGGCTGGACAGATGGCAATCAGATGGATGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCCTGA

8470 SerArgLysThr TyrArgI l eAspGly ySer Gl yGly nMetAl aI l eThr Val AspVal Gl uVal Al aSer AspThr P roHis sP roAl aArgI l eGly yLeuA

3201 ACTGTCAACTGGCAGAGGTGGCTGAGAGGGTGAACCTGGCTGGGCTTAGGCCCTCAGGAGAATACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTG
880▶ snCysGlnLeuAlaGlnValAlaGlnArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrp
BsrGI (3320)

3301 GGACCTGCCTCTGCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACATGGTCTCACCAGTGG
913▶ pAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGlyLeuAsnTyrGlyProHisGlnTrp
3401 AGGGGAGACTTCCAGTTCAACATCTCCAGGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGGGAACCTGGCTGA
947▶ ArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGlyuThrSerHisArgHisLeuLeuHisAlaGlyuGlyuGlyuThrTrpLeuA
Acc65I (3585)

3501 ACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCCAGTTATCTGTGGCAGGTACCACATCAGCT
980▶ snIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGlyPheGlnLeuSerAlaGlyArgTyrHisTyrGlnLe
MseI (3634)

3601 GGTGTGGTCCAGAAGTAAACCTGAGCTAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGC
1013▶ uValTrpCysGlnLys●●●
NheI (3626)

3701 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACACAACAATTCATTCTTTTATGTTTCAGG
HpaI (3766) MfeI (3775)

3801 TTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCCAAAT
EcoRI (3860)

3901 CAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCA
SspI (4101)

4001 TGGAGTTTAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTAGCTCTTCATTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTATGATAAAA
Swal (4116)

4101 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTA
4201 GTAGTTGGACTTAGGGAACAAGGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTT
141●●●AsnArgThrTyrLysLeuProIleLeuGly
4301 CCTCAATGGTGGTTTTGACCAGCTTGCATTATCTCAATGAGCACAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCATGCCCACAGGGGCT
130●uGlyIleThrThrLysValLeuLysGlyAsnMetGlyIleLeuValPheCysAspProAlaTyrAspSerIleLeuGlyuArgCysMetGlyCysProSer
BstXI (4410)

4401 GACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCGTTGCTCACAGCAGACCAATG
97●ValValArgIleSerArgAspValGlyuAspSerTyrProHisArgValAlaValIleThrAspPheAspLysGlnGlyAsnSerValAlaSerGlyIleA
StuI (4540)
Eco147I (4540)

4501 GCAATGGCTTCCAGCACAGACAGTACCCTGCCAATGTAGGCCCTCAATGTGGACAGCAGAGATGATCTCCCAGTCTTGGTCTGTGGCCGCCCGACAT
63●IleAlaGlyuAlaCysValThrValArgGlyIleTyrAlaGlyuIleHisValAlaSerIleIleGlyuGlyuThrLysThrArgIleAlaAlaGlyuValHis
BspHI (4688)

4601 GGTGCTTGTTCCTCATAGAGCATGGTATCTTCTCAGTGGCGACCTCCACAGCTCCAGATCTGCTGAGAGATGTTGAAGGCTTTCATGATGGCCCT
30●sHisLysAsnAspGlyuTyrLeuMetThrIleLysGlyuThrAlaValGlyuValLeuGlyuLeuAspGlnGlnSerIleAsnPheThrLysMet
XmnI (4679)

4701 CCTATAGTGAGTCGTATTATACTATGCCGATATACATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCCTAA
AseI (4747)

4801 ACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTCGCTCAATGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTT
SpeI (4901)

4901 ACTAGTCAAAACAACCTCCATTGACGTCAATGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCCAAACC
SnaBI (5031)
Eco105I (5031)

5001 GCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCACTGACTGGGCATAATGCCAGGCGGGCCATTT
NdeI (5135)

5101 ACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCCATTGACGTCAA
5201 TGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTACGCCAGGCGGGCCATTACCGTAA
PaeI (5324)
PstI (5317)
SdaI (5317)

5301 GTTATGTAACGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGG
5401 CTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGTTTTCCCCCTGGAAGCT
5501 CCCTCGTGCCTCTCTGTTCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGCCGCTTCTCATAGCTCACGCTGTAG
ApaLI (5644)

5601 GTATCTCAGTTCGGTGTAGGTGCTTCCGCTCAAGCTGGGCTGTGTGCAGAAACCCCGTTCAGCCGACCGCTGCGCCTTATCCGGTAATCATCGTCTT
5701 GAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGA
5801 AGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGTGGTGTAGCTTGTATC
5901 CGGCAAAACAACCACCGCTGGTAGCGGTGTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTT
EagI (6080)
PaeI (6064) Swal (6072) NotI (6080)

6001 ACGGGGTCTGACGCTCAGTGGAAACGAAAACCTACGTTAAGGGATTTTGGTATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTT
6101 ATTTTTCATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAACGAAACAACAACTAGCAAAATAGGCT
6201 GTCCCCAGTCAAGTGCAGTGCCAGAACATTTCTCTATCGAA