pSELECT-blasti-LacZ

A LacZ-expression plasmid selectable with blasticidin

Catalog code: psetb-lacz

https://www.invivogen.com/pselect-blasti

For research use only

Version 20I17-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-blasti-LacZ plasmid provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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

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.

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 DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

PLASMID FEATURES

First expression cassette

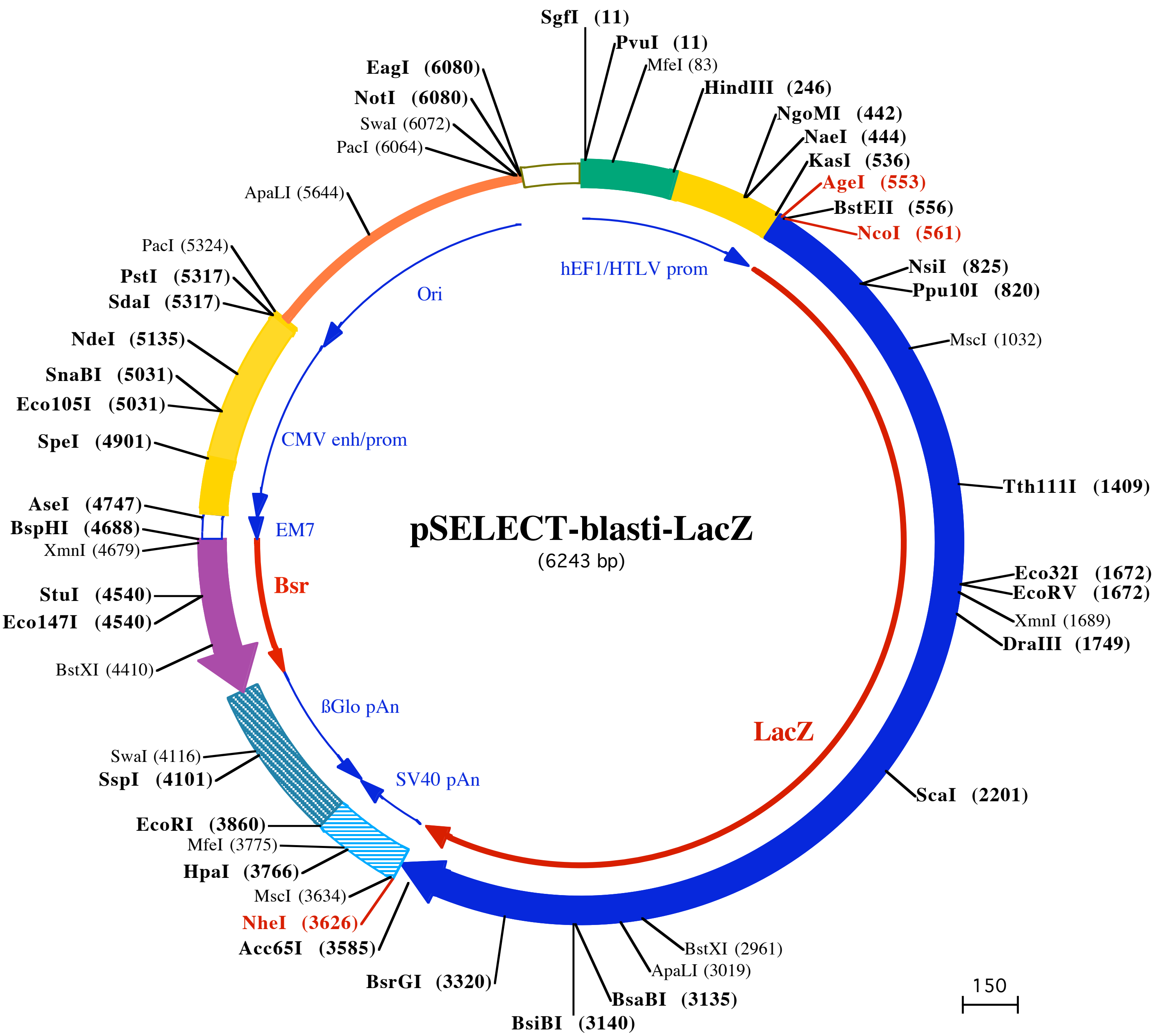
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting 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[3].
- **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*.
- **Blasti:** Resistance to Blasticidin 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 transcription[4].

References

1. Kim D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system.Gene 2: 217-223. **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.


pSELECT-blasti-LacZ
(6243 bp)
SgfI (11)
PvuI (11)
MfeI (83)
HindIII (246)
NgoMI (442)
NaeI (444)
KasI (536)
AgeI (553)
BstEII (556)
NcoI (561)
NsiI (825)
Ppu10I (820)
MscI (1032)
Tth111I (1409)
Eco32I (1672)
EcoRV (1672)
XmnI (1689)
DraIII (1749)
ScaI (2201)
BstXI (2961)
ApaLI (3019)
BsaBI (3135)
BsiBI (3140)
BsrGI (3320)
Acc65I (3585)
NheI (3626)
MscI (3634)
HpaI (3766)
MfeI (3775)
EcoRI (3860)
SspI (4101)
SwaI (4116)
BstXI (4410)
Eco147I (4540)
StuI (4540)
XmnI (4679)
BspHI (4688)
AseI (4747)
SpeI (4901)
Eco105I (5031)
SnaBI (5031)
NdeI (5135)
SdaI (5317)
PstI (5317)
PacI (5324)
ApaLI (5644)
PacI (6064)
SwaI (6072)
NotI (6080)
EagI (6080)
hEF1/HTLV prom
Ori
CMV enh/prom
EM7
Bsr
ßGlo pAn
SV40 pAn
LacZ
150

InvivoGen

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

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)

201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMI (442)
NaeI (444)

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

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

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGGACCCTGTTGTGCTGCAAAGGAGAGACTGGGAGAA
1 MetAspProValValLeuGlnArgArgAspTrpGluAs
601 CCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCCACCCTCCCTTTGCCTCTTGGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCCAGCCAGCAGCTC
13 nProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeu
701 AGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCCTGCCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCCAGAGGCTGACACTGTTG
47 ArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValV

Ppu10I (820)
NsiI (825)

801 TGGTGCCCAGCAACTGGCAGATGCATGGCTATGATGCCCCCATCTACACCAATGTCACCTACCCCATCACTGTGAACCCCCCTTTTGTGCCCACTGAGAA
80 alValProSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAs
901 CCCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGACCAGGATCATCTTTGATGGAGTCAACTCTGCCTTCCAC
113 nProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHis

MscI (1032)

1001 CTCTGGTGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGACCTCTCTGCCTTCCTCAGAGCTGGAGAGAACAGGCTGG
147 LeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuA
1101 CTGTCATGGTGCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCC
180 laValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysPr
1201 CACCACCCAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCAGA
213 oThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArg
1301 GACTACCTGAGAGTCACAGTGAGCCTCTGGCAAGGTGAGACCCAGGTGGCCTCTGGCACAGCCCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCT
247 AspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyGlyT

Tth111I (1409)

1401 ATGCTGACAGAGTCACCCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGC
280 yrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAl
1501 TGATGGCACCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTCAGAGAAGTCAGGATTGAGAATGGCCTGCTGCTGCTCAATGGCAAGCCTCTGCTCATC
313 aAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuLeuIle

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

1601 AGGGGAGTCAACAGGCATGAGCACCACCCTCTGCATGGACAAGTGATGGATGAACAGACAATGGTGCAAGATATCCTGCTAATGAAGCAGAACAACTTCA
347 ArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheA

DraIII (1749)

1701 ATGCTGTCAGGTGCTCTCACTACCCCAACCACCCTCTCTGGTACACCCTGTGTGACAGGTATGGCCTGTATGTTGTTGATGAAGCCAACATTGAGACACA
380 snAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluThrHi
1801 TGGCATGGTGCCCATGAACAGGCTCACAGATGACCCCAGGTGGCTGCCTGCCATGTCTGAGAGAGTGACCAGGATGGTGCAGAGAGACAGGAACCACCCC
413 sGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisPro
1901 TCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGGACATGGAGCCAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCCAGCAGACCTGTGC
447 SerValIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValG
2001 AGTATGAAGGAGGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCCATGTATGCCAGGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCCCAAGTG
480 lnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGlnProPheProAlaValProLysTr

ScaI (2201)

2101 GAGCATCAAGAAGTGGCTCTCTCTGCCTGGAGAGACCAGACCTCTGATCCTGTGTGAATATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAG
513 pSerIleLysLysTrpLeuSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLys
2201 TACTGGCAAGCCTTCAGACAGTACCCCAGGCTGCAAGGAGGATTTGTGTGGGACTGGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCT
547 TyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnGlyAsnProT
2301 GGTCTGCCTATGGAGGAGACTTTGGTGACACCCCCAATGACAGGCAGTTCTGCATGAATGGCCTGGTCTTTGCAGACAGGACCCCTCACCCTGCCCTCAC
580 rpSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuTh
2401 AGAGGCCAAGCACCAGCAACAGTTCTTCCAGTTCAGGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTTCAGGCACTCTGACAATGAGCTC
613 rGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeu
2501 CTGCACTGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGAGGTGCCTCTGGATGTGGCCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGC
647 LeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGlnGlyLysGlnLeuIleGluLeuProGluL
2601 TGCCTCAGCCAGAGTCTGCTGGACAACTGTGGCTAACAGTGAGGGTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACATCTCTGCATGGCA
680 euProGlnProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGl
2701 GCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTG
713 nGlnTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleGluLeu
2801 GGCAACAAGAGATGGCAGTTCAACAGGCAGTCTGGCTTCCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGGACCAATTCA
747 GlyAsnLysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheT

BstXI (2961)

2901 CCAGGGCTCCTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCCAATGCTTGGGTGGAGAGGTGGAAGGCTGCTGGACACTACCAGGC
780 hrArgAlaProLeuAspAsnAspIleGlyValSerGluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisTyrGlnAl

ApaLI (3019)

3001 TGAGGCTGCCCTGCTCCAGTGCACAGCAGACACCCTGGCTGATGCTGTTCTGATCACCACAGCCCATGCTTGGCAGCACCAAGGCAAGACCCTGTTCATC
813 aGluAlaAlaLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheIle

BsiBI (3140)
BsaBI (3135)

3101 AGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCCTGCAAGGATTGGCCTGA
847 SerArgLysThrTyrArgIleAspGlySerGlyGlnMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgIleGlyLeuA

3201 ACTGTCAACTGGCACAGGTGGCTGAGAGGGTGAACTGGCTGGGCTTAGGCCCTCAGGAGAACTACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTG
880 snCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTr

BsrGI (3320)

3301 GGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCCTCACCAGTGG
913 pAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrp

3401 AGGGGAGACTTCCAGTTCAACATCTCCAGGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTGGCTGA
947 ArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyThrTrpLeuA

Acc65I (3585)

3501 ACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCCAGTTATCTGCTGGCAGGTACCACTATCAGCT
980 snIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGlyArgTyrHisTyrGlnLe

MscI (3634)
NheI (3626)

3601 GGTGTGGTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGC
1013 uValTrpCysGlnLys•••

HpaI (3766) MfeI (3775)

3701 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGG

EcoRI (3860)

3801 TTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAAT

3901 CAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCA

SspI (4101)

4001 TGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAA

SwaI (4116)

4101 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTA

4201 GTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTT
141 •••AsnArgThrTyrLysLeuProIleLeuGl

4301 CCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCT
130 uGluIleThrThrLysValLeuLysGlyAsnMetGluIleLeuValPheCysAspProAlaTyrAspSerIleLeuGluArgCysMetGlyCysProSer

BstXI (4410)

4401 GACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCAATG
97 ValValArgIleSerArgAspValGluAspSerTyrProHisArgValAlaValIleThrAspPheAspLysGlnGlyAsnSerValAlaSerGlyIleA

StuI (4540)
Eco147I (4540)

4501 GCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCCTGATGGCCGCCCCGACAT
63 laIleAlaGluAlaCysValThrValArgGlyIleTyrAlaGluIleHisValAlaSerIleIleGluGlyThrLysThrArgIleAlaAlaGlyValHi

BspHI (4688)
XmnI (4679)

4601 GGTGCTTGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCT
30 sHisLysAsnAspGluTyrLeuMetThrIleLysGluThrAlaValGluValLeuGluLeuAspGlnGlnSerIleAsnPheThrLysMet

AseI (4747)

4701 CCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAA

4801 ACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTT

SpeI (4901)

4901 ACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACC

SnaBI (5031)
Eco105I (5031)

5001 GCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTT

NdeI (5135)

5101 ACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAA

5201 TGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAA

PacI (5324)
PstI (5317)
SdaI (5317)

5301 GTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGG

5401 CTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCT

5501 CCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAG

ApaLI (5644)

5601 GTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTT

5701 GAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGA

5801 AGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATC

5901 CGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCT

EagI (6080)
PacI (6064) SwaI (6072) NotI (6080)

6001 ACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTT

6101 ATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCT
6201 GTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA