

pBROAD3 kit

An optimized vector for mouse and rat transgenesis

Catalog code: kbroad3

For research use only

Version 20L07-MM

PRODUCT INFORMATION

Content:

- 20 μ g of pBROAD3-mcs provided as lyophilized DNA
- 20 μ g of pBROAD3-LacZnls provided as lyophilized DNA

Storage and Stability:

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

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA were purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

The pBROAD3 plasmid was designed for expression of a transgene in virtually all tissues of transgenic mice and rats. This feature is brought by the ROSA promoter. The murine ROSA26 promoter was initially identified by random retroviral gene trapping in mouse embryonic stem cells¹. This high CpG content promoter was shown to drive ubiquitous expression of the human placental alkaline phosphatase and enhanced green fluorescent protein during embryonic and postnatal development in mouse and rat².

A multiple cloning site (MCS) has been added downstream of the ROSA promoter for convenient cloning of your gene of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the *E. coli* region is flanked on either side by the well cutting 8 bp-recognizing restriction enzyme *Pac* I that enables linearization and easy excision of the *E. coli* region.

PLASMID FEATURES

- **mROSA prom:** This TATA-less promoter was found to be very effective *in vitro* in a very broad range of mammalian cell lines. The strength of the murine ROSA promoter is ascribed to the 10 potential Sp1 sites found within the CpG island extending from the core promoter to the first half of 5' untranslated region (5'UTR), the highest number of Sp1 sites ever recorded in any natural promoter. The 5'UTR contains an engineered intron of 350 bp which increases the transcription of the transgene³.

- **MCS (pBROAD3-mcs):** The multiple cloning site contains the following restriction sites:

Age I, *Bsp* LU111, *Bgl* II, *Hind* III, *Eco* RI, and *Msc* I

Age I is compatible with *Bsp* EI and *Sgr* AI.

Bsp LU111 is compatible with *Bsp* HI and *Nco* I.

Bgl II is compatible with *Bam* HI, *Bst* YI and *Bcl* I.

Eco RI is compatible with *Apo* I and *Mfe* I

- **LacZ- Δ CpG NLS (pBROAD3-LacZnls):** 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. A nuclear localization signal of SV40 large T has been inserted in the 5' end of the *lacZ* gene to allow the targeting of the chimeric protein to the nucleus. To reduce the immunogenicity of this bacterial gene, InvivoGen has engineered a synthetic *lacZnls* gene that is entirely free of CpG motifs, whereas the wild type *lacZ* gene contains 298 CpG dinucleotides.

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

- **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size but with the same activity as the longer Ori.

- **Amp:** The ampicillin resistance gene allows the selection of transformed *E. coli* carrying a pBROAD plasmid.

EXPERIMENTAL OUTLINE

Clone your transgene into pBROAD mcs



Select and isolate recombinant pBROAD



Linearize recombinant pBROAD with *Pac* I



Purify *Pac* I/*Pac* I fragment containing your transgene



Prepare DNA for microinjection



Generate transgenic lines

TECHNICAL SUPPORT

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

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

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3-622-34-80

E-mail: info@invivogen.com

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at $1\mu\text{g}/\mu\text{l}$, resuspend the DNA in $20\mu\text{l}$ of sterile H_2O . Store resuspended plasmid at -20°C .

Pac I linearization of recombinant pBROAD3:

1- Digest $10\mu\text{g}$ recombinant pBROAD3 plasmid with 1 to 5 units of Pac I restriction enzyme.

Note: Pac I may be purchased from New England Biolabs and used at 0.1-0.5 unit per μg plasmid DNA.

2- Incubate at 37°C for 1-2 hours.

3- Purify the fragment containing the ROSA26 prom-transgene- βGlo pAn cassette by agarose gel following your usual protocol.

Plasmid amplification and cloning

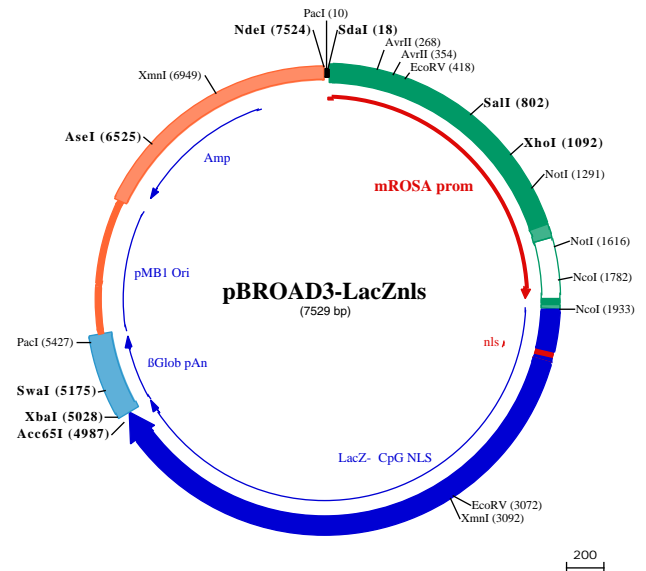
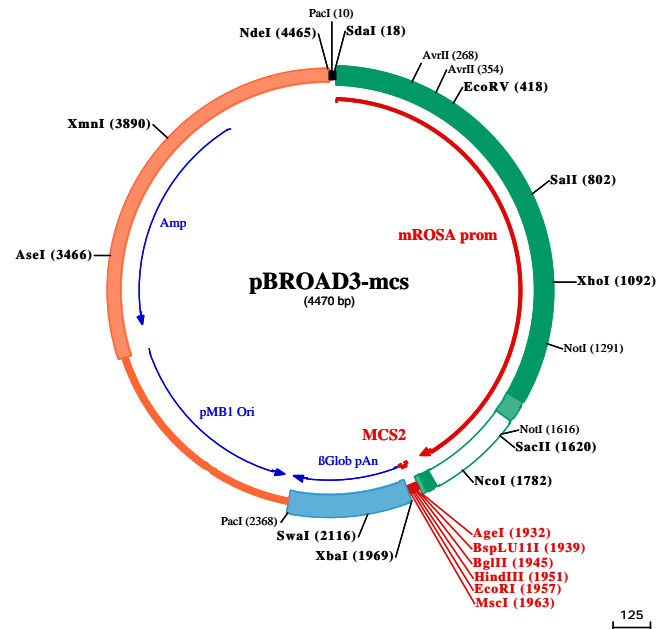
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5 α .

Ampicillin usage

Ampicillin (not provided) can be used for *E. coli* at $50\text{-}100\mu\text{g}/\text{ml}$ in liquid or solid media.

References:

1. Zambrowicz BP, et al. 1997. Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. *Proc Natl Acad Sci USA*. 94:3789-94.
2. Kisseberth WC, et al. 1999. Ubiquitous expression of marker transgenes in mice and rats. *Dev Biol*. 214:128-38.
3. Brinster RL, et al. 1988. Introns increase transcriptional efficiency in transgenic mice. *Proc Natl Acad Sci USA* 85(3):836-40
4. Yu J, Russell JE. 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.



TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

pBROAD3-mcs Sequence

PacI (10) SdaI (18)
1 GATCTCGACTTAATTAACCTGCAGGTGAAGACGTTACACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATCCACGCACCCCTGACCCAGGCCAGCAGG
101 CCGGGCTGCAGCATCAGTACACAGGAGAAAAGATCCTTATTCTTAAGAATGAGAAAAGCAAAGGCGCCGATAGAATAAATTAGCATAGAAGGGCTTTCC
201 CAGGAGTTAAAACCTTCTCTGAGCGATTACCTACTAAAACCAGGGCTTTTGGCCACTACCATTACCTAGGATCTTGGCTTGACGGATTTCATAGGGG
AvrII (268)
301 CATATCCCTCCCCCTCTTCTTTAGAGTCGTTCTTAAAAGATCGCTCTCCACGCCCTAGGCAGGGAAAACGACAAAATCTGGCTCAATTCAGGCTAGAAC
AvrII (354)
401 CCTACAAAATCAACAGGGATATCGCAAGGATACTGGGGCATAACCCACAGGAGTCCAAGAATGTGAGGTGGGGTGGCGAAGGTAATGTCTTTGGTGTG
EcoRV (418)
501 GGAAAAGCAGCAGCCATCTGAGATAGGAATCGAAAACAGAGGAGAGGCGTTTCAGGAAGATTATGGAGGGGAGGACTGGGCCCCACGAGCGACCAGAG
601 TTGTACAAGGCCGCAAGAACAGGGGAGGTGGGGGGCTCAGGGACAGAAAAAAGTATGTGTATTTTGGAGCAGGGTTGGGAGGCCTCTCTGAAAAA
701 GGTATAAACCTGGAGTAGGCAATACCAGGCAAAAAGGGGAGACCAGAGTAGGGGAGGGGAAGAGTCTTGACCCAGGGAAGACATAAAAAGGTAGTGG
801 SalI (802)
GGTCGACTAGATGAAGGAGAGCCTTTCTCTCTGGGCAAGAGCGGTGCAATGGTGTGTAAGGTAGCTGAGAAGCAGAAAAGGGCAAGCATCTTCTCTGCTA
901 CCAGGCTGGGGAGGCCAGGCCACGACCCGAGGAGAGGGAACGCAGGGAGACTGAGGTGACCCCTTCTTTCCCGGGGGCCGGTCTGTGGTTCCGGTG
1001 XhoI (1092)
TCTCTTTTCTGTTGGACCTTACCTTGACCCAGGCGCTGCCGGGGCCTGGGCCCGGGTGCAGCGCACGGCACTCCCGGGAGGCAGCGAGACTCGAGTTA
1101 GGGCCAAACGCGCGCCACGGCGTTTCTGCGCGGAATGGCCCTACCCTGAGGTGGGGTGGGGGCAAGAAAGCGGAGCGAGCCCGAGCGGGGAG
1201 NotI (1291)
GGGAGGGCCAGGGCGGAGGGGGCCGCACTACTGTGTTGGCGACTGGCGGACTAGGGTGCCTGAGTCTCTGAGCGCAGGGCGGGCGCGCCGCC
1301 CTCCCGCGCGCGGCGAGCGGGCAGCGCGGCGAGCTCACTCAGCCCGCTGCCGAGCGGAAACGCCACTGACCGCACGGGATTCCAGTGCCGGCGC
1401 CAGGGGACGCGGGACACGCCCTCCCGCGCGCCATTGGCTCTCCGCCACCGCCACACTTATTGGCGGTGCGCGCCAATCAGCGGAGGCTGC
1501 CGGGGCCGCTAAAGAAGAGGCTGTGCTTTGGGGCTCCGGCTCTCAGAGAGCCTCGGCTAGgtaggggatcgggactctggcgggagggcggttggtg
1601 SacII (1620)
cgtttggggggatggcgccgagcagcctccgagcgtggtggagcgttctgtgagacagcgggtacgagtcgtgacgctggaaggggcaagcgg
NotI (1616)
1701 gtggtgggaggaatgcggtccgacctgcagcaaccggagggggaggagaagggagcggaaaagtctccaccggagcggccatggctggggggggggg
NeoI (1782)
1801 gggcagcggaggagcgttccggccgagctctcgtcgtgattggcttctttctcccgctgtgtgaaaacacaattgtactaacctctctctctt
HindIII (1951)
1901 cctctcctgacagGTGTGAAACAGGAAGAGAACC GG T G A C A T G T A G A T C T A A G C T T G A A T T C T G G C C A T C T A G A A G C T C G C T T C T T G C T G C C A A T T T C
BspLU1I (1939) MscI (1963)
AgeI (1932) BglII (1945) EcoRI (1957) XbaI (1969)
2001 TATTAAGGTTCCCTTTGTTCCCTAAGTCCAATCTAACTGAGGATATTATGAAGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTT
2101 SwaI (2116)
TCATTGCAATGATGATTTAAATTTTCTGAATATTTTACTAAAAAGGGAATGTGGAGGTCAGTGCATTTAAACATAAAGAAATGAAGAGCTAGTTC
2201 AAACCTTGGGAAAATACATATATCTTAACTCCATGAAAGAAGGTGAGGCTGCAAAACAGCTAATGCACATTGGCAACAGCCCTGATGCCTATGCCTTA
2301 PacI (2368)
TTCATCCCTCAGAAAAGGATTCAAGTAGAGGCTTGATTGGAGGTTAAAGTTTGGCTATGCTGATTTTAAATAAAAACCGCTTCGGCGGTTTTTTTA
2401 TGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAA
2501 ATCGACGCTCAAGTCAGAGGTGCGAAACCCGACAGGACTATAAGATACCAGCGTTTCCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCCCT
2601 GCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGATGGTATCTCAGTTTCGGTGTAGGCTGTTCCG
2701 TCCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGGTCCAAACCCGGTAAACAGCACTTAT
2801 CGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG
2901 AAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACCACCGCTGGTAGCGGT
3001 GGTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAA
3101 ACTCACGTTAAGGGATTTTGGTATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATA
3201 TGAGTAAACTTGGTCTGACAGTTACCAATGCTTAACTAGTGGGACCTATCTCAGGATCTGTCTATTTCGTTTCATCCATAGTTGCTGACTCCCGCT
287 TrpHisLysIeLeuSerAlaGlyIeGluAlaIeGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrT
3301 GTGTAGATAACTACGATACGGGAGGCTTACCATCTGGCCCGAGTGTGCAATGATACCGGAGACCCAGCTCACGGCTCCAGATTTACGCAATAA
260 hrTyrIleValValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerGlyArgGluGlyAlaGlySerLysAspAlaIlePh

AseI (3466)

3401 ACCAGCCAGCCGGAAGGGCCGAGCCGAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTC
227 eTrpGlyAlaProLeuAlaSerArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspI leLeuGlnGlnArgSerAlaLeuThrLeuLeuGlu
3501 GCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTGGTTTGGTATGGCTTCATTCAGCTCCGGTCCCAACGA
194 GlyThrLeuLeuLysArgLeuThrAlaMetAlaValProMetThrThrAspArgGluAspAsnProI leAlaGluAsnLeuGluProGluTrpArgA
3601 TCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCAC
160 spLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluLysProGlyGlyI leThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSe
3701 TCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTGCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATA
127 rMetThrI leAlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValProSerTyrGluValLeuAspAsnGlnSerTyr

XmnI (3890)

3801 GTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAAGCTTTAAAAGTGCTCATCATTGGAACGTTCT
94 HisI leArgArgGlyLeuGlnGluGlnGlyAlaAspI leArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGluG
3901 TCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAAACCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTTCCCA
60 luProArgPheSerGluLeuI leLysGlySerAsnLeuAspLeuGluI leTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLe
4001 GCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATA
27 uThrGluProHisAlaPheValProLeuCysPheAlaAlaPhePheProI leLeuAlaValArgPheHisGlnI leSerMet
4101 TTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGATTTAGAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGGA
4201 AAAGTGCACCTGACGCTAAGAAACATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGCTCGCGCGTTTCGGTGATG
4301 ACGGTGAAAACCTCTGACACATGCAGTCCCAGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGG

NdeI (4465)

4401 TGTGGCGGGTGTGGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGACTGAGAGTGCACCATATG

pBROAD3-LacZlns Sequence

PacI (10) SdaI (18)
1 GATCTCGACTTAATTAAcctgcaGGTGAAGACGTTACACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATCCACGCACCCCTGACCCAGGCCAGCAGG

101 GCGGGCTGCAGCATCAGTACACAGGAGAAAGATCCTTATTCTCTAAGAATGAGAAAGGCAAAGGCGCCGATAGAATAAATTAGCATAGAAGGGGCTTTCC

201 CAGGAGTTAAAACTTTCTTCTGAGCGATTACCTACTAAAACCAGGGCTTTTGGCCACTACCATTTACCTAGGATCTTGGCTTGACAGGATTCATAGGGG

301 CATATCCCTCCCTCTTCTTTAGAGTCTTCTTAAAAGATCGCTCTCCACGGCTAGGCAGGGAAAACGACAAAATCTGGCTCAATTCAGGCTAGAAC

401 CCTACAAATTCAACAGGGATATCGCAAGGATACTGGGCATACGCCACAGGAGTCCAAGAATGTGAGGTGGGGTGGCGAAGGTAATGTCTTTGGTGTG

501 GGAAGCAGCAGCCATCTGAGATAGGAAGTGAAGAACAGAGGAGGCGTTCAGGAAGATTATGGAGGGGAGGACTGGGCCCCACAGCGACCAGAG

601 TTGTACAAGGCCCAAGAACAGGGGAGGTGGGGGCTCAGGGACAGAAAAAAGTATGTGTATTTGAGAGCAGGTTGGAGGCCTCTCTGAAAAG

701 GGTATAAACGTGGAGTAGGCAATACCCAGGCAAAAAGGGGAGACCAGAGTAGGGGAGGGGAAGAGTCTGACCAGGGAAGACATTA AAAAGGTAGTGG

801 GGTCCGACTAGATGAAGGAGAGCCTTTCTCTCTGGGCAAGCGGCTGCAATGGTGTGTAAGGTAGCTGAGAAGCAGAAAAGGGCAAGCATCTTCTCGCTA

901 CCAGGCTGGGAGGCCAGGCCACGACCCCGAGGAGAGGGAACGCAGGAGACTGAGGTGACCTTCTTTCCCGGGGCCCCGCTCGTGTGGTTCCGGT

1001 TCTCTTTTCTGTTGGACCCTTACCTTGACCCAGGCGCTGCCGGGCTGGGCCGGGCTGCGGCGCACGGCACTCCCGGAGGCAGCGAGACTCGAGTTA

1101 GGCCAAACGGCGCCACGGCTTTCTGCGCGGAATGGCCGTACCCGTGAGGTGGGGTGGGGGCGAGAAAAGGGGAGCGAGCCCGAGCGGGGGAG

1201 GGGGAGGGCCAGGGGCGGAGGGGCGGCACTACTGTGTTGGCGGACTGGGGGACTAGGGCTGCGTGAGTCTCTGAGCGCAGCGGGGCGGCGCCGCC

1301 CTCCCCGGCGCGCAGCGCGGCGGCGGCACTACTCAGCCCGCTGCCCGAGCGGAAACGCCACTGACCGCACGGGGATTCCAGTGCCTGGCGG

1401 CAGGGGACCGGGGACACGCCCTCCCGCGGCGCATTTGGCTCTCGCCACCGCCCCACACTTATGGCCGGTGGCCGCCAATCAGCGGAGGCTGC

1501 CGGGGCCGCTAAAGAAGAGGCTGTGCTTTGGGGTCCGGCTCCTCAGAGAGCTCGGCTAGgtaggggatcggggactctggcgggagggcggttgggtg

1601 cgtttcgggggatgggcgccgaggcagccctccgagcgtggtggagccgttctgtgagacagccgggtacgagtcgtgacgctggaaggggcaagcggg

1701 gtggtgggcaggaatcgggtccgacctgcagcaaccggagggggagggagaggggagcggaaaagtctccaccgggacgggcatggctcggggggggggg

1801 gggcagcgggagcgttccggccgagctcctgctgctgattggctctcttctcctcccgcctgtgtgaaacacaattgtactaaccttcttctctt

1901 cctctcctgacagGTGTGAACAGGAAGAGAACCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTG

2001 GCTGCCACCCCTCCCTTTGCCTCTGGAGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGTGGAGGTTTG

2101 CCTGGTTCCTGCCCTGAAGCTGTGCTGAGTCTGGCTGGAGTGTGACCTCCAGAGCGAGTCCCAAGAAGAAGAGAAAGTTGAGGCTGACACTGT

2201 TGTGGTGC AAGCAACTGGCAGATGCATGGCTATGATGCCCATCTACACCAATGTCACCTACCCATCACTGTGAACCCCTTTTGTGCCACTGAG

2301 AACCCTACTGGCTGCTACAGCCTGACCTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGCACAGGATCATCTTTGATGGAGTCAACTTGCCTCC

2401 ACCTCTGGTCAATGGCAGTGGGTTGGCTATGGCCAAGCAGCAGGCTGCCCTCTGAGTTTACCTCTGCCTTCTCAGAGCTGGAGAGAACAGGCT

2501 GGCTGTATGGTCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGCATGTGGAGGATGTCTGGCATCTTACAGGATGTGAGCCTGCTGCAAG

2601 CCCACCACAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCA

2701 GAGACTACCTGAGAGTCAGTGGCCTCTGGCAAGGTGAGACCCAGGTGGCTGCTGACAGAGTGGCAGAGGATGAGGATGAGAGAGGAGG

2801 CTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCAGCTGCTGCTGAGATCCCAACCTCTACAGGGCTGTTGTGGAGCTGACACT

2901 CCGTATGGCACCCCTGATGAAGCTGAAGCTGAGTGTGATGTTGAGTACAGAGAAGTCAGGATGAGAATGGCCTGCTGCTCAATGGCAAGCCTCTGCTCA

3001 TCAGGGGAGTCAACAGGCATGAGCACCCCTCTGCATGGACAAGTGATGGATGAACAGACAATGGTGAAGATATCCCTGTAATGAAGCAGAACAACTT

3101 CAATGCTGTGAGTGTCTCACTACCCCAACCACCTCTCTGGTACACCTGTGTGACAGGATGGCCTGTATGTTGATGAAGCCAACTTGAAGACA

3201 CATGGCATGGTCCCATGAACAGGCTCACAGATGACCCAGGCTGGCTGCTGACAGAGTGGCAGGATGACCCAGGATGAGGATGAGAGAGCAGCAACCC

3301 CCTCTGTGATCTCTGCTCTGGCAATGAGTCTGGACATGGAGCAACCATGATGCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGT

3401 GCAGTATGAAGGAGTGGAGCAGACACCAGCCACAGACATCATCTGCCCATGATGCCAGGTTGATGAGGACAGCCCTCCCTGCTGTGCCAAG

3501 TGGAGCATCAAGAAGTGGCTCTCTGCTGGAGAGCCAGACCTCTGATCTGTGTAATGACACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCA

AvrII (268)
AvrII (354)
EcoRV (418)
SalI (802)
XhoI (1092)
NotI (1291)
NotI (1616)
NcoI (1782)
NcoI (1933)
EcoRV (3072) XmnI (3092)

1 MetAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeu
23 AsnProThrPhyCysTrpSerLeuThrPheAsnValAspGluSerTrpLeuGlnGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheH
56 IaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaValProLysLysLysArgLysValGluAlaAspThrVa
89 IValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGlu
156 isLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLe
189 uAlaValMetValLeuArgTrpSerAspGlySerTrpLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgSerValSerLeuHisLys
223 ProThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuA
256 rgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyG
289 yTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThr
323 AlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuLeu

3601 AGTACTGGCAAGCCTTCAGACAGTACCCAGGCTGCAAGGAGGATTGTGTGGGACTGGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACC
556 ys Tyr Trp Gln Ala Phe Arg Gln Tyr Pro Arg Leu Gln Gly Gly Phe Val Trp Asp Trp Val Asp Gln Ser Leu I Le Lys Tyr Asp Glu Asn Gly Asn Pr
3701 CTGGTCTGCCTATGGAGGAGACTTTGGTGACACCCCAATGACAGGCAGTTCTGCATGAATGGCCTGGTCTTTGCAGACAGGACCCCTCACCTGCCCTC
589 o Trp Ser Ala Tyr Gly Gly Asp Phe Gly Asp Thr Pro Asn Asp Arg Gln Phe Cys Met Asn Gly Leu Val Phe Ala Asp Arg Thr Pro His Pro Ala Leu
3801 ACAGAGGCCAAGCACAGCAACAGTTCTCCAGTTCAGGCTGTCTGGACAGACCATTTGAGGTGACATCTGAGTACCTTCAGGCACCTTCGACAATGAGC
623 Thr Glu Ala Lys His Gln Gln Gln Phe Phe Gln Phe Arg Leu Ser Gly Gln Thr I Le Glu Val Thr Ser Glu Tyr Leu Phe Arg His Ser Asp Asn Glu L
3901 TCCTGCAGTGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGAGGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGA
656 eu Leu His Trp Met Val Ala Leu Asp Gly Lys Pro Leu Ala Ser Gly Val Pro Leu Asp Val Ala Pro Gln Gly Lys Glu Leu I Le Glu L
4001 GCTGCCTCAGCCAGAGTCTGCTGGACAACCTGTGGTAAACAGTGAGGGTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGAGGCCACATCTCTGCATGG
689 u Leu Pro Gln Pro Glu Ser Ala Gly Gln Leu Trp Leu Thr Val Arg Val Val Gln Pro Asn Ala Thr Ala Trp Ser Glu Ala Gly His I Le Ser Ala Trp
4101 CAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCCCTGCCTGCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGC
723 Gln Gln Trp Arg Leu Ala Glu Asn Leu Ser Val Thr Leu Pro Ala Ala Ser His Ala I Le Pro His Leu Thr Thr Ser Glu Met Asp Phe Cys I Le Glu L
4201 TGGGCAACAAGAGATGGCAGTTCAACAGGCAGTCTGGCTTCTCTCAGATGGATTGGAGACAAGAAGCAGCTCTCACCCCTCTCAGGGACCAATT
756 eu Gly Asn Lys Arg Trp Gln Phe Asn Arg Gln Ser Gly Phe Leu Ser Gln Met Trp I Le Gly Asp Lys Lys Gln Leu Leu Thr Pro Leu Arg Asp Gln Ph
4301 CACCAGGCTCTCTGGACAATGACATTGGAGTGTCTGAGGCCACAGGATTGACCAAAATGCTTGGTGGAGAGGTGGAAGGCTGTGGACACTACCAG
789 e Thr Arg Ala Pro Leu Asp Asn Asp I Le Gly Val Ser Glu Ala Thr Arg I Le Asp Pro Asn Ala Trp Val Glu Arg Trp Lys Ala Ala Gly His Tyr Gln
4401 GCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACCCCTGGCTGATGCTTTCTGATCACCACAGCCCATGCTTGGCAGCACAAGGCAAGACCCCTGTTC
823 Ala Glu Ala Ala Leu Leu Gln Cys Thr Ala Asp Thr Leu Ala Asp Ala Val Leu I Le Thr Thr Ala His Ala Trp Gln His Gln Gly Lys Thr Leu Phe I
4501 TCAGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCAGATGGATGGATGGAGGTGGCTCTGACACACCTCACCTGCAACACCTCACCTGAGCCT
856 I Le Ser Arg Lys Thr Tyr Arg I Le Asp Gly Ser Gly Gln Met Ala I Le Thr Val Asp Val Glu Val Ala Ser Asp Thr Pro His Pro Ala Arg I Le Gly Le
4601 GAACTGCAACTGGCAGCTGGCTGAGAGGGTGAAGTGGCTGGGCTTGGCCCTCAGGAGAACTACCCTGACAGGCTGACAGCTGCCTGCTTTCAGACG
889 u Asn Cys Gln Leu Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu Gly Pro Gln Glu Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp Arg
4701 TGGACCTGCCCTGTCTGACATGTACACCCCTATGTTTCCCTTCTGAGAATGGCTGAGGTGGCACCAGGAGCTGAACTATGGCTCCACAGT
923 Trp Asp Leu Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe Pro Ser Glu Asn Gly Leu Arg Cys Gly Thr Arg Glu Leu Asn Tyr Gly Pro His Gln T
4801 GGAGGGGAGACTTCCAGTCAACATCTCCAGTACTCTCAGCAACAGCTCATGGAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGAAACCTGGCT
956 rp Arg Gly Asp Phe Gln Phe Asn I Le Ser Arg Tyr Ser Gln Gln Gln Leu Met Glu Thr Ser His Arg His Leu Leu His Ala Glu Glu Gly Thr Trp Le

Acc65I (4987)

4901 GAACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTTTGGTCTCCTTCTGTCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCACATACAG
989 u Asn I Le Asp Gly Phe His Met Gly I Le Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala Glu Phe Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln

XbaI (5028)

5001 CTGGTGTGGTCCAGAAGTAAACCTAATCTAGAAGCTCGCTTCTTCTGTGTCCAATTTCTATTAAGGTTCTTTGTTCCTTAAGTCCAACCTACTAACT
1023 Leu Val Trp Cys Gln Lys . . .

SwaI (5175)

5101 GGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTTATTGCAATGATGATTTAAATTTATTTCTGAATATTTTTAC

5201 TAAAAAGGAATGTGGGAGGTCAGTGCATTTAAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATACACTATATCTTAAACTCCATGAAAG

5301 AAGGTGAGGCTGCAACAGCTAATGCACATTGGCAACAGCCCTGATGCCTATGCCTTATTTCATCCCTCAGAAAAGGATTCAAGTAGAGGCTTGATTTGG

PacI (5427)

5401 AGGTTAAAGTTTTGCTATGCTGTATTTAATTAATAAACCCGCTTCGGCGGGTTTTTTTATGCATGTGAGCAAAAAGCCAGCAAAAAGCCAGGAACCGTAA

5501 AAAGGCCCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTA

5601 TAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTCGCGCTTACCGGATACCTGTCCGCCCTTCTCCCTTCGGGAA

5701 CGGTGGCGCTTCTCATA GCTCACGCTG TAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGCTGTGTGCACGAACCCCGCTTCAGCCCA

5801 CGCTGGCCTTATCCGGTAACATCGTCTTGAGTCCAACCGGTAAGACAGCACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG

5901 AGGTATGTAGCGGTGTACAGAGTTCTGAAGTGGTGGCCTAACCTACCGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

6001 CCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACCCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACCGCGAGAAAAAA

6101 AGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAACGAAAACCTCACGTTAAGGGATTTTGGTTCATGAGATTATCAAAAAGG

6201 ATCTTACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTG

287 Trp His Lys I Leu Leu Ser

6301 AGGCACCTATCTCAGGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCGTCTGTAGATAACTACGATACGGGAGGCTTACCATCTGGCCC

280 r Ala Gly I Le Glu Ala I Le Gln Arg Asn Arg Glu Asp Met Thr Ala Gln Ser Gly Thr Thr Tyr I Le Val Val I Le Arg Ser Pro Lys Gly Asp Pro Gly

6401 CAGTGTGCAATGATACCGGAGACCCAGCTCACCAGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGCGGAGCCGAGAAGTGGCTCGCA

247 Leu Ala Ala I Le I Le Gly Arg Ser Gly Arg Glu Gly Ala Gly Ser Lys Asp Ala I Le Phe Trp Gly Ala Pro Leu Ala Ser Arg Leu Leu Pro Gly Ala V

AseI (6525)

6501 ACTTTATCCGCCCTCCATCCAGTCTATTAATTTGTTGCCGGAAAGTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGGCCAACGTTGTTGCCATTGCTACAG

213 al Lys Asp Ala Glu Met Trp Asp I Le Leu Gln Gln Arg Ser Ala Leu Thr Leu Leu Glu Gly Thr Leu Leu Lys Arg Leu Thr Thr Ala Met Ala Val Pr

6601 GCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTATTGCTCAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGC

180 o Met Thr Thr Asp Arg Glu Asp Asn Pro I Le Ala Glu Asn Leu Glu Pro Glu Trp Arg Asp Leu Arg Thr Val His Asp Gly Met Asn His Leu Phe Ala

6701 GTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTTCTTACTGTCTATG

147 Thr Leu Glu Lys Pro Gly Gly I Le Thr Thr Leu Leu Leu Asn Ala Ala Thr Asn Asp Ser Met Thr I Le Ala Ala Ser Cys Leu Glu Arg Val Thr Met G

6801 CCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCTTCTGAGAATAGTGTATGCGCGGACCGAGTGTCTTGTCCCGCGCTCAATAC

113 ly Asp Thr Leu His Lys Glu Thr Val Pro Ser Tyr Glu Val Leu Asp Asn Gln Ser Tyr His I Le Arg Arg Gly Leu Gln Glu Gln Gly Ala Asp I Le Ar

XmnI (6949)

6901 GGGATAATACCGCCACATAGCAGAACCTTAAAAAGTCTCATCTTTGGAAAACGTTCTTCGGGGGAAAACTCTCAAGGATCTTACCCTGTTGAGATC

80 g Ser Leu Val Ala Gly Cys Leu Leu Val Lys Phe Thr Ser Met Met Pro Phe Arg Glu Glu Pro Arg Phe Ser Glu Leu I Le Lys Gly Ser Asn Leu Asp

7001 CAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTTCAGCATCTTTACTTTCCACAGCGTTCTGGGTGAGCAAAAACGGAAGGCAAAAATGCCGCA

47 Leu Glu I Le Tyr Gly Val Arg Ala Gly Leu Gln Asp Glu Ala Asp Lys Val Lys Val Leu Thr Glu Pro His Ala Phe Val Pro Leu Cys Phe Ala Ala P

7101 AAAAAGGAATAAGGGCGACAGGAAATGTTGAATACTCATACTCTCTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCATGAGCGGAT

13 he Phe Pro I Le Leu Ala Val Arg Phe His Gln I Le Ser Met

7201 ACATATTTGAATGATTTAGAAAAATAAACAATAGGGGTTCCCGGCACATTTCCCGAAAAAGTCCACCTGACGCTTAAGAAACCATTTATTATCATGAC

7301 ATTAACCTATAAAAAATAGCGGTATCACAGGCCCTTTCGCTCTCGCGGTTTCGGTGATGACGGTGAACACCTCTGACACATGACGCTCCCGGAGACGGTC

7401 ACAGCTTGTCTGAAGCGGATGCCGGGAGCAGACAAGCCGTCAGGGCGCTCAGCGGGTGTGGCGGGTCTCGGGGCTGGCTTAACTATGCGGCATCAG

NdeI (7524)

7501 AGCAGATTGACTGAGAGTGCACCATATG