

pFUSE-SEAP-hG1-Fc

Plasmid designed for the expression of a SEAP-Fc Fusion protein

Catalog # pfuse-hg1sp

For research use only

Version 22H30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-SEAP-hG1-Fc 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 stored at -20°C and is stable 3 months.

- Resuspended DNA should be stored at -20°C and is stable up to 1 year.

- 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

pFUSE-Fc is a family of plasmid developed to facilitate the construction of Fc-Fusion proteins by fusing a sequence encoding a given protein to the Fc region of an immunoglobulin.

pFUSE-Fc plasmids yield high levels of Fc-Fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, CHO cells, monkey COS cells and human embryonic kidney (HEK)293 cells. These cells are commonly used in protein purification systems.

pFUSE-SEAP-hIgG1-Fc plasmids allow the production of SEAP-Fc fusion proteins. This plasmid can be used to make recombinant SEAP-Fc fusion proteins or can be used as a transfection control in experiments with other pFUSE-hFc constructs. Quantification of SEAP-Fc expression can be determined using QUANTI-Blue™ Solution (cat. code: rep-qbs).

PLASMID FEATURES

• **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.

• **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophotometer at 405 nm.

• **hIgG1 Fc (human)**: The Fc region of human IgG1 comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently.

Human IgG1 displays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.

• **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.

• **CMV enh / hFerL prom**: This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin[®]-resistance gene in mammalian cells.

• **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.

• **Zeo**: Resistance to Zeocin[®] is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same resistance gene confers 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.

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 or in 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.

TECHNICAL SUPPORT

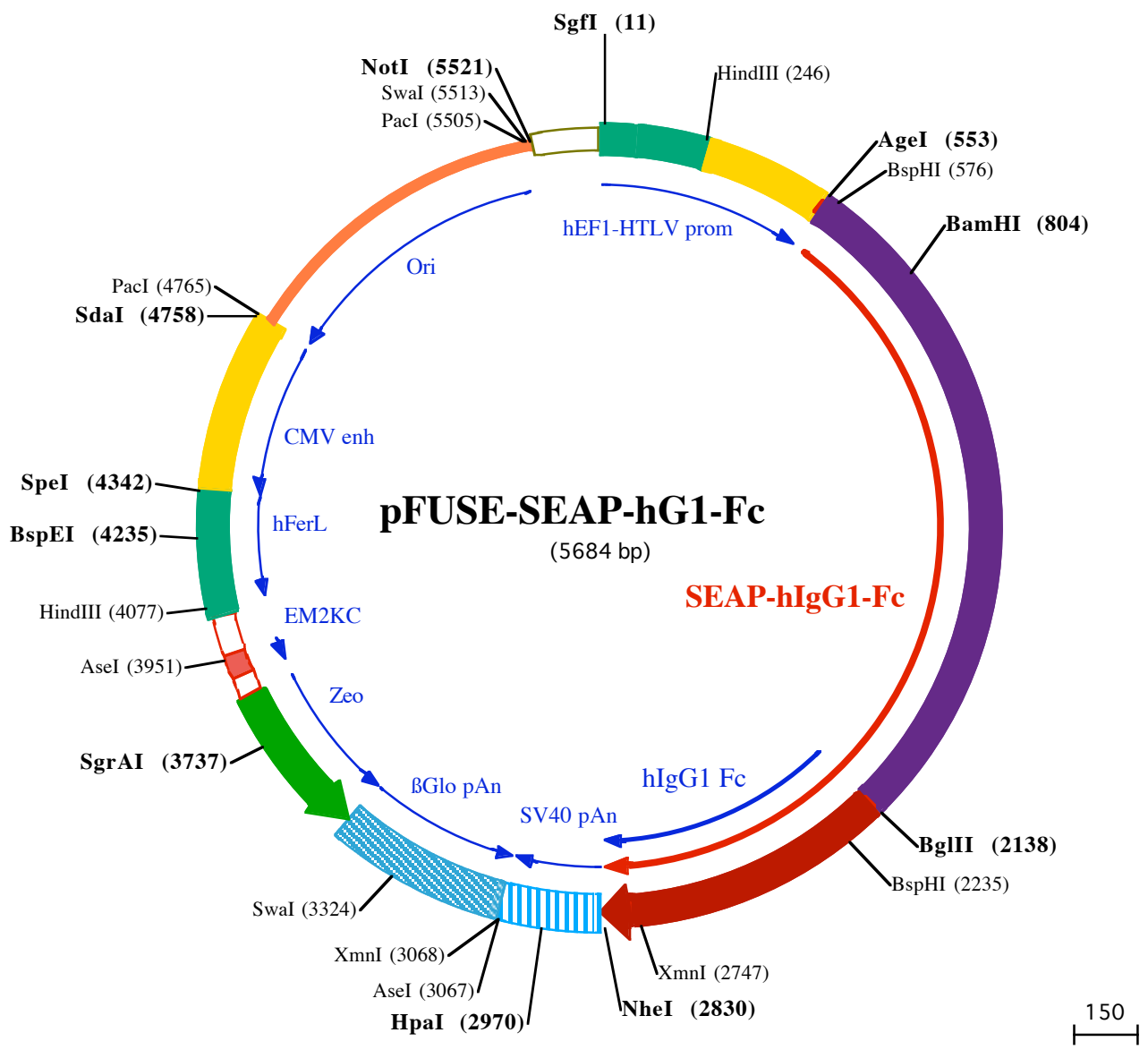
InvivoGen USA (Toll-Free): 888-457-5873

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

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

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com



SgfI (11)
1 GGATCTGCGATCGCTCCGGTCCCGTCCAGTGGGAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGGGGTAAGTGGAAAGTGATGTCGTGACTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGGC

HindIII (246)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGGCCGCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACTCGAGGCC
301 GCCATCCACGCGGTTGAGTCGCGTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTGCCTCCGCTCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGCGCTCCCTTGGAGCCTACCTAGACTCAGCGGGCTCTCCAGCCTTTGCTGACCTGCTTGTCAACTCTACGCTTTGTTTCGTTT

AgeI (553) BspHI (576)
501 TCTGTTCTGGCGCTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTTTCAGCTGAGGAGGCACATCATGATTCTGGGGCCCTGCATGCT
1MetI LeuLeuGI yProCysMeLe
601 GCTGCTGCTGCTGCTGGCCTGAGGCTACAGTCTCCCTGGGCATCATCCAGTGTAGGAGGAGAACCAGGACTTCTGGAACCGCGAGGAGCCGGAG
8P LeuLeuLeuLeuLeuLeuGI yLeuArgLeu nLeuSer LeuGI yI eI eP roVal GI uGI uGI uAsnProAspPheTrpAsnArgGI uAI aAI aGI u
701 GCCCTGGGTCCCGCAAGAGCTGCAGCTGCACAGACGCCCAAGCAACTCATCTTCTGGGCGATGGGATGGGTGTCTACGCTGACAGCTG
42Al aLeuGI yAI aAI aLysLysLeuGI nProAI aGI nThrAI aAI aLysAsnLeuI eI ePheLeuGI yAspGI yMetGI yValI SerThrValI ThrAI aA

BamHI (804)
801 CCAAGTCTTAAAGGGCAGAAGAAGGACAACTGGGGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTGTGCCAAGACATAAATGT
75I aArgI eLeuLysGI yGI nLysLysAspLysLeuGI yProGI uI eProLeuAI aMeTaspArgPheProTyrValAI aLeuSerLysThrTyrAsnVa
901 AGACAAATATGTCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGTCAAGGGCACTTCCAGACCATTTGAGTGCAGCGCCCGCTTT
108I AspLysHisValProAspSerGI yAI aThrAI aThrAI aThrLeuCysGI yValLysGI yAsnPheGI nThrI eGI yLeuSerAI aAI aAI aArgPhe
1001 AACAGTGAACACGACAGCCGCGCAACGAGGTCTCTCGTGTGAATCGGCCAAGAAAGCAGGGAAGTCACTGGGAGTGGTAACCACACACGAGTGC
142AsnGI nCysAsnThr ThrArgGI yAsnGI uValI eSerValMeTasnArgAI aLysLysAI aGI yLysSerValGI yValI ThrThrThrArgValI G
1101 AGCACGCTCCGACCGCCACCTACGCCACAGGTGAACCGCAACTCTCGGACCGCCGACGTGCCTGCCTCGGCCAGGAGGGTGCAGGAG
175I nHisAI aSerProAI aGI yThrTyrAI aHisThrValAsnArgAsnTrpTyrSerAspAI aAspValI ProAI aSerAI aArgGI nGI uGI yCysGI nAs
1201 CATCGCTACCGCAGCTCATCTCCAACATGGACATTGATGTATCCTGGGTGGAGGCCAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCA
208pI eAI aThrGI nLeuI eSerAsnMeTaspI eAspValI eLeuGI yGI yGI yArgLysTyrMeTpheArgMeTGI yThrProAspProGI uTyrPro
1301 GATGACTACAGCAAGTGGGACAGGCTGGACGGGAAGATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGTTGCCGGTATGTGTGAACCGCACTG
242AspAspTyrSerGI nGI yThrArgLeuAspGI yLysAsnLeuValGI nGI uTrpLeuAI aLysArgGI nGI yAI aArgTyrValI TrpAsnArgThrG
1401 AGCTCATGCGAGCTTCCCTGGACCCGCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGA
275I uLeuMeTGI nAI aSerLeuAspProSerValI ThrHisLeuMeTGI yLeuPheGI uProGI yAspMeTysTyrGI uI eHisArgAspSerThrLeuAs
1501 CCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGTGAGCAGGAACCCCGCGCTTCTTCTTCTCGTGGAGGGTGGTGCATCGACACCGT
308pProSerLeuMeTGI uMeTThrGI uAI aAI aLeuArgLeuLeuSerArgAsnProArgAsnGI yPhePheLeuPheValGI uGI yGI yArgI eAspHisSGI y
1601 CATCAAGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTCGACGACCCATTGAGAGGGCGGGCCAGCTCACACGAGGAGGACACGCTGA
342I HisHisSGI uSerArgAI aTyrArgAI aLeuThrGI uThrI eMeTaspAspAI aI eGI uArgAI aGI yGI nLeuThrSerGI uGI uAspThrLeuS
1701 GCCTCGTCACTCGACACTCCACGCTTCTCCTTGGAGGCTACCCCTCGGAGGGAGCTCCATCTTCCGGCTGGCCCTGGCCAGGCGCGGACAG
375erLeuVal ThrAI aAspHisSerHisValI PheSerPheGI yGI yTyrProLeuArgGI ySerSerI ePheGI yLeuAI aProGI yLysAI aArgAspAr
1801 GAAGGCTACACGGTCTCTATACGGAACGGTCCAGGCTATGTGCTCAAGGACCGCGCCCGCGGATGTTACGAGAGCGAGAGCGGGAGCCCGGAG
408yLysAI aTyrThrValLeuLeuTyrGI yAsnGI yProGI yTyrValLeuLysAspGI yAI aArgProAspValThrGI uSerGI uSerGI ySerProGI u
1901 TATCGGACAGCTCAGCAGTGGCCCTGGACGAAGAGACCACGACGAGGAGGACGTGGCGGTGTTGCGCGCGGCCCGAGCCGACCTGGTTACAGCGG
442TyrArgGI nGI nSerAI aValProLeuAspGI uGI uThrHisAI aGI yGI uAspValAI aValPheAI aArgGI yProGI nAI aHisLeuValHisSGI yV
2001 TGCAGGACGACCTTACATGCGCAGCTGATGGCTTCCGCGCTGCCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCCGCCAGCCACCGCAGC
475pI GI nGI uGI nThrPheI eAI aHisValMeTAl aPheAI aAI aCysLeuGI uProTyrThrAI aCysAspLeuAI aProProAI aGI yThrThrAspAI

BglII (2138)
2101 CGCGCACCGGGGCGTCCCGTCCAAAGCTGTGGATGATCTGACAAAACACACATGCCACCGTGGCCAGCACCTGAACTCCTGGGGGAGCCGTCA
508aAI aHisProGI yArgSerLysArgSerLysArgSerLysArgSerLysThrHisSThrCysProProCysProAI aProProAI aGI yThrThrAspAI
1AspLysThrHisSThrCysProProCysProAI aProGI uLeuLeuGI yGI yProSer

BspHI (2235)
2201 GTCTTCTCTTCCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCTGAGGTCACATGCGTGGTGGACGTGAGCCACGAAGACCTGAGG
542ValI PheLeuPheProProLysProLysAspThrLeuMeT I eSerArgThrProGI uValI ThrCysValI ValI ValI AspValI SerHisSGI uAspProGI uV
20ValI PheLeuPheProProLysProLysAspThrLeuMeT I eSerArgThrProGI uValI ThrCysValI ValI ValI AspValI SerHisSGI uAspProGI uV
2301 TCAAGTTCAACTGGTACGTGGACGGCTGGAGGTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTACAACAGCAGCTACCTGTGGTCAAGCTCT
575aI LysPheAsnTrpTyrValI AspGI yValI GI uValI HisAsnAI aLysThrLysProArgGI uGI uGI nTyrAsnSerThrTyrArgValI ValI SerValI Le
53aI LysPheAsnTrpTyrValI AspGI yValI GI uValI HisAsnAI aLysThrLysProArgGI uGI uGI nTyrAsnSerThrTyrArgValI ValI SerValI Le
2401 CACGCTCTGCACCGAGTGGCTGAATGGCAAGGAGTACAAGTCAAGGTTCCCAACAAAGCCCTCCAGCCCATCAGAAACCATCTCCAAAGCC
608uThrValI LeuHisSGI nAspTrpLeuAsnGI yLysGI uTyrLysCysLysValI SerAsnLysAI aLeuProAI aProI eGI uLysThrI eSerLysAI a
86uThrValI LeuHisSGI nAspTrpLeuAsnGI yLysGI uTyrLysCysLysValI SerAsnLysAI aLeuProAI aProI eGI uLysThrI eSerLysAI a
2501 AAAGGGACGCCGAGAACACAGGTGTACACCTGCCCCATCCCGGAGGAGATGACCAAGAACAGGTGACCAAGAACAGGTGACGCTGACCTGCAAGGCTTCT
642LysGI yGI nProArgGI uProGI nValI TyrThrLeuProProSerArgGI uGI uMeTThrLysAsnGI nValI SerLeuThrCysLeuValI LysGI yPheT
120LysGI yGI nProArgGI uProGI nValI TyrThrLeuProProSerArgGI uGI uMeTThrLysAsnGI nValI SerLeuThrCysLeuValI LysGI yPheT
2601 ATCCAGCGACATCGCGTGGAGTGGGAGCAATGGGACGCCGAGAACACTACAAGACCAGCCCTCCCGTGTGGACTCCGACCGGCTCTTCTTCT
675yProProSerAspI eAI aValI GI uTrpGI uSerAsnGI yGI nProGI uAsnAsnTyrLysThrThrProProValI LeuAspSerAspGI ySerPhePheLe
153yProProSerAspI eAI aValI GI uTrpGI uSerAsnGI yGI nProGI uAsnAsnTyrLysThrThrProProValI LeuAspSerAspGI ySerPhePheLe

XmnI (2747)
2701 CTACAGCAAGTCCAGTGGACAAGAGCAGGTGGCAGCAGGGAAAGCTTCTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCCTACACGCAAG
708uTyrSerLysLeuThrValAspLysSerArgTrpGI nGI nGI yAsnValI PheSerCysSerValMeThiSGI uAI aLeuHisAsnHisSThrThrGI nLys
186uTyrSerLysLeuThrValAspLysSerArgTrpGI nGI nGI yAsnValI PheSerCysSerValMeThiSGI uAI aLeuHisAsnHisSThrThrGI nLys

NheI (2830)
2801 AGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACCAACAATGAGTCAAGAAAA
742SerLeuSerLeuSerProGI yLys
742SerLeuSerLeuSerProGI yLys
220SerLeuSerLeuSerProGI yLys

HpaI (2970)
2901 ATGCTTTATTTGTAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTATTTATGTTT

AseI (3067)
XmnI (3068)
3001 CAGGTTCAAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAAACTTTAAAC
3101 CTCCAAATCAAGCTCTACTTGAATCCTTTCTGAGGGATGAATAAGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCAC

3201 TTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTTGAAGTACTCTTCATTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTT

Swal (3324)

3301 TAGTAAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCC

3401 CCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCTGCC

125•••AspGlnGluAlaVal

3501 ACAAGTGCACGCAAGTTGCCGGCCGGTCCGCGAGGCGAAGTCCCGCCCCACGGCTGCTCGCGATCTCGGTCATGGCCGGCCGGAGGCGTCCGGGA

118AlaPheHisValCysAsnGluAlaProAspArgLeuAlaPheGluArgGlyTrpProGlnGluGlyLeuGluThrMetAlaProGlySerAlaAspArgPh

3601 AGTTCGTGGACACGACTCCGACACTCGGCTACAGCTCTCAGGCCGCGCACCACCCAGGCCAGGTTGTGTCGGCCACCTGGTCTGGAC

85LeuAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGluAspGlnVal

SgrAI (3737)

3701 CGCGTGTGAACAGGTCACGTCGTCGCGGACCACCGCGAAGTCTCCTCCACGAAGTCCCGGAGAACCAGCCGCGTCCGAGAACTCGACC

52AlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAla

3801 GCTCCGGCAGCTCGCGCGCGTGGAGCAGCGAAGCGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggttagtac

18AlaGlyAlaValAspArgAlaThrLeuValIleProValAlaSerThrLeuLysAlaMet

AseI (3951)

3901 aattgCTATAGTGTATTATATACTATGCAGATATACTATGCCAATGATTAATTGTCAAAGTGGCTGCAGgggttcatagtgcacttttctgcac

HindIII (4077)

4001 tgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTCACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGAC

4101 CGCGAACTGCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCAGCGCCCTCGGAGGCAGGGCGCTCGGGGAGGCTAGCGGCCAATCTCGGGTG

BspEI (4235)

4201 GCAGGAGGCGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCC

SpeI (4342)

4301 AGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAACAACAACTCCATTGACGTCATGGGGTGGAGACTTGGAAATCCCCGTGA

4401 GTCAAACCGCTATCCAGCCCATGATGTACTGCCAAAACCGCATCATGGAATAGCGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCC

4501 CATAAGTTCATGTAAGTGGGATAATGCCAGGCGGGCCATTTACCGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCCA

4601 AGTGGGCAAGTTTACCGTAAATACTCCACCATTGACGTCATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCATGGGGC

PacI (4765)

SdaI (4758)

4701 GGGGTCGTTGGGCGGTACGACAGGCGGGCCATTTACCGTAAGTTATGTAACGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCA

4801 GGAACCGTAAAAAGCCGCGTTGCTGCGCTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC

4901 GACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTGTCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTC

5001 CCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCAGAACCCCGC

5101 TTCAGCCCAGCGCTGCCCTTATCCGGTAACTATCGCTTGAGTCCAACCCGGTAAGACAGCACTTATCGCCACTGGCAGCAGCACTGGTAACAGGAT

5201 TAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG

5301 AAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAACCACCCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGC

5401 GCAGAAAAAAGGATCTCAAGAAGATCTTTGATCTTTCTACGGGCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAG

PacI (5505) Swal (5513) NotI (5521)

5501 TTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGTTTTTTGTGTAATCGTAACATAACGCTCTCC

5601 ATCAAACAACAAAGCAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA