pFUSE-SEAP-mG1-Fc

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

Catalog code: pfuse-mg1sp

https://www.invivogen.com/pfuse-seap-mg1-fc

For research use only

Version 22H30-MM

PRODUCT INFORMATION

Contents

• 20 µg of **pFUSE-SEAP-mG1-Fc** plasmid provided as lyophilized DNA

• 1 ml of Zeocin® (100 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 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.
- Expression of SEAP-mG3Fc was confirmed by using QUANTI-Blue™ Solution.
- SEAP-mG3Fc protein was purified using protein G affinity chromatography following manufacturer's protocol.

GENERAL PRODUCT USE

pFUSE-SEAP-Fc plasmids express a SEAP-Fc fusion protein generated by fusing the gene encoding for human secreted alkaline phosphatase (SEAP) and the Fc region of an immunoglobulin G (IgG).

pFUSE-SEAP-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, Chinese hamster ovary (CHO) cells, monkey COS cells and human embryonic kidney (HEK)293 cells. These cells are commonly used in protein purification systems.

SEAP-Fc fusion proteins are secreted and can be easily detected in the supernatant of pFUSE-SEAP-Fc-transfected cells by using QUANTI-Blue™ Solution, a SEAP detection medium.

<u>WARNING:</u> mIgG1-Fc has a very low affinity to Protein A and Protein G making it difficult to purify.

PLASMID FEATURES

• **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.

• **SEAP-mIgG1-Fc** was generated by fusing the gene encoding for human SEAP with the Fc region of mouse IgG3. This region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the SEAP and Fc moieties, allowing each part of the molecule to function independently.

• **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.

• **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[4].

1. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
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. 8(1):466-72.
3. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58.
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.

InvivoGen
www.invivogen.com

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 H_2O. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 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.

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-12
QUANTI-Blue™ Solution	rep-qbs

InvivoGen
www.invivogen.com

SgfI (11)
NotI (5514)
SwaI (5506)
PacI (5498)
HindIII (246)
AgeI (553)
BspHI (576)
BamHI (804)
hEF1-HTLV prom
PacI (4758)
SdaI (4751)
Ori
SnaBI (4465)
CMV enh
pFUSE-SEAP-mG1-Fc
(5677 bp)
SEAP-mIgG1-Fc
SpeI (4335)
hFerL
BspEI (4228)
StuI (4172)
Eco147I (4172)
HindIII (4070)
EM2KC
AseI (3944)
Zeo
Eco147I (1805)
StuI (1805)
SgrAI (3730)
ßGlo pAn
mIgG1-Fc
SV40 pAn
BglII (2138)
BsrGI (2162)
BbrPI (2247)
SwaI (3317)
BsrGI (2511)
XmnI (3061)
AseI (3060)
NheI (2823)
HpaI (2963)

150
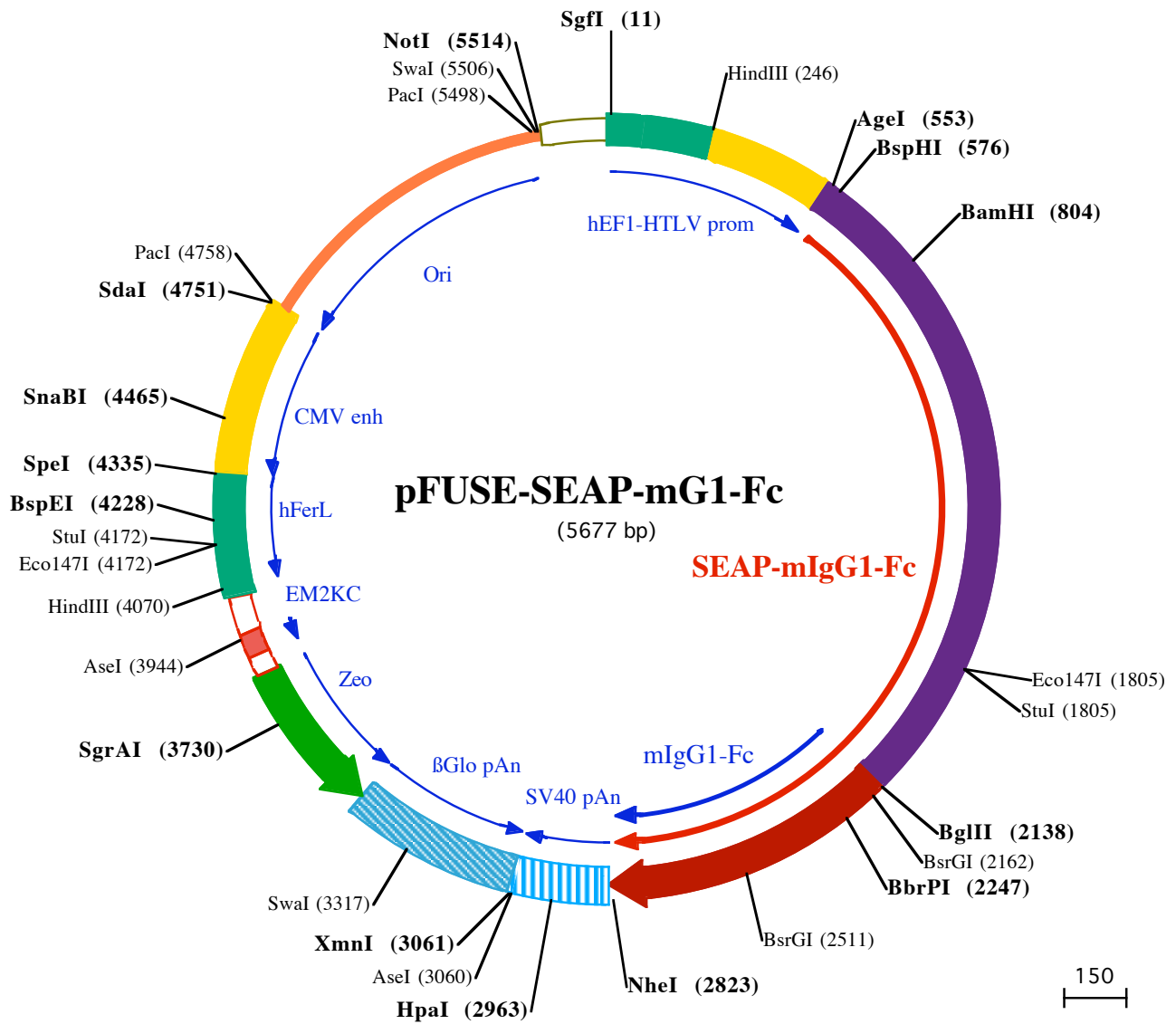
SgfI (11)
```
  1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
```

HindIII (246)
```
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
```

AgeI (553)   BspHI (576)
```
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTTCAGCTGAGGAGGCACATCATGATTCTGGGGCCCTGCATGCT
                                                                                    1▶MetIleLeuGlyProCysMetLe

601 GCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAG
  8▶uLeuLeuLeuLeuLeuGlyLeuArgLeuGlnLeuSerLeuGlyIleIleProValGluGluGluAsnProAspPheTrpAsnArgGluAlaAlaGlu

701 GCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTG
 42▶AlaLeuGlyAlaAlaLysLysLeuGlnProAlaGlnThrAlaAlaLysAsnLeuIleIlePheLeuGlyAspGlyMetGlyValSerThrValThrAlaA
```

BamHI (804)
```
801 CCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGT
 75▶laArgIleLeuLysLysGlyGlnLysLysAspLysLeuGlyProGluIleProLeuAlaMetAspArgPheProTyrValAlaLeuSerLysThrTyrAsnVa

901 AGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTT
108▶lAspLysHisValProAspSerGlyAlaThrAlaThrAlaTyrLeuCysGlyValLysGlyAsnPheGlnThrIleGlyLeuSerAlaAlaAlaArgPhe

1001 AACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGC
142▶AsnGlnCysAsnThrThrArgGlyAsnGluValIleSerValMetAsnArgAlaLysLysAlaGlyLysSerValGlyValValThrThrThrArgValG

1101 AGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGA
175▶lnHisAlaSerProAlaGlyThrTyrAlaHisThrValAsnArgAsnTrpTyrSerAspAlaAspValProAlaSerAlaArgGlnGluGlyCysGlnAs

1201 CATCGCTACGCAGCTCATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCA
208▶pIleAlaThrGlnLeuIleSerAsnMetAspIleAspValIleLeuGlyGlyGlyArgLysTyrMetPheArgMetGlyThrProAspProGluTyrPro

1301 GATGACTACAGCCAAGGTGGGACAGGGAAGAATCTGGCTGGCAGGGATGGCTGGCGAGGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTG
242▶AspAspTyrSerGlnGlyGlyThrArgLeuAspGlyLysAsnLeuValGlnGluTrpLeuAlaLysArgGlnGlyAlaArgTyrValTrpAsnArgThrG

1401 AGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGA
275▶luLeuMetGlnAlaSerLeuAspProSerValThrHisLeuMetGlyLeuPheGluProGlyAspMetLysTyrGluIleHisArgAspSerThrLeuAs

1501 CCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTTCGTGGAGGGTGGTCGCATCGACCACGGT
308▶pProSerLeuMetGluMetThrGluAlaAlaLeuArgLeuLeuSerArgAsnProArgGlyPhePheLeuPheValGluGlyGlyArgIleAspHisGly

1601 CATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGA
342▶HisHisGlySerArgAlaThrTyrArgAlaLeuThrGluThrIleMetPheAspAspAlaIleGluArgAlaGlyGlnLeuThrSerGluGluAspThrLeuS

1701 GCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAG
375▶erLeuValThrAlaAspHisSerHisValPheSerPheGlyGlyTyrProLeuArgGlySerSerIlePheGlyLeuAlaProGlyLysAlaArgAspAr
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StuI (1805)
Eco147I (1805)
```
1801 GAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAG
408▶gLysAlaTyrThrValLeuLeuTyrGlyAsnGlyProGlyTyrValLeuLysAspGlyAlaArgProAspValThrGluSerGluSerGlySerProGlu

1901 TATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCG
442▶TyrArgGlnGlnSerAlaValProLeuAspGluGluThrHisAlaGlyGluAspValAlaValPheAlaArgGlyProGlnAlaHisLeuValHisGlyV

2001 TGCAGGAGCAGACCTTCATAGCGCACGTCATGGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGCACCACCGACGC
475▶alGlnGluGlnThrPheIleAlaHisValMetAlaPheAlaAlaCysLeuGluProTyrThrAlaCysAspLeuAlaProProAlaGlyThrThrAspAl
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BglII (2138)   BsrGI (2162)
```
2101 CGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATAGATCTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCC
508▶aAlaHisProGlyArgSerArgSerLysArgLeuAspArgSerGlyCysLysProCysIleCysThrValProGluValSerSerValPheIlePhePro
  1▶GlyCysLysProCysIleCysThrValProGluValSerSerValPheIlePhePro
```

BbrPI (2247)
```
2201 CCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGT
542▶ProLysProLysAspValLeuThrIleThrLeuThrProLysValThrCysValValValAspIleSerLysAspAspProGluValGlnPheSerTrpP
 20▶ProLysProLysAspValLeuThrIleThrLeuThrProLysValThrCysValValValAspIleSerLysAspAspProGluValGlnPheSerTrpP

2301 TTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCA
575▶heValAspAspValGluValHisThrAlaGlnThrGlnProArgGluGluGlnPheAsnSerThrPheArgSerValSerGluLeuProIleMetHisGl
 53▶heValAspAspValGluValHisThrAlaGlnThrGlnProArgGluGluGlnPheAsnSerThrPheArgSerValSerGluLeuProIleMetHisGl

2401 GGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAG
608▶nAspTrpLeuAsnGlyLysGluPheLysCysArgValAsnSerAlaAlaPheProAlaProIleGluLysThrIleSerLysThrLysGlyArgProLys
 86▶nAspTrpLeuAsnGlyLysGluPheLysCysArgValAsnSerAlaAlaPheProAlaProIleGluLysThrIleSerLysThrLysGlyArgProLys
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BsrGI (2511)
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2501 GCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTA
642▶AlaProGlnValTyrThrIleProProProLysGluGlnMetAlaLysAspLysValSerLeuThrCysMetIleThrAspPhePheProGluAspIleT
120▶AlaProGlnValTyrThrIleProProProLysGluGlnMetAlaLysAspLysValSerLeuThrCysMetIleThrAspPhePheProGluAspIleT

2601 CTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAA
675▶hrValGluTrpGlnTrpAsnGlyGlnProAlaGluAsnTyrLysAsnThrGlnProIleMetAspThrAspGlySerTyrPheValTyrSerLysLeuAs
153▶hrValGluTrpGlnTrpAsnGlyGlnProAlaGluAsnTyrLysAsnThrGlnProIleMetAspThrAspGlySerTyrPheValTyrSerLysLeuAs

2701 TGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCACTCT
708▶nValGlnLysSerAsnTrpGluAlaGlyAsnThrPheThrCysSerValLeuHisGluGlyLeuHisAsnHisHisThrGluLysSerLeuSerHisSer
186▶nValGlnLysSerAsnTrpGluAlaGlyAsnThrPheThrCysSerValLeuHisGluGlyLeuHisAsnHisHisThrGluLysSerLeuSerHisSer
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NheI (2823)
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2801 CCTGGTAAATGATCCCAGTGTCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTT
742▶ProGlyLys•••
220▶ProGlyLys•••
```

HpaI (2963)
```
2901 ATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTC
```

AseI (3060)
XmnI (3061)
```
3001 AGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAA
```

```
3101 TCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTC

3201 ATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAA

                     SwaI (3317)
3301 ATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTT

3401 AGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGT
                                                       125◄•••AspGlnGluGluValaValaPheHi
3501 GCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGT
     116◄sValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThr
3601 GGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTG
     83◄SerValValGluSerTrpGluaTyrLeuGluAspLeuGlyAlaArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerI
                 SgrAI  (3730)
3701 ATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGG
     49◄IlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAl
3801 CGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaattgCT
     16◄aValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet                                              ◄
                                                           AseI (3944)
3901 ATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgccacttttcctgcactgcccca

                                                                          HindIII (4070)
4001 tctcctgcccacccttccccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGAA
                                                                          ◄
                                                            StuI (4172)
                                                            Eco147I (4172)
4101 CTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCGGTGGCAGGAG

                            BspEI  (4228)
4201 GCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGT

                       SpeI  (4335)
4301 TGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAAC
                                            ◄
                                                               SnaBI  (4465)
4401 CGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGG

4501 TCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGC

4601 AGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCG

                                                            PacI (4758)
                       SdaI  (4751)
4701 TTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCG
                                                               ◄
4801 TAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGA

4901 CTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGG

5001 GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCC

5101 CGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA

5201 GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG

5301 TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAA

                                                                          PacI (5498)
5401 AAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTA

        SwaI (5506)     NotI  (5514)
5501 ACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAA
5601 CAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
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