

# pFUSE-SEAP-hIgG4-Fc

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

Catalog # pfuse-hg4sp

For research use only

Version # 08H29-SV

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-SEAP-hIgG4-Fc plasmid provided as lyophilized DNA  
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

### 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 *E. coli* Fast-Media® Zeo 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

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-hIgG2-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 utilizing InvivoGen's SEAP Reporter Assay Kit (rep-sap) or QUANTI-Blue™ (rep-qb1 or rep-qb2).

## PLASMID FEATURES

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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<sup>2</sup>. 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.
- **hIgG4 Fc (human):** The Fc region of human IgG4 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.

- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA<sup>3</sup>.
- **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<sup>4</sup>.

## 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<sub>2</sub>O. 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® Zeo is available separately: #fas-zn-1 (liquid), #fas-zn-s (agar).

### 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.

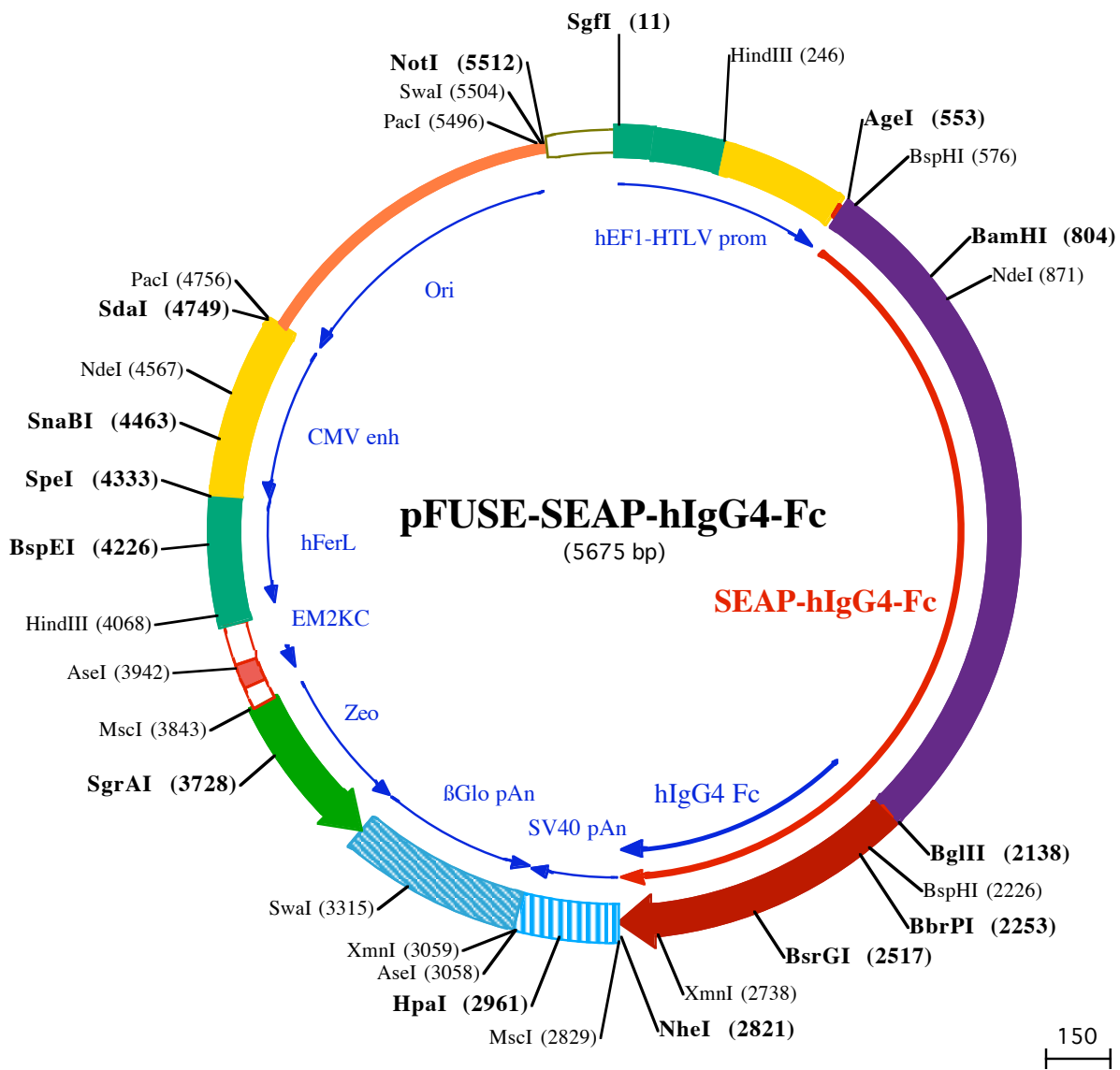
**Note:** Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

### 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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SgfI (11)  
1 GGATCTCGCATCGCTCCGGTCCCGTGCAGTGGCGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTGGCAATTGAACGGGTGCCTA  
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC  
HindIII (246)  
201 GTGAACGTTCTTTTTCCGCAACGGGTTTGCCTCCGAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTGCCTCGCCGCTTAGGTAAGTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCGGCGCTCCCTTGGAGCCTACCTAGACTACGCGGGCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTGTTTCTGTTT  
AgeI (553) BspHI (576)  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCCCTACCTGAGATCACCGGTTACGTCAGGAGGCACATGATTTCTGGGGCCCTGCATGCT  
1MetI I eLeuGI yProCysMeLe  
601 GCTGCTGCTGCTGCTGGCCTGAGGTACAGTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCGAGGCAGCCGAG  
8uLeuLeuLeuLeuLeuGI yLeuArgLeuGI nLeuSerLeuGI yI eI eP roVal GI uGI uGI uAsnProAspPheTrpAsnArgGI uAI aAI aGI u  
701 GCCCTGGTGCCTCCGCAAGAGCTGCAGCTGCACAGACAGCCCAAGCAACTCATCATCTTCTGGCGATGGGATGGGGGTCTACGCTGACAGCTG  
42AI aLeuGI yAI aAI aLysLysLeuGI nProAI aGI nThrAI aAI aLysAsnLeuI I eI ePheLeuGI yAspGI yMetGI yVal Ser Thr Val Thr AI aA  
BamHI (804) NdeI (871)  
801 CCAGGATCCTAAAAGGGCAGAAGAAGGACAACTGGGGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTGTCCAAGACATAAATGT  
75I aArgI I eLeuLysGI yGI nLysLysAspLysLeuGI yProGI uI eP roLeuAI aMe tAspArgPheP roTyrVal AI aLeuSer LysThr TyrAsnVa  
901 AGACAAATATGTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTTGGCTTGAAGTGCAGCCGCGCTTT  
108I AspLysHi sVal P roAspSer GI yAI aThr AI aThr AI aTyrLeuCysGI yVal LysGI yAsnPheGI nThr I I eGI yLeuSer AI aAI aAI aArgPhe  
1001 AACAGTGAACACGACACCGCGCAACGAGGTATCTCCGTGATGAATCGGCCAAGAAAGCAGGAAGTCAAGTGGAGTGGTAACCACACAGAGTGC  
142AsnGI nCysAsnThr Thr ArgGI yAsnGI uVal I I eSer ValMe tAsnArgAI aLysLysAI aGI yLysSer Val GI yVal I Val Thr Thr ArgVal I G  
1101 AGCAGCCTGCACCGCGCACCTACGCCACACCGTGAACCGCAACTGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGAGGAGGGTGCAGGA  
175I nHi sAI aSer P roAI aGI yThr TyrAI aHi sThr Val AsnArgAsnTrpTyrSerAspAI aAspVal P roAI aSer AI aArgGI nGI uGI yCysGI nAs  
1201 CATCGCTACCGAGCTCATCTCAACATGGACATTGATGTATCCTGGTGGAGGCCAAAGTACATGTTTCGCATGGGAAACCCAGACCTGAGTACCCA  
208pI I eAI aThr GI nLeuI I eSerAsnMe tAspI I eAspVal I I eLeuGI yGI yGI yArgLysTyrMe tPheArgMe tGI yP roAspP roGI uTyrP ro  
1301 GATGACTACAGCAAGTGGGACAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCTGGTATGTGTGAACCGCACTG  
242AspAspTyrSer GI nGI yGI yThr ArgLeuAspGI yLysAsnLeuVal GI nGI uT rpLeuAI aLysArgGI nGI yAI aArgTyrVal I TrpAsnArgThr G  
1401 AGCTATGACGGCTTCCCTGGACCCGCTGTGTACCATCTCATGGCTCTTTGAGCCTGGAGACATGAATACGAGATCCACCGAGACTCCACACTGGA  
275I uLeuMe tGI nAI aSer LeuAspP roSer Val I ThrHi sLeuMe tGI uLysPheGI uP roGI yAspMe tLysTyrGI uI I eHi sArgAspSer Thr LeuAs  
1501 CCCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCCTGCTGAGCAGAAACCCCGCGCTTCTTCTTCTCGTGGAGGGTGGTGCATCGACACCGGT  
308pP roSer LeuMe tGI uMe tThr GI uAI aAI aLeuArgLeuLeuSer ArgAsnP roArgGI yPhePheLeuPheVal GI uGI yGI yArgI I eAspHi sGI y  
1601 CATCAGGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACAGCGAGGAGGACAGCCTGA  
342Hi sHi sGI uSer ArgAI aTyrArgAI aLeuThr GI uThr I I eMe tPheAspAspAI aI eGI uArgAI aGI yGI nLeuThr Ser GI uGI uAspThr LeuS  
1701 GCCTGCTACTGCCGACACTCCACGCTTCTCCTCGGAGGCTACCCCTGCAGGGAGTCCATCTTGGGCTGGCCCTGGCAAGGCCCGGGACAG  
375er LeuVal Thr AI aAspHi sSer Hi sVal I PheSerPheGI yGI yTyrP roLeuArgGI ySer Ser I I ePheGI yLeuAI aP roGI yLysAI aArgAspA  
1801 GAAGCCTACACGGTCCCTATACGGAACGGTCCAGGCTATGTCTCAAGGACGGCGCCCGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGGAG  
408yLysAI aTyrThr Val LeuLeuTyrGI yAsnGI yP roGI yTyrVal I LeuLysAspGI yAI aArgP roAspVal Thr GI uSer GI uSer GI ySer P roGI u  
1901 TATCGGACGAGCTCAGCAGTGCCTCGGACGAGAGCCACGACGAGGAGCGTGGCGGTGTTTCGCGCGGGCCAGCGGCACCTGGTTACAGCGCG  
442TyrArgGI nGI nSer AI aVal P roLeuAspGI uGI uThrHi sAI aGI yGI uAspVal AI aVal I PheAI aArgGI yP roGI nAI aHi sLeuVal I Hi sGI yV  
2001 TGCAGGAGCAGACCTCATAGCCGACGTCATGGCTTCCGCGCTGCCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCGCGCACCCAGCGC  
475aI GI nGI uGI nThr PheI I eAI aHi sValMe tAI aPheAI aAI aCysLeuGI uP roTyrThr AI aCysAspLeuAI aP roP roAI aGI yThr ThrAspAI  
BglIII (2138)  
2101 CGCGCACCCGGGGCGTCCCGTCCAAGCGTCTGGATAGATCTCCCATGCCCCATGCCCCAGCAGCTGAGTTCCTGGGGGACCATCAGTCTTCTCTG  
508aAI aHi sP roGI yArgSer LysArgLeuAspArgSer P roP roCysP roAI aP roGI uPheLeuGI yGI yP roSer Val PheLeu  
1P roP roCysP roSer CysP roAI aP roGI uPheLeuGI yGI yP roSer Val PheLeu  
BspHI (2226) BbrPI (2253)  
2201 TTCCCCCAAACCAAGGACACTCATGATCTCCCGGACCCTGAGGTGACGTGGTGGTGGTGGACGTGAGCCAGGAAGACCCGAGGTCCAGTTCA  
542PheP roP roLysP roLysAspThr LeuMe tI I eSer ArgThr P roGI uVal I Thr CysVal I Val I Val I AspVal I Ser GI nGI uAspP roGI uVal I GI nPheA  
20PheP roP roLysP roLysAspThr LeuMe tI I eSer ArgThr P roGI uVal I Thr CysVal I Val I Val I AspVal I Ser GI nGI uAspP roGI uVal I GI nPheA  
2301 ACTGTCAGTGGATGGCGTGGAGTGCATAATGCAAGCAAGCCGCGGAGGAGCAGTTCAACAGCAGTACCGTGTGGTCCCTCCACTCCACTCCCT  
575snTrpTyrVal AspGI yVal GI uVal I Hi sAsnAI aLysThr LysP roArgGI uGI uGI nPheAsnSer Thr TyrArgVal I Val Ser Val I LeuThr Val I Le  
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2401 GCACCAGGACTGGCTGAACGGCAAGTACAAGTCTCAACAAAGCCCTCCCTCCTCATCGAGAAACCACTCCAAAGCCAAAGGGCAG  
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86uHi sGI nAspTrpLeuAsnGI yLysGI uTyrLysCysLysVal I SerAsnLysGI yLeuP roSer Ser I I eGI uLysThr I I eSer LysAI aLysGI yGI n  
BsrGI (2517)  
2501 CCCCAGAGCCACAGGTGTACACCCTGCCCCATCCAGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCG  
642P roArgGI uP roGI nVal I TyrThr LeuP roP roSer GI nGI uGI uMe tThr LysAsnGI nVal I Ser LeuThr CysLeuVal I LysGI yPheTyrP roSer A  
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2601 ACATCGCGTGGAGTGGAGAGCAATGGGACGCGGAGACAACACTACAAGACCCAGCCTCCCTGGTGGACTCCGAGCGGCTCTTCTTCTACAGCAG  
675spl I eAI aVal I GI uTrpGI uSerAsnGI yGI nP roGI uAsnAsnTyrLysThr Thr P roP roVal I LeuAspSer AspGI ySer PhePheLeuTyrSer Ar  
153spl I eAI aVal I GI uTrpGI uSerAsnGI yGI nP roGI uAsnAsnTyrLysThr Thr P roP roVal I LeuAspSer AspGI ySer PhePheLeuTyrSer Ar  
XmnI (2738)  
2701 GCTAACCTGGACAAGAGCAGGTGGCAGGAGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACAGAAAGAGCCTCTCC  
708gLeuThr Val AspLysSer ArgTrpGI nGI uGI yAsnVal I PheSer CysSer Val I Me tHi sGI uAI aLeuHi sAsnHi sTyrThr GI nLysSer LeuSer  
186gLeuThr Val AspLysSer ArgTrpGI nGI uGI yAsnVal I PheSer CysSer Val I Me tHi sGI uAI aLeuHi sAsnHi sTyrThr GI nLysSer LeuSer  
MscI (2829)  
2801 CTGTCTCCGGTAAATGAGTGCAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACCCACAACCTAGAATGCAGTGAAAAAATGCTTTAT  
742LeuSer P roGI yLys...  
220LeuSer P roGI yLys...  
NheI (2821)  
HpaI (2961)  
2901 TTGTGAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTGAACAAAGTTAAACAACAACAAATTCATTTATGTTTACAGTTTAC  
AseI (3058)  
XmnI (3059)  
3001 GGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTAATTTCTAAAAATACAGCATAGCAAACTTTAACTCCAAATC

3101 AAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTTGACGCTCACCTTCTTTTCAT  
3201 GGAGTTTAAGATATAGTGATTTTCCCAAGGTTTGAAC TAGCTCTTCATTTCTTTATGTTTAAATGCACTGACCTCCACATTCCTTTTATAGTAAAAA  
Swal (3315)  
3301 ATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAG  
3401 TAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGC  
125↓●●AspGlnGluAlaValPheHisV  
3501 ACGCAGTTGCCGGCGGGTCCGCGAGGGCAACTCCCGCCCCACGGTGTCTGCGGATCTGGTCATGGCCGGCCGGAGGCGTCCGGAAAGTTCGTGG  
115↓aI CysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpProGlnGluIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSer  
3601 ACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACCCAGCCAGGGGTGTTGTCCGGCACCACTGGTCTGGACCGCTGAT  
82↓rValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGluAspGlnValAlaSerIle  
SgrAI (3728)  
3701 GAACAGGGTCACGTCGTCGCCGACACCCGGCGAAGTCGCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCG  
49↓PheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaVal  
MseI (3843)  
3801 ACGTCGCGCGCGGTGAGCACCGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggttagtacaattgCTAT  
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AseI (3942)  
3901 AGTGAGTTGTATTACTATGCAGATATACTATGCCAATGATTAATTGTCAAAC TAGGGCTGCAGgggttcagtgtccacttttctgactgccccatc  
HindIII (4068)  
4001 tcctgccaccctttccaggcatagacagtcagtgacttacCAAAC TACAGGAGGAGAAAGCAGAAGCTTGAGACAGACCCCGGGACCGCCGAAC T  
4101 GCGAGGGGACGTGGCTAGGGCGGCTCTTTTATGGTGCCTCGGCCCTCGGAGGACGGCGCTCGGGGAGGCTAGCGGCCAATCTGCGGTGGCAGGAGGC  
BspEI (4226)  
4201 GGGGCCGAAGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTG  
SpeI (4333)  
4301 TGAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAACAACACTCCCATGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCG  
SnaBI (4463)  
4401 CTATCCACGCCATTGATGTACTGCCAAAACGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCT  
NdeI (4567)  
4501 ATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCAATTGACGTCAATAGGGGGGTAAGTGGCATATGATACACTTGTACTGCCAAGTGGGCA  
4601 TTTACCGTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGCGGGGGTCGTT  
PacI (4756)  
SdaI (4749)  
4701 GGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACCGTA  
4801 AAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCAAAACCCGACAGGACT  
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PacI (5496)  
5401 AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGATTTTGGTCATGGCTAGTTAATTAAC  
Swal (5504) NotI (5512)  
5501 ATTTAAATCAGCGGCGCAATAAAATATCTTTATTTTATTACATCTGTGTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACA  
5601 AAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAA