

pFUSE-SEAP-hG3Fc

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

Catalog # pfuse-hg3sp

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-SEAP-hG3Fc 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.
- Expression of SEAP-hG3Fc was confirmed by using QUANTI-Blue™ Solution.
- SEAP-hG3Fc 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. SEAP-Fc fusion proteins can be easily purified by single-step protein G affinity chromatography.

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-hG3Fc** was generated by fusing the gene encoding for human SEAP with the Fc region of human 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 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*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

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 J.C. 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 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.

TECHNICAL SUPPORT

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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.

Purification of SEAP-hG3Fc protein

The following protocol describes the purification of SEAP-hG3Fc protein produced by 293 cells using Protein G affinity chromatography.

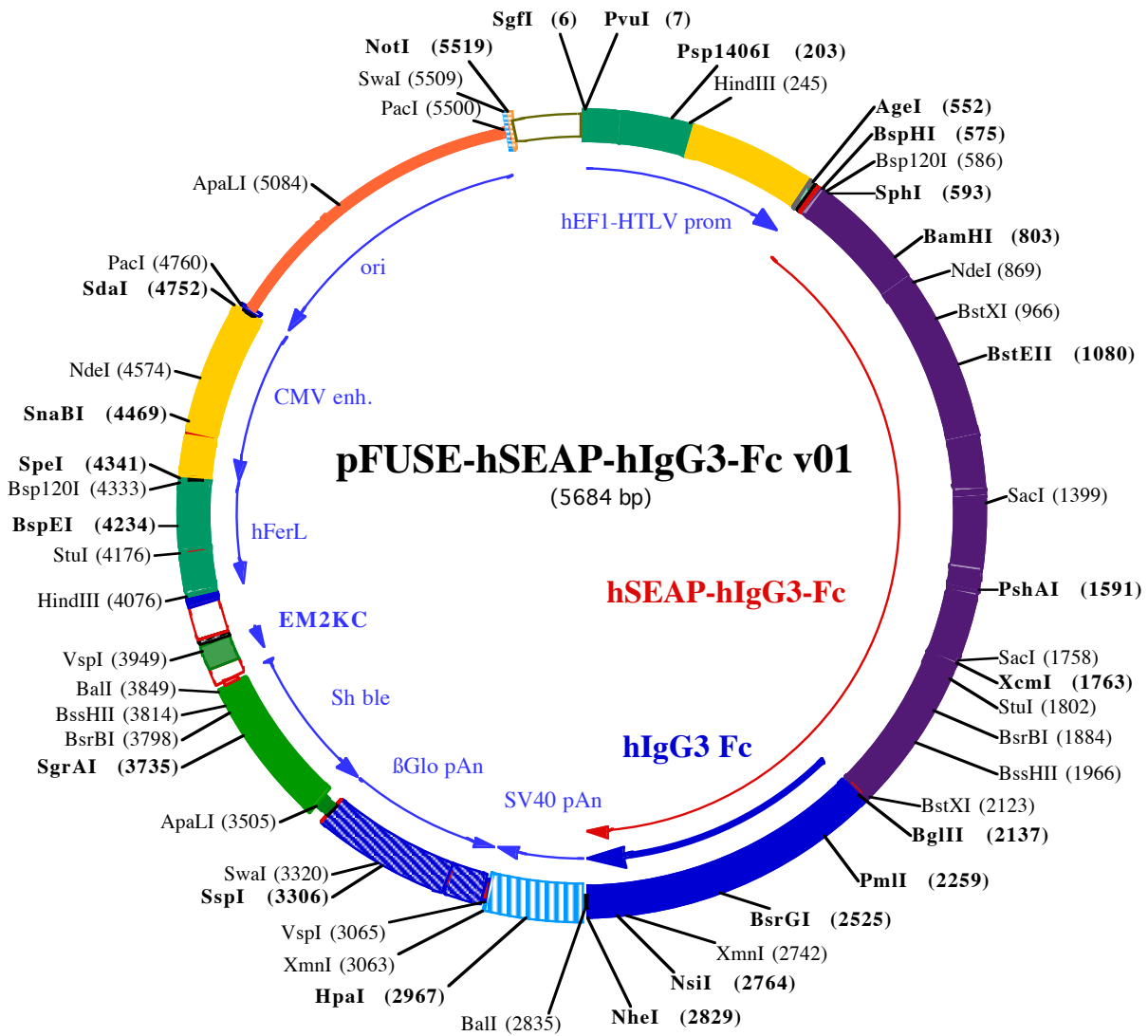
- 1- Seed 3.5x10⁶ 293 cells in a 100 mm plate containing 6 ml of DMEM supplemented with 10% FBS.
- 2- Transfect cells with 750 µl of pFUSE-SEAP-hG3Fc/LyoVec™ complexes at a ratio of 1:6 prepared by mixing 7.5 µg pFUSE-SEAP-hG3Fc and 750 µl reconstituted LyoVec™ following the LyoVec™ protocol.
- 3- After 16 hours transfection, replace the medium with a serum-free medium such as PRO 293a-CDM (Biowitaker-Cambrex).
- 4- After 72 hours transfection, collect supernatant.
- 5- Purify protein using Protein G affinity chromatography such as Hi Trap Protein G HP (Amersham Biosciences) following manufacturer's protocol.

RELATED PRODUCTS

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

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150

PvuI (7)
SgfI (6)
 1 GGATCTGCGATCGCTCCGGTCCCGTCCAGTGGGACAGCGCACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGTGGCAATTGAACGGTGCCTA
 101 GAGAAGGTGGCGGGGTAAACTGGGAAAGTGATGTCTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACGTATATAAGTGCAGTAGTCGCCG

Psp1406I (203) **HindIII (245)**
 202 TGAACGTTCTTTTTCGAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTTCACGCGCCCGCCCTACCTGAGGCCGCG
 303 CATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGTGCCTCCGCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGG
 404 CTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTCTTTCTG

AgeI (552) **BspHI (575)** **Bsp120I (586)** **SphI (593)**
 505 TTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTTTCAGCTGAGGAGCACATCATGATTCTGGGGCCCTGCATGCTGCTGC
 606 TGCTGCTGCTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGGAGGAGCCGAGGCCCTG
 101▶ L L L L L G L R L Q L S L G I I P V E E E N P D F W N R E A A E A L
 707 GGTGCCCAAGAAGCTGCAGCCTGCACAGACAGCCGAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGTGTCTACGGTGACAGCTGCCAGGAT **BamHI (803)**
 44▶ G A A K K L Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I
 808 CCTAAAAGGGCAGAAGAAGACAACCTGGGGCTGAGATACCCCTGGCTATGGACCGCTTCCATATGTGGCTGTCCAAGACATACAATGTAGACAAC
 77▶ L K G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N V D K
 909 ATGTGCCAGACAGTGGAGCCACAGCCAGCGCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATGGCTTGTAGTGCAGCCGCGCTTTAACAGTGC
 111▶ H V P D S G A T A T A Y L C G V K G N F Q T I G L S A A A R F N Q C
 1010 AACACGACACGGCAACGAGGTCATCTCCGTGATGAATCGGGCAAGAAGCAGGGAAGTCAAGTGGGAGTGGTAACCACACAGAGTGCAGCACGCCCTC
 145▶ N T T R G N E V I S V M N R A K K A G K S V G V V T T T R V Q H A S
 1111 GCCAGCCGCACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCTGCCTCGGCCCGCCAGGAGGGTGCAGGACATCGCTACGC
 178▶ P A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T
 1212 AGCTCATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGAAACCCAGACCTGAGTACCCAGATGACTACAG
 212▶ Q L I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y S
 1313 CAAGGTGGGACAGGCTGGACGGGAAGATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTCCCGGATGTGTGGAACCGCACTGAGCTCATGCAGGC
 246▶ Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q A
 1414 TTCCCTGGACCGCTGTGACCCATCTCATGGGTCTCTTTGAGCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGG
 279▶ S L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P S L M
 1515 AGATGACAGAGGCTGCCCTGCCCTGTGAGCAGGAACCCCGCGGCTTCTTCTCTTCTGAGGGTGGTGCATCGACACGGTTCATCACGAAAGCAGG
 313▶ E M T E A A L R L L S R N P R G F F L F V E G G R I D H G H H E S R
 1616 GCTTACCGGGCACTGACTGAGACGATCATGTTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCGAGGAGGACACGCTGAGCCTCGTCACTGCCGA
 347▶ A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L V T A D
 1717 CCACTCCACGTCTTCTCCTCGGAGGCTACCCCTCGGAGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCGGGACAGGAAGGCCCTACACGGTCC
 380▶ H S H V F S F G G Y P L R G S S I F G L A P G K A R D R K A Y T V
 1818 TCCTATACGGAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCGGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGCAGTCAAG
 414▶ L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R Q Q S A
 1919 GTGCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCCGCGCGGCCCGCAGGCGCACCTGTTTACGGCGTGCAGGAGCAGACCTTCAT
 448▶ V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E Q T F I
 2020 AGCGCAGTCATGGCCTTCGCCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCGCGGACACCGGACCGCGCACCCGGGGGGTCC
 481▶ A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P G R S
 2121 GGTCAAAGCGTCTGGATAGATCTGACACACCTCCCGGTGCCAAGGTGCCAGCACCTGAAGTCTGGGAGGACCGTCACTTCTTCTTCCCCAAAA
 515▶ R S K R L D R S D T P P P C P R C P A P E L L G G P S V F L F P P K
 2222 CCAAGGATACCCCTTATGATTTCCCGACCCCTGAGGTACGCTGCTGGTGGGACGTGAGCCACGAAGACCCCGAGGTCAGTTCAGTGGTACGTGGGA
 27▶ P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F K W Y V D
 549▶ P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F K W Y V D
 2323 CGGCGTGGAGGTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTCAACAGCAGCTTCCGTGGTTCAGCGCTCCTCACCGCTGCACCGACTGG
 60▶ G V E V H N A K T K P R E E Q F N S T F R V V S V L T V L H Q D W
 582▶ G V E V H N A K T K P R E E Q F N S T F R V V S V L T V L H Q D W
 2423 CTGAAGGGCAAGGATACAAGTCAAGTCTCCAACAAGCCCTCCAGCCCATCGAGAAACCATCTCCAAAACCAAGGACAGCCCGGAGAACCA
 94▶ L N G K E Y K C K V S N K A L P A P I E K T I S K T K G Q P R E P Q
 616▶ L N G K E Y K C K V S N K A L P A P I E K T I S K T K G Q P R E P Q
 2524 GGTGTACACCTGCCCATCCCGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCTGGTCAAAGGCTTCTACCCGACGACATCGCCGTGGAG
 127▶ V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E
 649▶ V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E

2624 TGGGAGAGCAGCGGGCAGCCGGAGAACAAC TACAACACCACGCCTCCCATGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAA
161▶ W E S S G Q P E N N Y N T T P P M L D S D G S F F L Y S K L T V D K
683▶ W E S S G Q P E N N Y N T T P P M L D S D G S F F L Y S K L T V D K
XmnI (2742) NsiI (2764)
2725 GAGCAGGTGGCAGCAGGGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGCTTACGCGAGAAGGCCTCCCTGTCTCCGGGTAAT
194▶ S R W Q Q G N I F S C S V M H E A L H N R F T Q K S L S L S P G K
716▶ S R W Q Q G N I F S C S V M H E A L H N R F T Q K S L S L S P G K
Ball (2835)
NheI (2829)
2826 GAGTGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATG
228▶ •
750▶ •
HpaI (2967)
2927 CTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGT
VspI (3065)
XmnI (3063)
3028 TTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCT
3129 TTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTA
SspI (3306) SwaI (3320)
3230 TTTTCCAAGGTTTGAAGTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAAATATTAGAAAATAATTTAAATAC
3331 ATCATTGCAATGAAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAG
ApaLI (3505)
3432 GAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGACGCAAGTTGCCGGCCGGTTCGCG
125▶ • D Q E E A V F H V C N G A P D R
3533 CAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTATGCGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGCCGT
108▶ L A F E R G W P Q E G I E T M A P G S A D R F N T S V V E S W E A Y
3634 ACAGCTGTCCAGCCGCGCACCCACACCCAGGCCAGGGTGTGTCGGCACCACTGGTCTGGACCGCTGATGAACAGGGTACGTCGTCCCGACC
74▶ L E D L G R V W V W A L T N D P V V Q D Q V A S I F L T V D D R V
SgrAI (3735) BsrBI (3798) BssHII (3814)
3735 ACACCGCGAAGTCTCTCCACGAAGTCCCGGGAGAACCAGCCGGTCCGAGAACTCGACCCGCTCCGGGACGTCGCGCGGTGAGCACCGGAAC
40▶ V G A F D D E V F D R S F G L R D T W F E V A G A V D R A T L V P V
Ball (3849)
3836 GGCACTGGTCAACTTGGCCATGATGGCTCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGAT
7▶ A S T L K A M
3▶ I A G
VspI (3949)
3936 ATACTATGCCAATGATTAATTGTCAAAC TAGGGCTGCAGgggttcatagtgccacttttctgcactgccccatctcctgccaccctttccaggcataga
HindIII (4076)
4037 cagtcaagtacttacCAAACCTCACAGGAGGAGAAGGCAGAGCTTGAGACAGCCCGGGACCGCCGAACCTGCGAGGGGACGTGGCTAGGGCGGCTTCT
StuI (4176) BspEI (4234)
4138 TTTATGGTGGCCGGCCCTCGGAGGCAGGGCGCTCGGGAGGCCTAGCGGCCAATCTGCGTGGCAGGAGCGGGGCCGAAGCGCTGCTGACCAATCCG
Bsp120I (4333)
4239 GAGCACATAGGAGTCTACGCCCCCGCCCCAAAGCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTTGGGGCC
SpeI (4341)
4340 TGACTAGTCAAAACAACCTCCATTGACGTCAATGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAAAC
SnaBI (4469)
4441 CGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAGGTCATGTACTGGGCATAATGCCAGCGGGCCATT
NdeI (4574)
4542 ACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTGTACTGCCAAGTGGCAGTTTACCCTAAATACTCCACCACTGACGTCAAT
4643 GGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTATTATTGACGTCAATGGCGGGGGTCTTGGCGGTCAGCCAGCGGGCCATTACCCTAAGT
PacI (4760)
SdaI (4752)
4744 TATGTAACGCCTGCAGGTTAATTAAAGAACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACCGTAAAAAGCCGCTTGTGCGCTTTTTCCATAGGCTC
4845 CGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACAGGCGTTTCCCTCGAAGCTCCCT
4946 CGTGGCTCTCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGATGATC
ApaLI (5084)
5047 TCAGTTCGGTGTAGGTGCTTCCGCTCAAGCTGGCTGTGTCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTGATCC
5148 AACCCGGTAAGACACGACTTATCGCCACTGCGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTG
5249 GCCTAACTACGGTACTACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAA
5350 AAACCACCGCTGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCT
PacI (5500) SwaI (5509) NotI (5519)
5451 GACGCTCAGTGAACGAAACCTACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTA
5552 CATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCAGTGC
5653 AAGTGCAGGTGCCAGAACATTTCTCTATCGAA