pFUSE-SEAP-rFc

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

Catalog # pfuse-rsp

Version 20K04-MM

PRODUCT INFORMATION

Content:
- 20 µg of **pFUSE-SEAP-rFc** 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-rFc was confirmed by using QUANTI-Blue™ Solution
- SEAP-rFc protein was purified using protein G affinity chromatography.

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 A or protein G affinity chromatography.

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-rFc** was generated by fusing the gene encoding for human SEAP with the Fc region of rabbit IgG. 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*.

• **ßGlo 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 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.

RELATED PRODUCTS

Product	Catalog Code
Agar Protein G/ Agarose	gel-agg-2
QUANTI-Blue™ Solution	rep-qbs

InvivoGen

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pFUSE-SEAP-rFc v01
(5670 bp)

SgfI **(171)**
NotI **(-1)**
SwaI (5660)
PacI (5651)
PvuI **(172)**
Psp1406I **(368)**
HindIII (410)
Bsu36I (456)
ApaLI (5235)
AgeI **(717)**
BspHI (740)
SphI **(758)**
Bsu36I (786)
PacI (4911)
SdaI **(4903)**
BamHI **(968)**
NdeI (4725)
NdeI (1034)
SnaBI **(4620)**
BstXI (1131)
BstEII **(1245)**
SpeI **(4492)**
BspEI **(4385)**
SacII (4242)
HindIII (4227)
SacI (1564)
AseI (4100)
SacII (1719)
PshAI **(1756)**
BalI (4000)
BssHII (3965)
SgrAI **(3886)**
SacI (1923)
XcmI **(1928)**
ApaLI (3656)
BssHII (2131)
SwaI (3471)
SspI **(3457)**
BstXI (2288)
BglII **(2302)**
PmlI **(2316)**
BspHI (2387)
AseI (3216)
XmnI **(3214)**
HpaI **(3118)**
BalI (2986)
NheI **(2980)**
NcoI **(2684)**
FspI **(2486)**

ori
hEF1-HTLV prom
CMV enh
hFerL
EM2KC
Sh ble
ßGlo pAn
SV40 pAn
SEAP-rFc
rFc

150
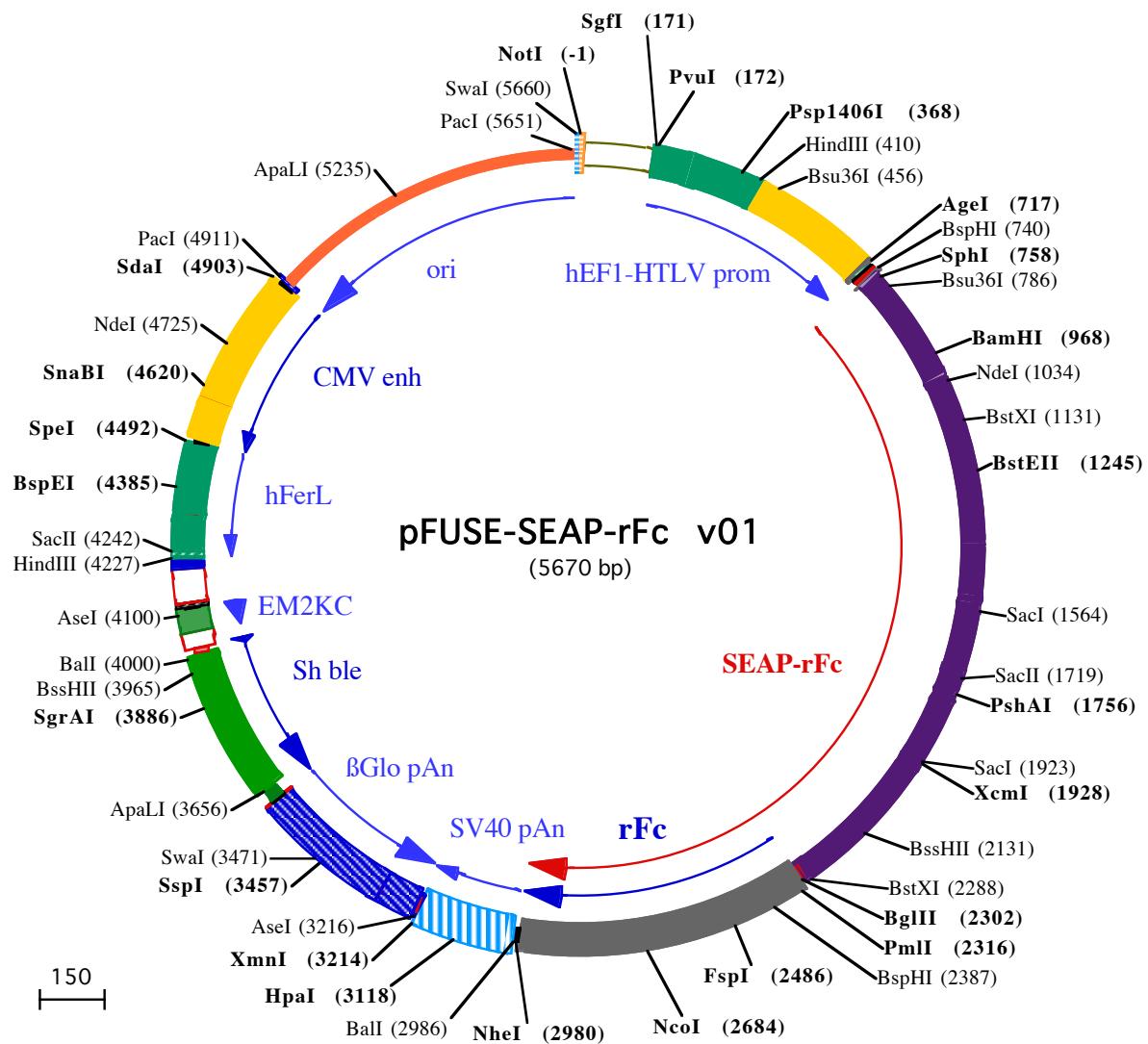
NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA

PvuI (172)
SgfI (171)

101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA

201 GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATG

Psp1406I (368)

301 TCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (410)   Bsu36I (456)

401 AACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCT

501 CCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

AgeI (717)   BspHI (740)   SphI (758)   Bsu36I (786)

701 GGCGCCTACCTGAGATCACCGGTTCAGCTGAGGAGGCACATCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGC

1▶ M I L G P C M L L L L L L L G L R L Q

801 TCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACA

20▶ L S L G I I P V E E E N P D F W N R E A A E A L G A A K K L Q P A Q

BamHI (968)

901 GACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTG

53▶ T A A K N L I I F L G D G M G V S T V T A A R I L K G Q K K D K L

NdeI (1034)

1001 GGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCA

87▶ G P E I P L A M D R F P Y V A L S K T Y N V D K H V P D S G A T A

BstXI (1131)

1101 CGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCAT

120▶ 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 N T T R G N E V I

BstEII (1245)

1201 CTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACG

153▶ 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 P A G T Y A H T

1301 GTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTG

187▶ 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 Q L I S N M D I

1401 ATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGG

220▶ 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 Q G G T R L D G

SacI (1564)

1501 GAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACC

253▶ 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 S L D P S V T

1601 CATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGC

287▶ 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 E M T E A A L

SacII (1719)   PshAI (1756)

1701 GCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGA

320▶ 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 A Y R A L T E

1801 GACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCC

353▶ 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 H S H V F S

XcmI (1928)
SacI (1923)

1901 TTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTC

387▶ 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 L L Y G N G

2001 CAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGA

420▶ 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 V P L D E E

BssHII (2131)

2101 GACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGCC

453▶ 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 A H V M A

BstXI (2288)

2201 TTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGG

487▶ 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 R S K R L

BglII (2302)   PmlI (2316)   BspHI (2387)

2301 ATAGATCTAGCAAGCCCACGTGCCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCAC

1▶ S K P T C P P P E L L G G P S V F I F P P K P K D T L M I S

520▶ D R S S K P T C P P P E L L G G P S V F I F P P K P K D T L M I S

FspI (2486)

2400 GCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGTGCGCACCGCCG

31▶ R T P E V T C V V V D V S Q D D P E V Q F T W Y I N N E Q V R T A R

553▶ R T P E V T C V V V D V S Q D D P E V Q F T W Y I N N E Q V R T A R

2500 GCGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTG

64▶ P P L R E Q Q F N S T I R V V S T L P I A H Q D W L R G K E F K C

586▶ P P L R E Q Q F N S T I R V V S T L P I A H Q D W L R G K E F K C

NcoI (2684)

2599 CAAAGTCCACAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCCC

97▶ K V H N K A L P A P I E K T I S K A R G Q P L E P K V Y T M G P P

619▶ K V H N K A L P A P I E K T I S K A R G Q P L E P K V Y T M G P P

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2699 CGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAG
 131▶ R  E  E  L  S  S  R  S  V  S  L  T  C  M  I  N  G  F  Y  P  S  D  I  S  V  E  W  E  K  N  G  K  A
 653▶ R  E  E  L  S  S  R  S  V  S  L  T  C  M  I  N  G  F  Y  P  S  D  I  S  V  E  W  E  K  N  G  K  A
2799 AGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGCGGGGCG
 164▶ E  D  N  Y  K  T  T  P  A  V  L  D  S  D  G  S  Y  F  L  Y  S  K  L  S  V  P  T  S  E  W  Q  R  G
 686▶ E  D  N  Y  K  T  T  P  A  V  L  D  S  D  G  S  Y  F  L  Y  S  K  L  S  V  P  T  S  E  W  Q  R  G
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                                                                                                BalI (2986)
                                                                                         NheI (2980)
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2898 ACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGAGCTAGCTGGCCAGACAT
 197▶ D  V  F  T  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  I  S  R  S  P  G  K  •
 719▶ D  V  F  T  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  I  S  R  S  P  G  K  •
2998 GATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACC
```
                        HpaI (3118)
```
3098 ATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACC
```
                                           AseI (3216)
                                        XmnI (3214)
```
3198 TCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAA
3298 GGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAA
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                                                    SspI (3457)   SwaI (3471)
```
3398 CTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAA
3498 TAAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAA
```
                                                                 ApaLI (3656)
```
3598 ATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCC
                                           125◀ •  D  Q  E  E  A  V  F  H  V  C  N  G  A  P  D  R  L  A  F  E  R
3698 GCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAG
 103◀ 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  L  E  D  L
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                                                                              SgrAI (3886)
```
3798 GCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAG
  70◀ 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  V  G  A  F
```
                                                              BssHII (3965)
```
3898 TCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGGCGGTGAGCACCGGAACGGCACTGGTCA
  36◀ 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  A  S  T  L
```
       BalI (4000)
```
3998 ACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCC
   3◀ K  A  M
         3◀ I  A  G
```
       AseI (4100)
```
4097 AATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgccacttttcctgcactgccccatctcctgcccacccttttcccaggcatagacagtcagtg
```
                      HindIII (4227)   SacII (4242)
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4197 acttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGT
4297 GCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACA
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                                                                                   BspEI (4385)
                                                                          SpeI (4492)
```
4397 TAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTA
4497 GTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCAT
```
                          SnaBI (4620)
```
4597 CATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCG
```
                          NdeI (4725)
```
4697 TCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGA
4797 AAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTA
```
                                PacI (4911)
                SdaI (4903)
```
4897 TGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
4997 GCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT
5097 CGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTAT
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                                    ApaLI (5235)
```
5197 CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT
5297 CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
5397 GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGC
5497 AAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG
```
                                            PacI (5651)   SwaI (5660)
```
5597 GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA
```