

pFUSE-SEAP-rtFc

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

Catalog # pfuse-rtsp

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-SEAP-rtFc 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-rtFc was confirmed by using QUANTI-Blue™ Solution.
- SEAP-rtFc 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 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-rtFc** was generated by fusing the gene encoding for human SEAP with the Fc region of **rat IgG2b**. 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

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@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 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

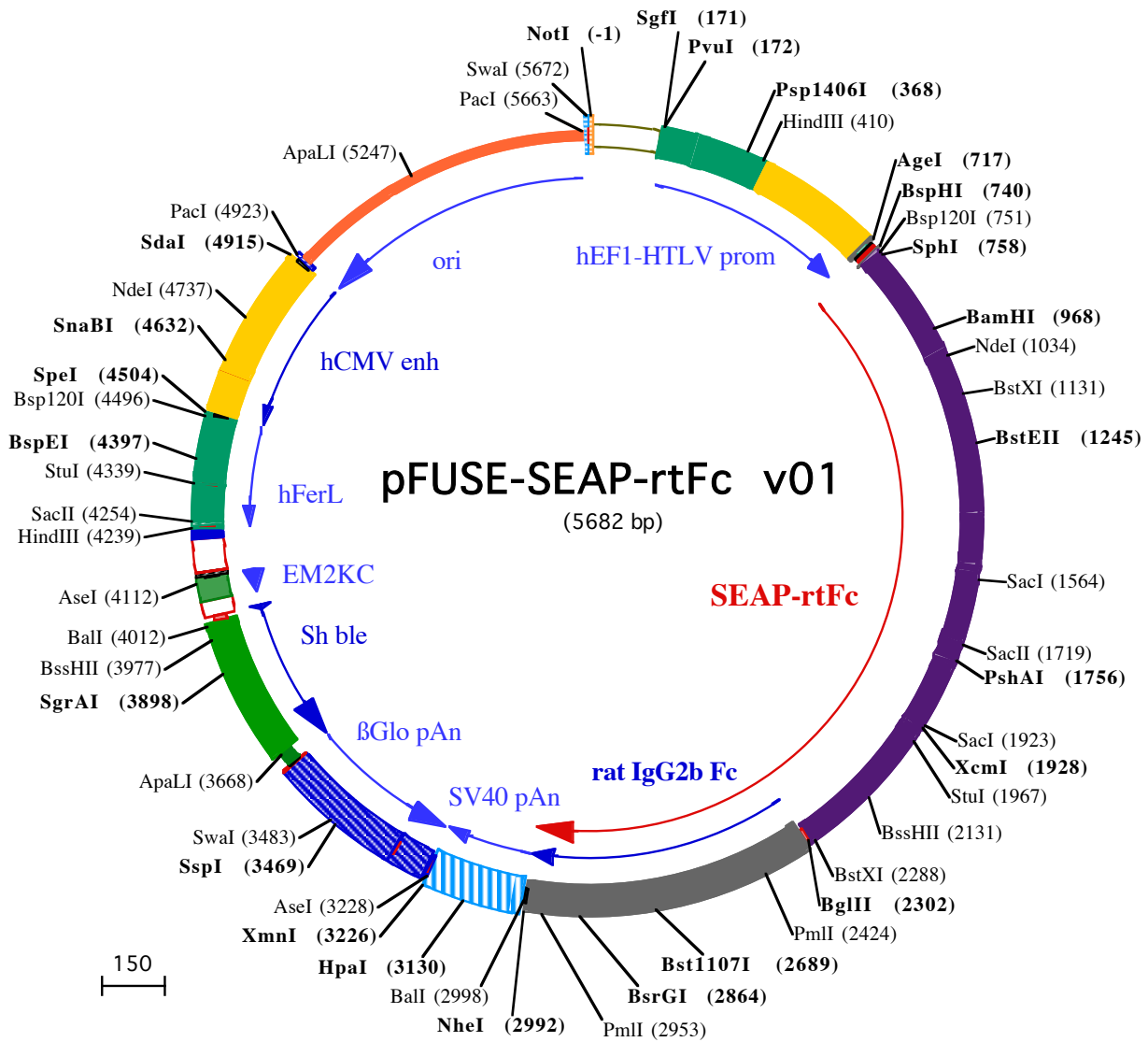
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

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

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 Hong Kong: +852 3622-3480
E-mail: info@invivogen.com



NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA

PvuI (172)
SgfI (171)
101 AAACAACTAGCAAAATAGGCTGTCCCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTCGATCGCTCCGGTCCCGTCACTGGGCA

201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCCGCAATTGAACGGTGCCTAGAGAAGTGGCGGGGTAAGTGGGAAAGTGTG

Psp1406I (368)
301 TCGTGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCGGTGAACGTTCTTTTTTCGCAACGGGTTTCCGCCAG

HindIII (410)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACACGCGCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCCT

501 CCCCCTGTGGTCCCTCTGAACTGCGTCCGCCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTCCTGACCCTGCTTCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCCCGTTACAGATCCAAGCTGTGACC

AgeI (717) **BspHI (740)** **Bsp120I (751)** **SphI (758)**
701 GGGCGCTACCTGAGATCACCGGTTTCAGCTGAGGAGCACATCATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGGCCCTGAGGCTACAGC

801 TCTCCCTGGGCATCATCCCAGTTGAGGAGAGAACC CGACTTCTGGAACCGGAGGACGCCGAGGCCCTGGGTGCCGCAAGAAGCTGCAGCCTGCACA

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 GACAGCCCAAGAACCTCATCATCTTCTGGCGATGGATGGGGGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTG

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 GGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCA

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 CGGCCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTTGTGAGTGCAGCCGCCGCTTAAACAGTGCAACACGACACGGGCAACGAGGTCAT

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 CTCCGTGATGAATCGGCCAAGAAAGCAGGGAAGTCACTGGGATGGTAACCACACACGAGTGACAGCAGCCTGCCAGCCGGCACCTACGCCACACG

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 GTGAACCGCAACTGGTACTCGGACCGGACGTGCCTGCCTCGCCCGCCAGGAGGGTGCACAGGACATCGCTACGCAGCTCATCTCAACATGGACATTG

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 ATGTGATCCTGGTGGAGGCCGAAAGTACATGTTTCGATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCAAGTGGGACCAGGCTGGACGG

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 GAAGAATCTGGTGCAAGGAATGGCTGGCGAAGCGCCAGGGTCCCGGTATGTGTGAACCGCACTGAGCTCATGCAGGTTCCCTGGACCGTCTGTGACC

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 CATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCTGATGGAGATGACAGAGGCTGCCTGC

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 GCCTGCTGAGCAGGAACCCCGCGCTTCTTCTCTCTGTTGGGGTGGTGCATCGACCAGGTCATCACGAAAGCAGGGCTTACCGGCACTGACTGA

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 GACGATCATGTTGACGACGCCATTGAGAGGGCGGCCAGCTCACCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCACGCTTCTTCC

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)** **StuI (1967)**
1901 TTCGAGGCTACCCCTGCGAGGGAGCTCCATCTTCCGGCTGGCCCCGGCAAGGCCGGACAGGAAGGCTACACGGTCTCTATACGAAACGGT

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 CAGGCTATGTCTCAAGGACGGCCCGCCGGATGTTACCAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGCAGTCAAGTCCCTGGACGAAGA

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

2101 GACCCACGAGGCGAGGACGTGGCGGTGTTCCGCGCGGCCCGCAGGCGACCTGTTTACGGCGTGAGGAGCAGACCTTATAGCGACGTCATGGCC

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

2201 TTCGCGCCTGCCTGGAGCCCTACCCGCTGCGACCTGGCGCCCCCGCCGACCACCGAGCCGCGACCCGGGGCGTCCCGTCCAAAGCTCTGG

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

BglIII (2302)
2301 ATAGATCTCTTACATGCCCTACATGTCAAAATGCCAGTTCTTGAACCTTGGGTGGACCATCTGCTTTCATCTTCCGCAAAAGCCAAAGGACATCCT

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

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

PmlI (2424)
2401 CTTGATCTCCAGAACGCAAGGTCACGTGTGGTGGTGTGAGCGAGGAGGCCGACGTCAGTTCAGCTGGTTTGTGAACAACGTAGAAGTA

31▶ L I S Q N A K V T C V V V D V S E E E P D V Q F S W F V N N V E V

553▶ L I S Q N A K V T C V V V D V S E E E P D V Q F S W F V N N V E V

2501 CACACAGCTCAGACACAACCCCGTGGAGGACGAGTACAACAGCACCTTCAAGTGGTCACTGCCCCATCCAGCACCAGGACTGGATGAGCGGCAAGG

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

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

587 H T A Q T Q P R E E Q Y N S T F R V V S A L P I Q H Q D W M S G K
Bst1107I (2689)
2601 AGTTCAAATGCAAGGTCAACAACAAGCCCTCCCAAGCCCATCGAGAAAACCATCTCAAACCCAAAGGGCTAGTCAGAAAACACAGGTATACGTCAT
98▶ E F K C K V N N K A L P S P I E K T I S K P K G L V R K P Q V Y V M
620▶ E F K C K V N N K A L P S P I E K T I S K P K G L V R K P Q V Y V M
2701 GGGTCCACCGACAGAGCAGTTGACTGAGCAAAACGGTCAGTTTGACCTGCTTGACCTCAGGCTTCCCTCCTAACGACATCGGTGTGGAGTGGACCAGCAAC
131▶ G P P T E Q L T E Q T V S L T C L T S G F L P N D I G V E W T S N
653▶ G P P T E Q L T E Q T V S L T C L T S G F L P N D I G V E W T S N
BsrGI (2864)
2801 GGGCATATAGAAAAGAACTACAAGAACCAGGAGCAGTGATGGACTCTGACGGTCTTTCTTCATGTACAGCAAGCTCAATGTGAAAAGGAGCAGGTGGG
165▶ G H I E K N Y K N T E P V M D S D G S F F M Y S K L N V E R S R W
687▶ G H I E K N Y K N T E P V M D S D G S F F M Y S K L N V E R S R W
Ball (2998)
2901 ATAGCAGAGCGCCCTTCGTCTGCTCCGTGGTCCACGAGGGTCTGCACAATCACCACGTGGAGAAAGACATCTCCCGGCTCCGGGTAATGAGCTAGCTG
198▶ D S R A P F V C S V V H E G L H N H V E K S I S R P P G K •
720▶ D S R A P F V C S V V H E G L H N H V E K S I S R P P G K •
NheI (2992)
3001 GCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTA
HpaI (3130)
3101 TTTGTAACCATTATAAGTCGAATAAACAAGTTAAACAACAATTGCATTCATTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCA
AseI (3228)
XmnI (3226)
3201 AGTAAAACCTCTACAAATGTGGTATGAATTAATTCAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGG
3301 GATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGTATTTCCCA
SspI (3469) SwaI (3483)
3401 AGGTTTGAAGTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAAATAATTTAAATACATCATTG
3501 CAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACCT
ApaLI (3668)
3601 TTAATAGAAATTGGACAGCAAGAAGCGAGCTTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCGGCGGGGTGCGCGAGGG
125▶ D Q E E A V F H V C N G A P D R L A
3701 CGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTTCATGGCCGGCCGGAGGCGTCCCGAAAGTTCGTGGACACGACCTCCGACCCTCGCGCTACAG
106▶ 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 L
SgrAI (3898)
3801 CTCGTCCAGGCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCCTGGTCTGGACCAGCGTGTGAACAGGGTACGCTGCTCCCGGACCACA
73▶ 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 V
BssHII (3977)
3901 CCGGCAAGTCGTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCAGAACTCGACCCGCTCCGGCGACGTCGCGCGGTTGAGCACCAGGAAACGG
39▶ 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 A
Ball (4012)
4001 CACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagagaaggttagtacaattgCTATAGTGAGTTGTATTACTACTGCAGATA
6▶ S T L K A M
3▶ I A G
AseI (4112)
4100 TACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgcccattttctgactgccccatctctgccccacctttccaggcataga
HindIII (4239) SacII (4254)
4200 cagtcaagtacttacAAACTCACAGGAGGAGAAGGCAGAACTTGAGACAGACCCGCGGACCCGCAACTGCGAGGGGACGTGGCTAGGGCGGCTTC
StuI (4339) BspEI (4397)
4300 TTTTATGGTGCGCCGGCCCTCGAGGCAGGGCGCTCGGGGAGGCCTAGCGCCAATCTGCGGTGGCAGGAGGGCGGGCCGAAGGCCGTGCTGACCAATC
Bsp120I (4496)
4400 CGGAGCACATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTTGGGG
SpeI (4504)
4500 CCCTGACTAGTCAAAACAACTCCATTGACGCTCAATGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCA
SnaBI (4632)
4600 AAACCGCATCATCATGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTTCATGTAAGTGGGCATAATGCCAGGCGGGC
NdeI (4737)
4700 CATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCAAGTGGCAGTTTACCGTAAATACTCCACCCATTGAC
4800 GTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCATGGCGGGGGTCTTGGCGGTCAGCCAGCGGGCCATTTAC
PacI (4923)
SdaI (4915)
4900 CGTAAGTTATGTAACCCCTGCAGGTTAATTAAAGACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACCGTAAAAGGCCGCTTGTGGCGTTTTTCC
5000 ATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGACTATAAAGATACAGGCGTTTCCCTCGG
5100 AAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCGCCTTTCTCCCTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGC
ApaLI (5247)
5200 TGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAAGCTGGGCTGTGTGCACGAAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATC
5300 GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGGTT
5400 CTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCT
5500 TGATCCGGCAACAAACCCGCTGGTAGCGGTGGTTTTTTTGGTGAAGCAGCAGATTACGCCAGAAAAAAGGATCTAAGAAGATCCTTTGATCT
PacI (5663) SwaI (5672)
5600 TTTCTACGGGCTGACGCTCAGTGAACGAAACTCACGTTAAGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA