

# pFUSE-SEAP-hG4-Fc

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

Catalog # pfuse-hg40sp

For research use only

Version 22H30-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-SEAP-hG4-Fc 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 for 3 months.
- 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.

## 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 using QUANTI-Blue™ Solution (cat. code: rep-qbs).

## 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.

## 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.

### 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

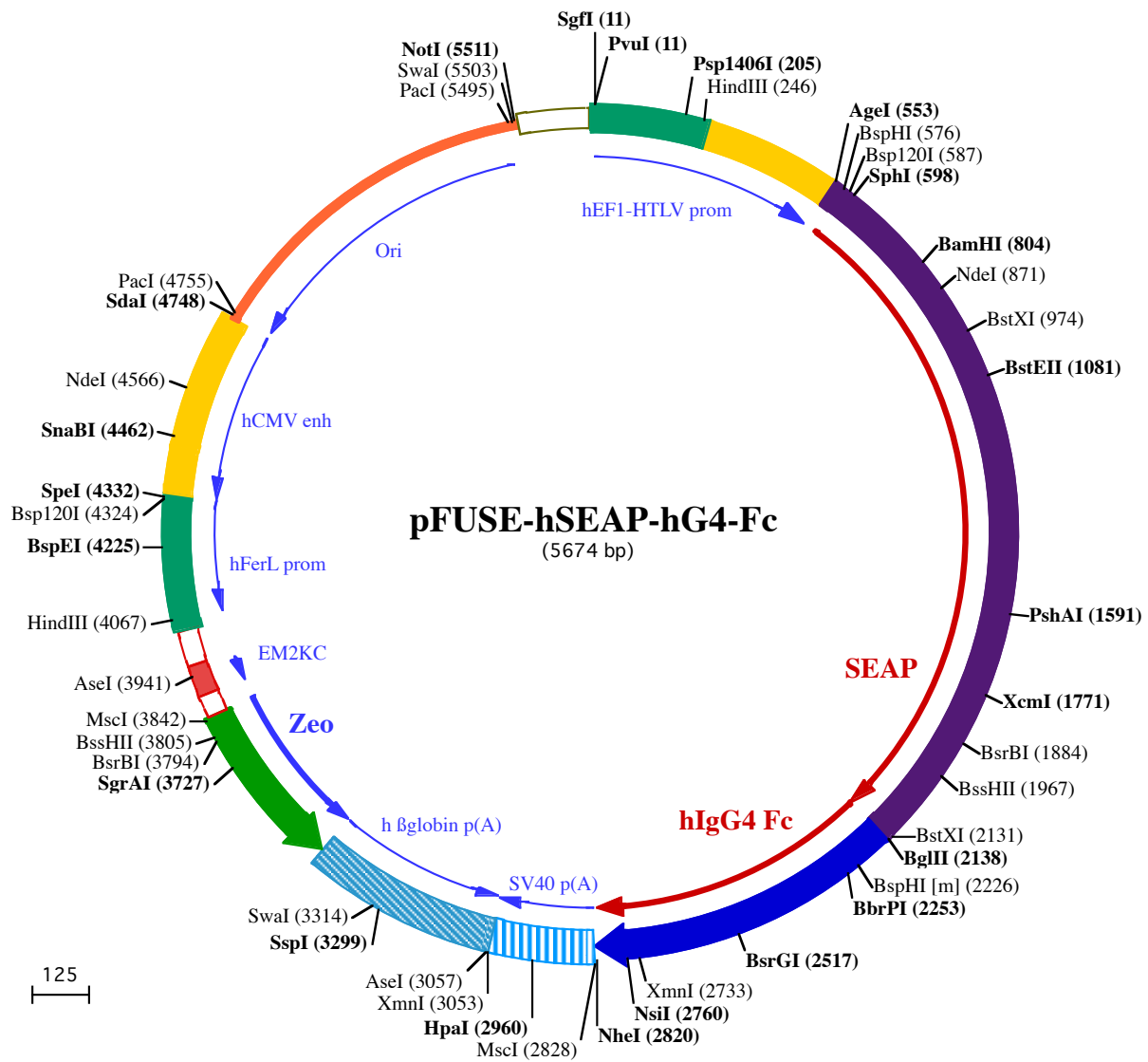
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

### 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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PvuI (11)  
SgfI (11)  
1 GGATCTGCATCGCTCCGGTGCCGTCAGTGGGAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA  
101 GAGAAAGTGGCGCGGGTAAACTGGAAAAGTATGTCGTGACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (205) HindIII (246)  
201 GTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCTCCTGAACTGCGTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
401 GGGCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

AgeI (553) BspHI (576) Bsp120I (587) SphI (598)  
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTTACAGTCTGAGGAGGCACATCATGATTCTGGGGCCCTGCATGCT  
601 GCTGCTGCTGCTGCTGCTGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCCGAGGAGCCGAG  
80 L 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  
701 GCCCTGGTGGCCCAAGAAGCTGCAGCTGCACAGACAGCCCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTG  
42 A L 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  
801 CCAGGATCCTAAAAGGGCAGAAGAAGGACAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGT  
75 A R I 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  
BamHI (804) NdeI (871) BstXI (974)  
901 AGACAAACATGTGCCAGACAGTGGAGCCACAGCCAGCGCTACCTGTGCGGGTCAAGGGCAACTCCAGACCATTGGCTTGTGAGTGCAGCGCCCGCTTT  
108 D K 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  
1001 AACCAGTGAACACGACACGCGCAACGAGGTCTCCGTGATGAATCGGGCAAGAAAGCAGGGAAGTCAAGTGGGAGTGGTAACCAACACACGAGTGC  
142 N Q C 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  
1101 AGCAGCCTGCCAGCCGACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACCGGACGTGCCTCGGCCCGCCAGGAGGGGTGCCAGGA  
175 Q H A S 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  
1201 CATCGCTACGAGCTCATCTCCAACATGGACATTGATGTGATCCTGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCA  
208 I A T Q L I S N M D I D V I L G G R K Y M F R M G T P D P E Y P  
1301 GATGACTACAGCCAAGTGGGACAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCAAGCGCCAGGTTGCCGGTATGTGTGAACCGCACTG  
242 D D Y S 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  
1401 AGCTCATGCAAGCTTCCCTGGACCGTCTGTGACCCATCTCATGGTCTCTTTGAGCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGA  
275 E L M Q A 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  
BstEII (1081) PshAI (1591)  
1501 CCCCTCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCGCGGCTTCTTCTCTTCTGAGGAGGTGGTCGCATCGACCAGGT  
308 P S L M 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  
1601 CATCAGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGCCAGCTCACCAGCGAGGAGGACAGCGTGA  
342 H H E S R 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  
XcmI (1771) BsrBI (1884)  
1701 GCCTGCTACTGCCGACCACTCCACGCTTCTCTTCCGAGGCTACCCCTGCGAGGAGCTCCATCTTCCGGCTGGCCCCTGGCAAGGCCCGGACAG  
375 S L V T A D 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  
1801 GAAGGCCTACACGGTCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAG  
408 K A Y T V 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  
BssHII (1967)  
1901 TATCGGCAGCAGTCAAGCAGTCCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGCGGTTCGCGCGCGCCCGAGGCGCACCTGGTTCACGGCG  
442 Y R Q Q S A 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  
2001 TGCAGGACGACCTTATAGCGCAGCTCATGGCTTCCGCGCTGCCTGGAGCCCTACACCGCTGCACCTGGCGCCCCCGCGGACCAACCGACGC  
475 V Q E Q T F I 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  
BstXI (2131) BglIII (2138)  
2101 CGCGACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATAGATCTCCCCATGCCATCATGCCAGCACCTGAGTTCCTGGGGGACCATCAGTCTTCTCTG  
508 A H P G R S R S K R L D R P P C P S C P A P E F L G G P S V F L  
BspHI [m] (2226) BbrPI (2253)  
2201 TTCCCCCAAACCCAAGGACACTCTCATGATCTCCCGGACCCTGAGTCAAGTCAAGTGGTGGTGGTGGAGTGGAGCAGGAAGACCCCGAGGTCAGTTCA  
20 F P P K P K D T L M I S R T P E V T C V V V D V S Q E D P E V Q F  
2301 ACTGGTACGTGGATGGCTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAAGCTCCTCACGCTCT  
53 N W Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V L T V L  
2401 GCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCAACAAAGGCTCCCGTCTCCATCGAGAAAACCATCTCAAAAGCAAAGGGCAG  
86 H Q D W L N G K E Y K C K V S N K G L P S S I E K T I S K A K G Q  
BsrGI (2517)  
2501 CCCCAGAGCCACAGGTGTACACCTGCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTACCCAGCG  
120 P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F Y P S  
2601 ACATCGCGTGGAGTGGGAGAGCAATGGGACGCGGAGACAACACTACAAGACCAGCCTCCGTGCTGGACTCCGACGGCTCTTCTCTCTACAGCAG  
153 D I A V E W E S N G O P E N N Y K T T P P V L D S D G S F F L Y S R

XmnI (2733) NsiI (2760)  
2701 GCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACAGAAGAGCCTCTCC  
186▶ L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K S L S

MscI (2828)  
NheI (2820)  
2801 CTGTCTCIGGGTAAATAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATT  
220▶ L S L G K •

HpaI (2960)  
2901 TGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATGTTTCAGGTTCCAGG

AseI (3057)  
XmnI (3053)  
3001 GGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCCAATCA  
3101 AGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTTCTTTTCATG

SspI (3299)  
3201 GAGTTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATAGTAAAAA

SwaI (3314)  
3301 TTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGT

3401 AGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGCA  
125◀ • D Q E E A V F H V

3501 CGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGA  
115◀ C N G A P D R 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

3601 CACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGCTGATG  
82◀ V V E S W E A Y 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

SgrAI (3727) BsrBI (3794)  
3701 AACAGGGTCACGTGCTCCCGACCACACCGGCGAAGTCTGCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGGTCCAGAACTCGACCGCTCCGGCGA  
48◀ F L T V D D R V 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

BssHII (3805) MscI (3842)  
3801 CGTCGCGCGGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCTCctgtcaggagaggaaagagaagaaggtagtacaattgCTATA  
15◀ D R A T L V P V A S T L K A M

AseI (3941)  
3901 GTGAGTTGTATTACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAggggtcatagtgccacttttctgactgccccatct

HindIII (4067)  
4001 cctgcccaccctttcccagcatagacagtccagtgacttacCAAACCTACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCGAAGCTG  
4101 CGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCAGCCGCGCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTCGGTGGCAGGAGGGC

BspEI (4225)  
4201 GGGCCGAAGGCCGTGCTGACCAATCCGGAGCAGATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGT

SpeI (4332)  
Bsp120I (4324)  
4301 GAAATGGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAACCAAACCTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGC

SnaBI (4462)  
4401 TATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCA

NdeI (4566)  
4501 TGTAAGTGGCATAATGCCAGGCGGGCCATTTACCGTCAATGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCAGT  
4601 TTACCGTAAATACTCCACCCATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATATTGACGTCAATGGGCGGGGCTCGTTG

PacI (4755)  
SdaI (4748)  
4701 GCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAA  
4801 AAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAAGGTGGCGAAACCCGACAGGACTA  
4901 TAAAGTACCAGCGTTTTCCCTGGAAGTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAA  
5001 GCGTGGCGCTTTCTCATAGCTCAGCTGAGGTATCTCAGTTCGGTGTAGGCTGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGA  
5101 CCGCTGCGCTTATCCGGTAACTATCGTCTTGGTCCAAACCGGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG

5201 AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA

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5301 CCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCCTGGTAGCGGTGGTTTTTTTGTTCGCAAGCAGCAGATTACGCGCAGAAAAA

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5401 AGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACA

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PacI (5495) SwaI (5503)

**NotI (5511)**

5501 TTAAATCAGCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAA

5601 AACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGAAGTGCAAGTGCCAGGTGCCAGAACATTTCTCTATCGAA