

# pSELECT-zeo-mSEAP-CpG-free

An expression plasmid coding for a CpG-free murine SEAP gene

Catalog code: psetz-mseap

<https://www.invivogen.com/pselect-zeo-seap-cpg-free>

For research use only

Version 24G18-MM

## PRODUCT INFORMATION

### Contents:

- 20 µg of pSELECT-zeo-mSEAP-CpG-free 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.
- Resuspended DNA should be stored at -20 °C and is stable for 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 full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

## GENERAL PRODUCT USE

pSelect-zeo plasmids contain genes that have been chemically synthesized. The DNA sequence of these genes was modified by optimizing the codon usage, reducing or eliminating the CpG motifs and avoiding secondary DNA structures without changing the amino acid sequence of the wild type proteins.

### pSELECT-zeo plasmids may be used:

**To subclone the synthetic gene into another vector.** To facilitate subcloning, the murine SEAP gene is flanked by two unique restriction sites: Age I at the 5' end and Nhe I at the 3' end.

**As a gene reporter plasmid.** pSELECT-zeo is a mammalian expression plasmid selectable in *E. coli* and mammalian cells with Zeocin®, as the *Sh ble* gene in the second expression cassette is driven by the eukaryote CMV enhancer/promoter in tandem with the bacterial EM7 promoter.

## PLASMID FEATURES

• ori: a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

### First expression cassette

• hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1alpha (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.

• mSEAP CpG-free: Synthetic murine secreted alkaline phosphatase gene. InvivoGen has synthesized a CpG-free murine SEAP gene. The native mSEAP has 65 CpG-motifs.

• 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>.

### Second expression cassette

- CMV enh/prom: The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- Zeo: Resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing 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>.

1. Kim D. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system *Gene* 91(2):217-23.
2. Takebe, Y. *et al.*, 1988. R 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.* 1:466-72.
3. Carswell S. & Alwine J., 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. 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.

## 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.

## TECHNICAL SUPPORT

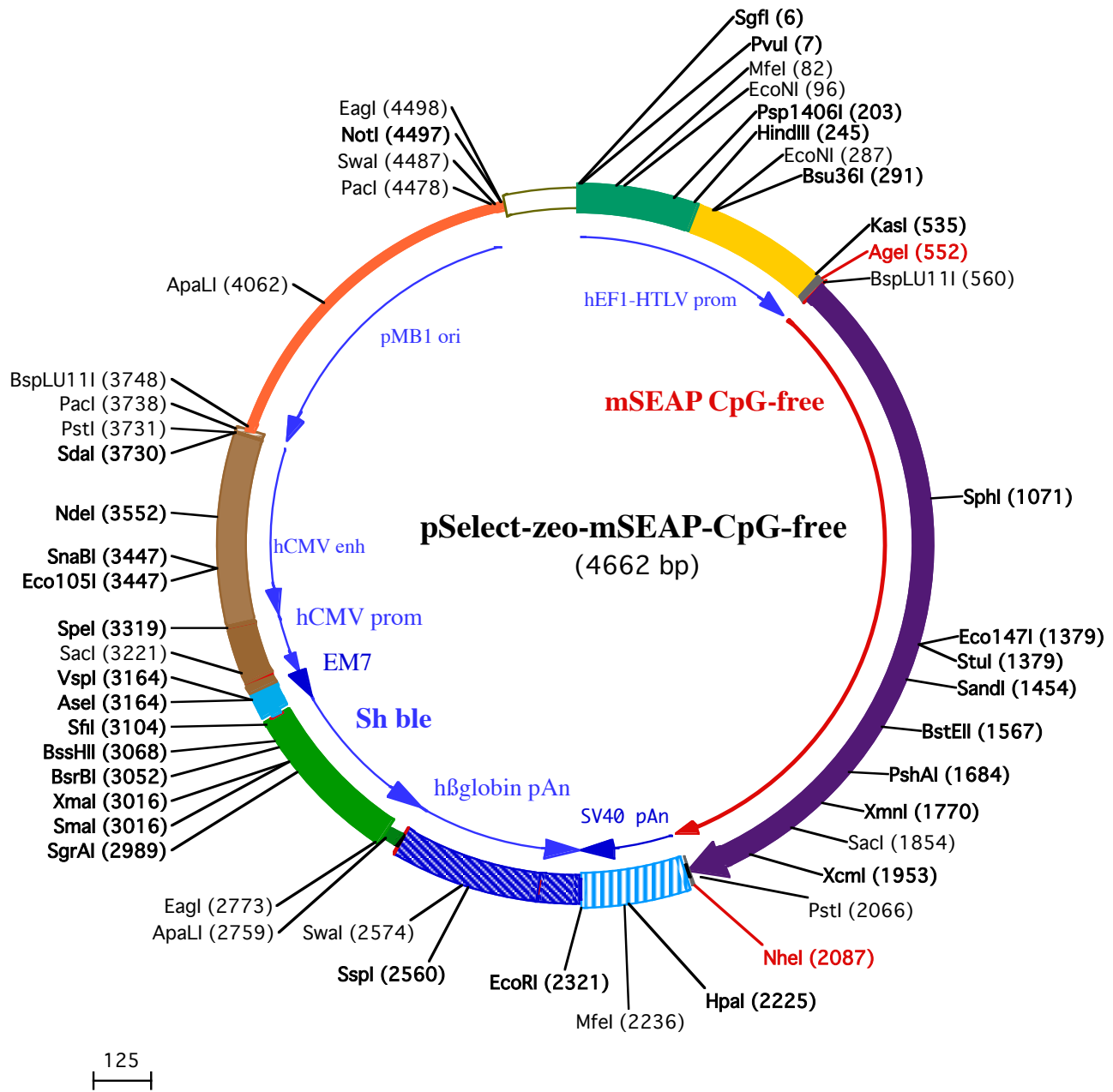
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**PvuI (7)** **SgfI (6)** **MfeI (82)** **EcoN I (96)**  
 1 GGATCTGCGATCGCTCCGGTGCCTGTCAGTGGGAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA  
 101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGCTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

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**Bsu36I (29)**  
**Psp1406I (203)** **HindIII (245)** **EcoNI (287)**  
 201 GTGAACGTTCTTTTTGCGAACGGGTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTTCACGGCGCCGCCGCCCTACCTGAGGCC  
 301 GCCATCCACGCGGTTGAGTTCGGCTTCTGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACC  
 401 GGGCCTTTGTCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCTCAACTCTACGCTTTTGTTCGTTT

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**KasI (535)** **AgeI (552)** **BspLU11I (560)**  
 501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTCAACATGTGGGTGCCTGTCTGCTATTGCTGGCTTAAGTCT  
 601 TCAAGTTTGCCCAAGTGTCTTCTGTGGAGGAGGAGAATCCTGCTTTTGAATAGGAAGGAGCTGAAGCCTTGGATGCAGCAAGAAGCTCAAGCCC  
 13▶ Q V C P S V I P V E E E N P A F W N R K A A E A L D A A K K L K P  
 701 ATTCAGACATCTGCAAGAATCTTGTATCCTCATGGGTGATGGAATGGGTGTCTCACTGTAACAGCCAGGATTCTGAAGGGCCAGCAACAAGGTC  
 47▶ I Q T S A K N L V I L M G D G M G V S T V T A T R I L K G Q Q Q G  
 801 ATCTAGGCCAGAGACCCAGTTGGCAATGGACAGGTTCCCTCAGTGGCCCTTCCAAGACTTACAACACTGACAAGCAGATTCTGACTCTGCTGGGAC  
 80▶ H L G P E T Q L A M D R F P H M A L S K T Y N T D K Q I P D S A G T  
 901 AGGCACAGCATTCTGTGGAGTAAAAACCAACATGAAAGTCATTGGTCTTTGAGTCTGCTGACAGATTCAACAGTGAACACCCACATGGGGCAATGAA  
 113▶ G T A F L C G V K T N M K V I G L S A A A R F N Q C N T T W G N E

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**SphI (1071)**  
 1001 GTGGTCTCTGAATGCACAGGGCCAAAAAGCTGGGAAAAGTGTGGTGTGGTGAACACCTCTGTCCAGCATGCCTCTCTGCTGGAACCTTATGCC  
 147▶ V V S V M H R A K K A G K S V G V V T T T S V Q H A S P A G T Y A  
 1101 ACACAGTGAACAGAGGTTGGTACTCTGATGCTCAGATGCCTGCCTCAGCTTACAAGATGGCTGCAAGGACATCAGCACCCAGCTCATCTCAAAACATGGA  
 180▶ H T V N R G W Y S D A Q M P A S A L Q D G C K D I S T Q L I S N M D  
 1201 CATAGATGTCATCTTAGGGGTGGGAGAAAGTTCATGTTCCAAAGGGGACTCTGACCAGGAGTACCCACAGACAAAAGCAGGCTGGCACAAGATTA  
 213▶ I D V I L G G R K F M F P K G T P D Q E Y P T D T K Q A G T R L

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**StuI (1379)** **Eco147I (1379)**  
 1301 GATGGTAGGAACCTTGTGCAAGAGTGGCTGCCAAGCATCAGGGAGCAAGGTATGTCTGGAACAGGAGTGAAGTAAATCCAGGCCTCTTTGAACAGGTCG  
 247▶ D G R N L V Q E W L A K H Q G A R Y V W N R S E L I Q A S L N R S

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**SacI (1454)**  
 1401 TCACTCACCTAATGGGTTATTTGAGCCCAATGACATGAAGTATGAGATACACAGGGACCCTGCCAGGACCCTCTTAGCAGAAATGACTGAAGTTGC  
 280▶ V T H L M G L F E P N D M K Y E I H R D P A Q D P S L A E M T E V A

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**BstEII (1567)**  
 1501 TGTGAGGATGTTGCCAGAAATCAAAGGGTTTACCTCTTTGTTGAGGGGGGAAGGATTGATCATGGTCACCATGAGCAGTTGCTTACAGAGCCTTA  
 313▶ V R M L S R N P K G F Y L F V E G G R I D H G H H E T V A Y R A L

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**PshAI (1684)**  
 1601 ACTGAGGCTGTGATGTTTATTCTGCTGTGGACAAGGCTGACAAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCAAGCAGTCAATGTTT  
 347▶ T E A V M F D S A V D K A D K L T S E Q D T M I L V T A D H S H V

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**XmnI (1770)**  
 1701 TCTCCTTTGGGGCTACACCCAGAGGGGTGCTCAATCTTTGGCCTGGCCCTTTCAAGGCAGAAGTGGGAAGAGTTTACCTCCATCCTCTATGGGAA  
 380▶ F S F G G Y T Q R G A S I F G L A P F K A E D G K S F T S I L Y G N

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**SacI (1854)**  
 1801 TGGTCTGGGTACAAGTGCACAATGGGGCCAGAGTGTGTGACAGAAGAGGAGAGTCCCAACCACTACCAGCAGCAAGCAGCAGTCCCTCTTTCT  
 413▶ G P G Y K L H N G A R A D V T E E E S S N P T Y Q Q Q A A V P L S

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**XcmI (1953)**  
 1901 TCAGAAACCACTCTGGGAAGATGTGGCCATATTTGCCAGAGGCCCAAGCCACTTGGTGCATGGAGTTCAGGAGCAGAATTACATAGCTCATGTAA  
 447▶ S E T H S G E D V A I F A R G P Q A H L V H G V Q E Q N Y I A H V

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**PstI (2066)** **NheI (2087)**  
 2001 TGGCTTTTGTGCTTCTTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCGAGTCTCTGAGTAAGCCAGGCTAGAGCTAGCTGGCCAG  
 480▶ M A F A A C L E P Y T D C G L A S P A G Q S S A V S P G •  
 2101 ACATGATAAGATACATTGATGAGTTTGGACAACCACTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGT

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**HpaI (2225)** **MfeI (2236)**  
 2201 AACCATTAAGTGCATAAACAAGTTAAACAACAACAAATTCATTCTTTATGTTTCAGGTTTCAGGGGAGGTGTGGAGGTTTTTAAAGCAAGTAA

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**EcoRI (2321)**  
 2301 AACCTCTACAAATGGTATGGAATTTCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAA  
 2401 GGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTCTTTTATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGA

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**SspI (2560)** **Swal (2574)**  
 2501 CTAGCTCTTACTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATGATAAATATTCAGAAATAATTTAAATACATTCATGCAATGAAAA  
 2601 TAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGAACCTTTAATAGAA

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**ApaI (2759)** **EagI (2773)**  
 2701 ATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTCTGCCACAAAGTGCACGAGTTGCCGGCCGGTGCAGGGCGAACTCCC

2801 G C C C C C A C G G T G C T G C C G A T C T G G T C A T G G C C G G C C C G A G G C G T C C C G G A A G T T C G T G G A C A C G A C T C C G A C C A C T C G G C G T A C A G C T C G T C C A G

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 **SgrAI (2989)**

2901 G C C G C G C A C C C A C C C A G G C C A G G G T G T T G T C C G G C A C C A C T G G T C C T G G A C C G C G T G A T G A A C A G G G T C A C G T C G T C C C G G A C C A C C C G G C G A A G

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

**XmaI (3016)**  
**SmaI (3016)**  
**BsrBI (3052)** **BssHII (3068)**

3001 T C G T C C T C C A C G A A G T C C C G G G A G A A C C C G A G C C G G T C G G T C C A G A A C T C G A C C G T C C G G C G A C G T C G C G C G G T G A G C A C C G G A A C G G C A C T G G T C A

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

**SfiI (3104)** **VspI (3164)**  
**Asel (3164)**

3101 A C T T G G C C A T G A T G G C C C T C T A T A G T G A G T C G T A T T A T A C T A T G C C G A T A T A C T A T G C C G A T G A T T A A T T G T C A A A A C A G C G T G G A T G G C G T C T C C A G C

3 K A M

**SacI (3221)**

3201 T T A T C T G A C G G T T C A C T A A A C G A G C T C T G C T T A T A T A G A C C T C C C A C C G T A C A C G C C T A C C G C C A T T T G C G T C A A T G G G G C G G A G T T G T T A C G A C A T T

**SpeI (3319)**

3301 T G G A A G T C C C G T T G A T T A C T A G T C A A A A C A A A C T C C C A T T G A C G T C A A T G G G G T G G A G A C T T G G A A A T C C C C G T G A G T C A A A C C G T A T C C A C G C C C A

**SnaBI (3447)**  
**Eco105I (3447)**

3401 T T G A T G T A C T G C C A A A A C C G A T C A T C A T G G T A A T A G C G A T G A C T A A T A C G T A G A T G T A C T G C C A A G T A G G A A A G T C C C A T A A G G T C A T G T A C T G G G C A T

**NdeI (3552)**

3501 A A T G C C A G G C G G C C A T T T A C C G T C A T T G A C G T C A A T A G G G G C G T A C T T G G C A T A T G A T A C A C T T G A T G T A C T G C C A A G T G G G C A G T T T A C C G T A A A T A

3601 C T C C A C C A T T G A C G T C A A T G G A A A G T C C C T A T T G G C G T T A C T A T G G G A A C A T A C G T C A T T A T T G A C G T C A A T G G G C G G G G T C G T T G G G C G G T C A G C C A

**PacI (3738)**  
**PstI (3731)**  
**SdaI (3730)** **BspLU11I (3748)**

3701 G G C G G G C A T T T A C C G T A A G T T A T G T A A C G C T G C A G G T T A A T T A A G A A C A T G T G A G C A A A A G G C C A G A A A A G C C A G G A A C C G T A A A A G C C G C G T T

3801 G C T G G C G T T T T T C A T A G G C T C G C C C C C T G A C G A G C A T C A C A A A A T C G A C G C T C A A G T C A G A G G T G G C G A A A C C C G A C A G G A C T A T A A A G A T A C C A G

3901 G C G T T T C C C C T G G A A G C T C C C T C G T G C G C T C C T G T T C C G A C C C T G C C G T T A C C G G A T A C C T G T C C G C T T T C T C C T T C G G G A A G C G T G C G C T T T

**ApaLI (4062)**

4001 C T C A T A G T C A C G C T G T A G G T A T C T C A G T T C G G T G T A G G T C G T T C G C T C C A A G C T G G G C T G T G T G C A C G A A C C C C C G T T C A G C C C G A C C G C T G C G C T T

4101 A T C C G G T A A C T A T C G T T T G A G T C C A A C C G G T A A G A C A C G A C T T A T C G C C A C T G G C A G C A G C C A C T G G T A A C A G G A T T A G C A G A G C G A G G T A T G T A G G C

4201 G G T G T A C A G A G T T C T T G A A G T G G T G C C T A A C T A C G G T A C A C T A G A A G A A C A G T A T T T G G T A T C T G C G C T C T G C T G A A G C C A G T T A C C T T C G G A A A A

4301 G A G T T G G T A G C T T T G A T C C G G C A A A C A A A C C A C C G C T G G T A G C G G T G G T T T T T T G T T G C A A G C A G C A G A T T A C G C G C A G A A A A A A A G G A T C T C A A G A

**Swal (4487)** **EagI (4498)**  
**PacI (4478)** **NotI (4497)**

4401 A G A T C T T T G A T C T T T T C A C G G G T C T G A C G C T C A G T G G A A C G A A A A C T C A C G T T A A G G G A T T T T G T C A T G G C T A G T T A A T T A A C A T T T A A A T C A G C G

4501 G C C G C A A T A A A A T A T C T T T A T T T T C A T T A C A T C T G T G T T G G T T T T T G T G T G A A T C G T A A C T A A C A T A C G C T C C A T C A A A A C A A A A C G A A A C A A A A

4601 C A A A C T A G C A A A A T A G G C T G T C C C C A G T G C A A G T G C A G G T G C C A G A A C A T T T C T A T C G A A