

# pSELECT-zeo-mSEAP

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

Catalog code: psetz-mseap

For research use only

Version 20L01-MM

## PRODUCT INFORMATION

### Contents:

- 20 µg of pSELECT-zeo-mSEAP 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**

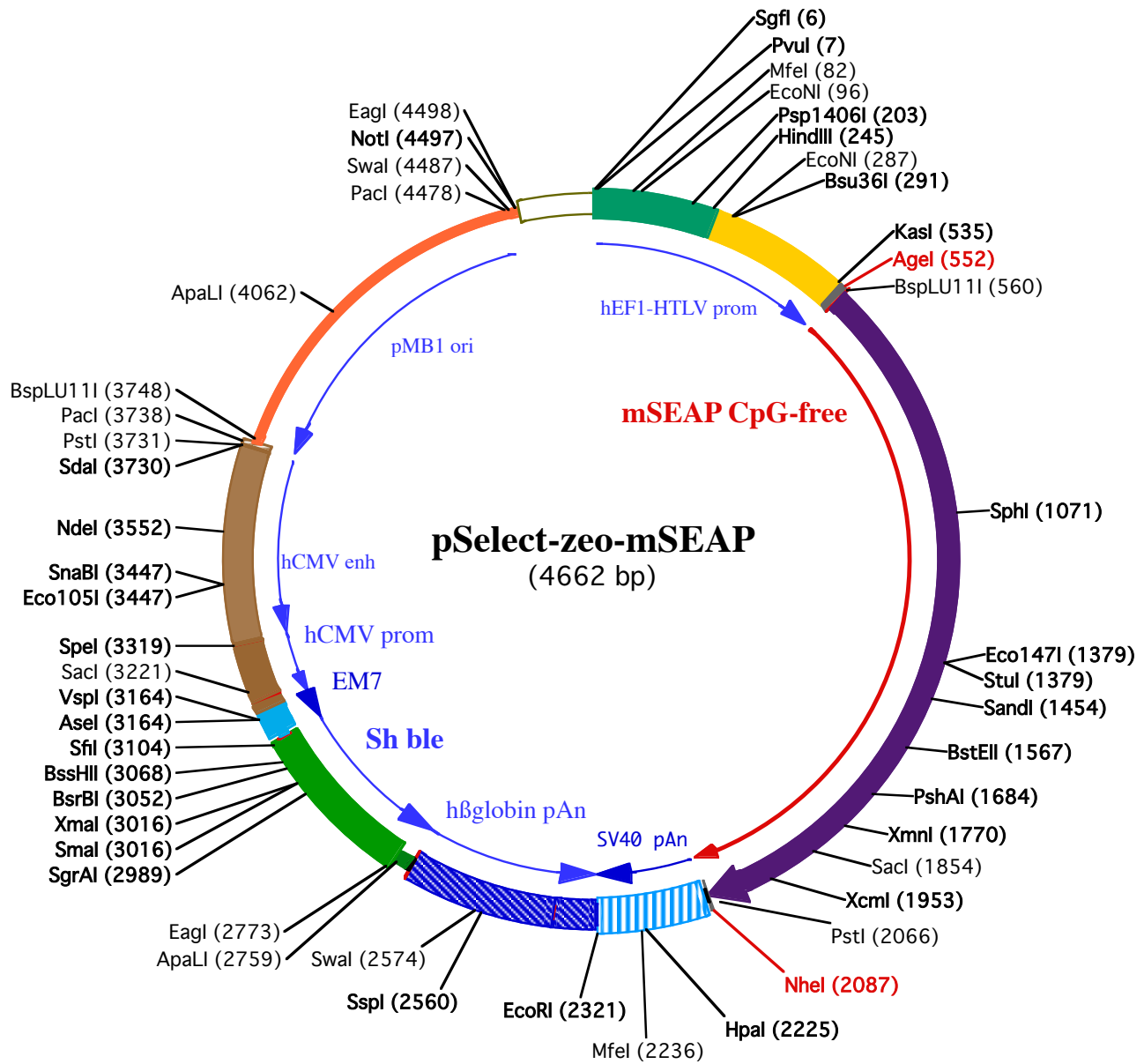
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

### **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

InvivoGen USA (Toll-Free): 888-457-5873  
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InvivoGen Europe: +33 (0) 5-62-71-69-39  
InvivoGen Hong Kong: +852 3622-3480  
E-mail: [info@invivogen.com](mailto:info@invivogen.com)



**PvuI (7)** **SgfI (6)** **MfeI (82)** **EcoN I (96)**  
 1 GGATCTGCGATCGCTCCGGTGCCTGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA  
 101 GAGAAGTGGCGCGGGTAAACTGGGAAAGTATGTCGTACTGGTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

**Bsu36I (29)**  
**Psp1406I (203)** **HindIII (245)** **EcoNI (287)**  
 201 GTGAACGTTCTTTTTCGCAACGGGTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCCTACCTGAGGCC  
 301 GCCATCCACGCGGTTGAGTTCGGCTTCTGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCCGTAGGTAAGTTAAAGCTCAGGTCGAGACC  
 401 GGGCCTTTGTCGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCTCAACTCTACGCTTTTTCGTTT

**KasI (535)** **AgeI (552)** **BspLU11I (560)**  
 501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTCAACATGTGGGTGCCTGTCTGCTATTGCTGGCTTAAGTCT  
 601 TCAAGTTTGCCCAAGTGTCTTCTGTGGAGGAGGAGAATCCTGCTTTTGAATAGGAAGGAGCTGAAGCCTTGGATGCAGCAAGAAGCTCAAGCCC  
 701 ATTGAGCATCTGCAAGAATCTTGTCTCCTCATGGTGTGGAATGGGTGTCTCCTGTAACAGCCAGGATTCTGAAGGGCCAGCAACAGGTC  
 801 ATCTAGGCCAGAGACCCAGTTGGCAATGGACAGGTTCCCTCAGTGGCCCTTCCAAGACTTACAACACTGACAAGCAGATTCTGACTCTGCTGGGAC  
 901 AGGCACAGCATTCTGTGGAGTAAAAACCAACATGAAAGTCAATGGTCTTTGAGTCTGCTGCAAGATTCAACAGTGAACACCCATGGGGCAATGAA  
 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

**SphI (1071)**  
 1001 GTGGTCTCTGAATGCACAGGCCAAAAAGCTGGGAAAAGTGTGGTGTGGTGAACACCTCTGTCCAGCATGCCTCTCTGCTGGAACCTTATGCC  
 1101 ACACAGTGAACAGAGGTTGGTACTCTGATGCTCAGATGCCTGCCTCAGCTTACAAGATGGCTGCAAGGACATCAGCACCCAGCTCATCAAAACATGGA  
 1201 CATAGATGTCATCTTAGGGGTGGGAGAAAGTTCATGTTCCAAAGGGGACTCCTGACCAGGAGTACCCACAGACAAAAGCAGGCTGGCACAAGATTA  
 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

**StuI (1379)** **Eco147I (1379)**  
 1301 GATGGTAGGAACCTTGTGCAAGAGTGGCTGCCAAGCATCAGGGAGCAAGGTATGTCTGGAACAGGAGTGAAGTAAATCCAGGCCTCTTTGAACAGTCTG  
 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

**SacI (1854)**  
 1801 TGGTCTGGGTACAAGTGCACAATGGGCGCAGAGTGTGACAGAAGAGGAGAGTCCCAACCACTACCAGCAGCAAGCAGCAGTCCCTCTTTCT  
 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

**BstEII (1567)**  
 1501 TGTGAGGATGTTGCCAGAAATCAAAGGGTCTACCTCTTTGTTGAGGGGGGAAGGATTGATCATGGTCACCATGAGCAGTTGCTTACAGAGCCTTA  
 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

**PshAI (1684)**  
 1601 ACTGAGGCTGTGATGTTGATTCTGCTGTGGACAAGGCTGACAACTGACCTCTGAGCAGGACACAATGATTCTAGTACTGCTGACCAAGTCAATGTTT  
 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

**XmnI (1770)**  
 1701 TCTCCTTTGGGGCTACCCAGAGGGGTGCTCAATCTTTGGCCTGGCCCTTTCAAGGCAGAAGTGGGAAGAGTTTACCTCCATCCTCTATGGGAA  
 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

**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

**PstI (2066)** **NheI (2087)**  
 2001 TGGCTTTTGTGCTTGTGGAGCCCTACACAGACTGTGGCTAGCCAGCCAGCAGGCGAGTCTCTGAGTAAAGCCAGGCTAGAGCTAGCTGGCCAG  
 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 ACATGATAAGATACATTGATGAGTTTGGACAACCACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGT

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

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

**SspI (2560)** **Swal (2574)**  
 2501 CTAGCTCTTACTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATAGTAAATATTCAGAAATAATTTAAATACATTCATGCAATGAAAA  
 2601 TAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTATGAGTTGGACTTAGGGAACAAGAACCTTTAATAGAA

**ApaLI (2759)** **EagI (2773)**  
 2701 ATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCTCTGCCACAAAGTGCACGAGTTGCCGGCCGGTTCGCCAGGGCGAACTCCC

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  
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 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)  
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 AseI (3164)  
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  
SacI (3221)  
3301 T G G A 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  
SpeI (3319)  
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  
SnaBI (3447)  
Eco105I (3447)  
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  
NdeI (3552)  
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)  
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  
SdaI (3730) BspLU11I (3748)  
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 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 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 C A C G A A 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 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)  
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 G 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  
PacI (4478) NotI (4497)  
4501 G C C G C A A T A A A A T A 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 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