pSELECT-zeo-hSEAP

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

Catalog code: psetz-hseap

For research use only

Version 20L01-MMv35

PRODUCT INFORMATION

Contents:

• 20 µg of pSELECT-zeo-hSEAP 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 unique restriction sites: Age I and Nco I at the 5' end, and Nhe I at the 3'end.

Note: Nco I encompasses the Start codon.

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 casssette 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[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.

• hSEAP CpG-free: Synthetic human secreted alkaline phosphatase gene. InvivoGen has synthesized a CpG-free human SEAP gene. The native hSEAP gene contains 109 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[3].

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*.

• ßGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

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_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.

TECHNICAL SUPPORT
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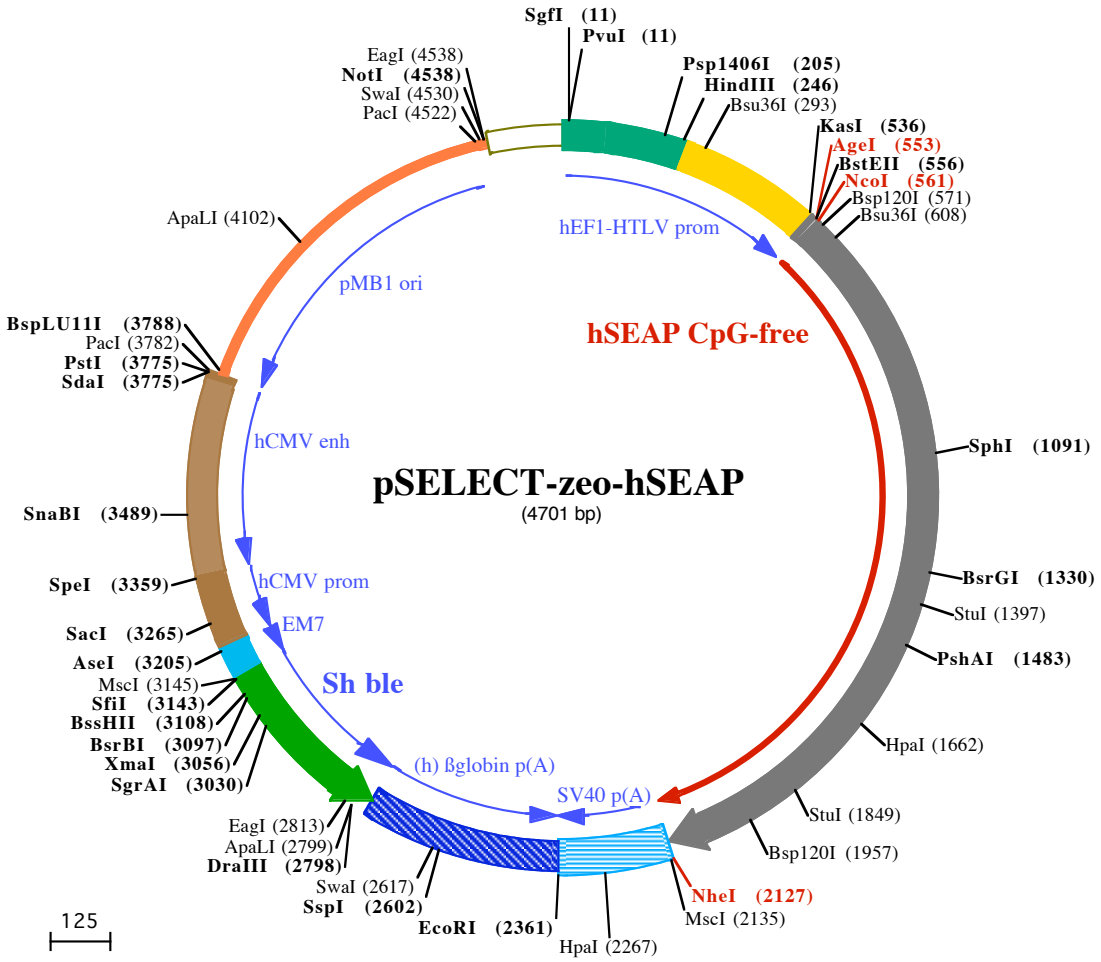

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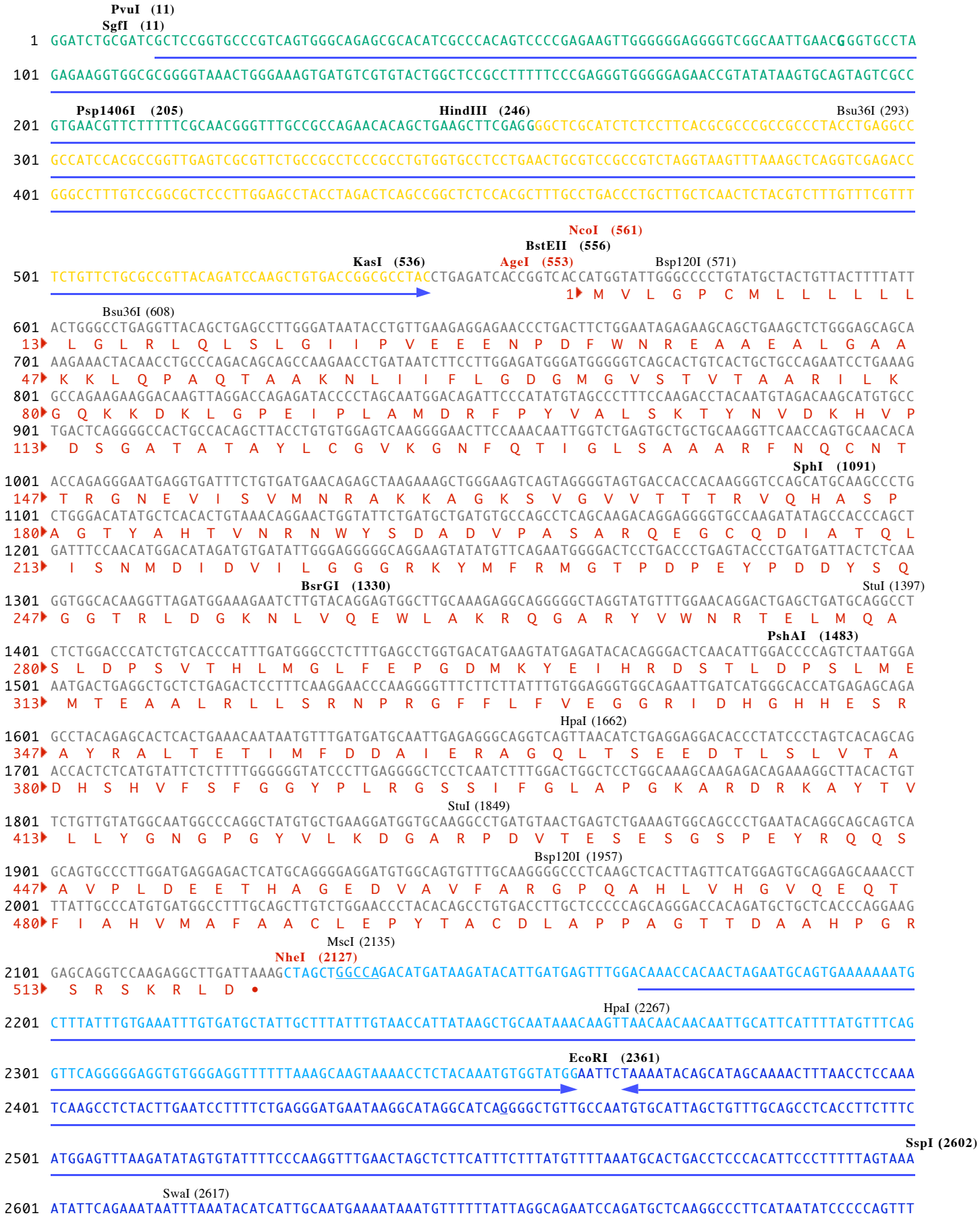
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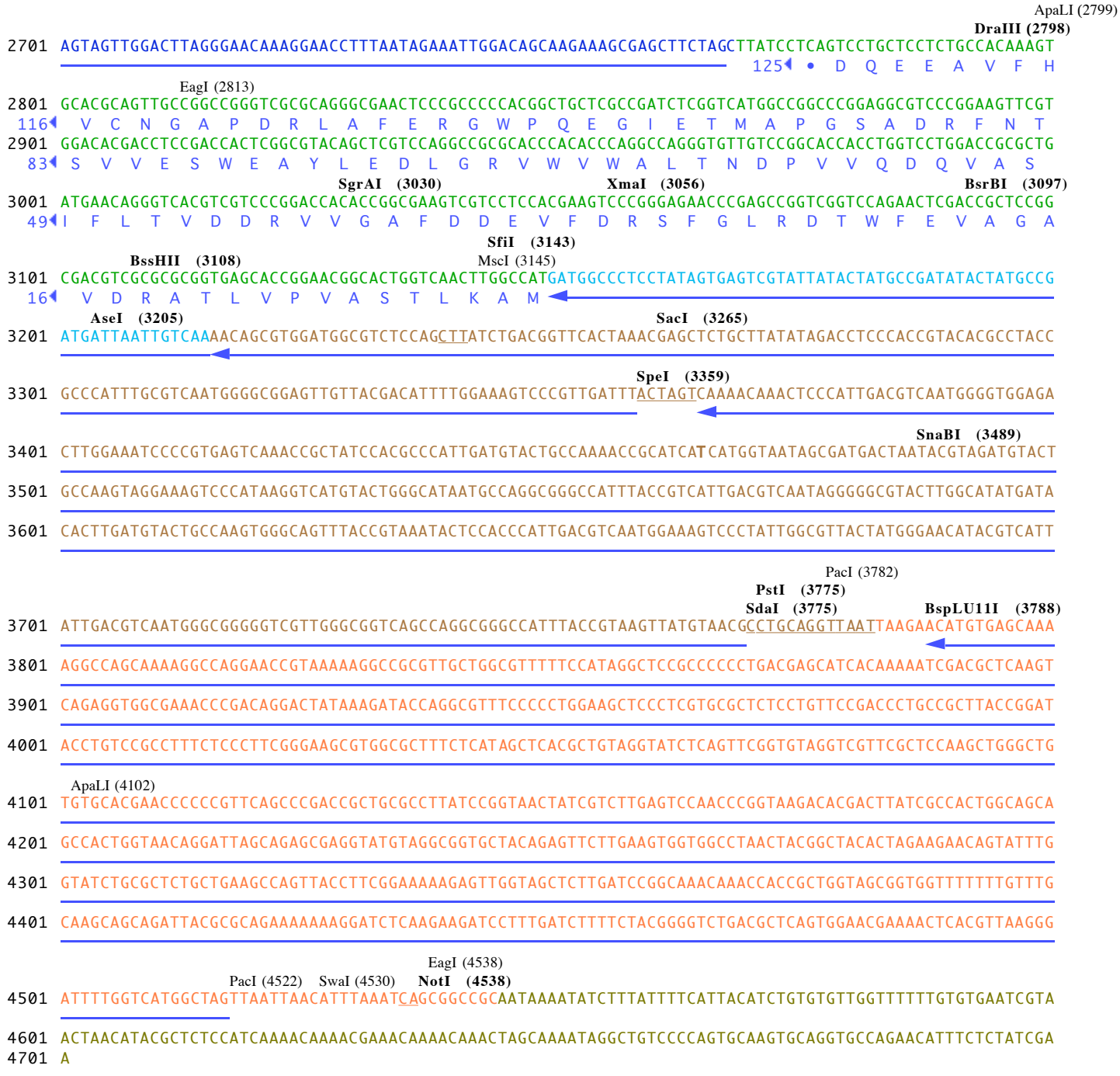
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