

# pSELECT-zeo-GFP::Sh

A plasmid encoding a CpG-free GFP-Zeocin resistance fusion gene

Catalog code: psetz-zgfpsh

For research use only

Version 23F26v03-JC

## PRODUCT INFORMATION

### Content:

- 20 µg of pSELECT-zeo-GFP::Sh 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 resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable 3 months at -20°C. Resuspended DNA is stable more than one year 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

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 GFP::Sh gene is flanked by two unique restriction sites: Nco I at the 5' end that encompasses the Start codon, 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

### 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.

• **GFP::Sh CpG-free:** InvivoGen has engineered a fusion gene between the red-shifted variant of the jellyfish GFP gene that encodes a green fluorescent protein and the *Sh ble* gene conferring Zeocin™ resistance. Both genes have been modified and contain no CpG motifs, whereas their wildtype counterparts contain 60 and 50 CpG motifs respectively. This GFP::Sh fusion protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

• **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.

### 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.W. *et al.* (1990). *Gene* 2: 217-223.

2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.

3. Carswell, S. & Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.

4. Yu J & Russell JE. (2001). *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 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

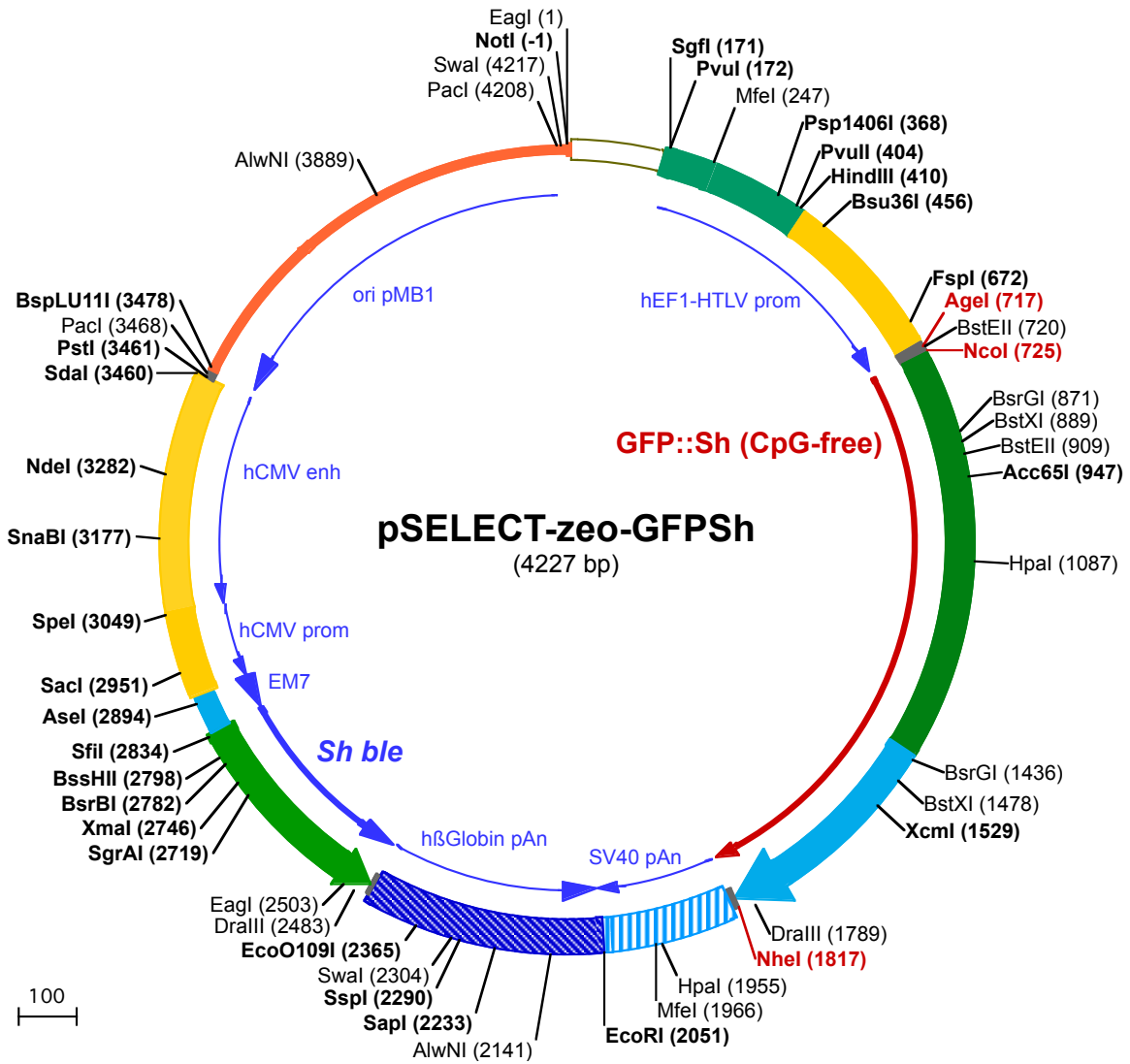
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EagI (1)  
NotI (-1)  
1 GCGGCCCAATAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACATAAGCTCTCCATCAAAACAAAACGAAACA  
PvuII (172)  
SgfI (171)  
101 AAACAACTAGCAAAATAGGCTGTCCCCAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGCA  
MfeI (247)  
201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTTCGGCAATTGAACGGTGCCTAGAGAAGTGGCGCGGGTAAACTGGGAAAGTATG  
Psp1406I (368)  
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG  
HindIII (410)  
PvuII (404) Bsu36I (456)  
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGCGCCCATCCACGCCGTTGAGTCGGCTTCTGCCGCT  
501 CCCGCTGTGGTCCCTGAACTGCGTCCGCCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTA  
FspI (672)  
601 GACTCAGCCGGCTCTCCACGTTTGCCTGACCCTGCTGCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACC  
BstEII (720)  
AgeI (717) NcoI (725)  
701 GCGCCTACCTGAGATCACCGTCCACCATGGTTCTAAGGAGAAGAACTCTTTACTGGTGTGTCCAATTCTGTTGAGCTGGATGGTGTGTGAATG  
1 M V S K G E E L F T G V V P I L V E L D G D V N  
BsrGI (871) BstXI (889)  
801 GCCACAAATTTCTGTGTCTGGTGAAGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCAATTTGTACAACAGGAAAGCTGCCAGTGCCTTG  
25 G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W  
BstEII (909) Acc65I (947)  
901 GCCAACTCTGGTACCACCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAA  
58 P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E  
HpaI (1087)  
1001 GGTATGTTCCAGGAGAGACAATCTTCTTAAGGATGATGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGTTAACAGAATTG  
92 G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I  
1101 AGCTGAAAGGCAATTGATTTTAAAGGAAGTGAACATTTCTGGTCCAAAGCTGGAGTACAACATAATTTCTACAATGTTTACATTATGCCAGATAAGCA  
125 E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q  
1201 GAGGAATGGAATTAAGGTAATTTAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTCCAGACATTACCAGCAGAACACCCCTATTGGTGAT  
158 R N G I K A N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D  
1301 GGCCAGTTCTCCTCCAGATAATCACTATCTCAGCACTCAATCTGCTGTGCTCAAGACCCTAATGAGAAAAGACCACATGGTCTCCTGGAGTTTG  
192 G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F  
BsrGI (1436) BstXI (1478)  
1401 TGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGGCCAAGTTGACCAGTGCTGTCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGT  
225 V T A A G I T L G M D E L Y K A K L T S A V P V L T A R D V A G A V  
XcmI (1529)  
1501 TGATTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCCCTGTTTCATCTCAGCAGTC  
258 E F W T D R L G F S R D F V E D D F A G V V R D D V T L F I S A V  
1601 CAGGACCAGTGGTCCGTCACAACCCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGGTGCTCCACCAACTCA  
292 Q D Q V V P D N T L A W V W V R G L D E L Y A E W S E V V S T N F  
DraIII (1789)  
1701 GGGATGCCAGTGGCCTGCCATGACAGAGATTGGAGAGCAGCCTGGGGGAGAGATTTGCCCTGAGAGACCAGCAGGCAACTGTGTGCACTTTGTGGC  
325 R D A S G P A M T E I G E Q P W G R E F A L R D P A G N C V H F V A  
NheI (1817)  
1801 AGAGGAGCAGGACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTAAAAAATGCTTTATTTG  
358 E E Q D •  
HpaI (1955) MfeI (1966)  
1901 TGAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAACAAGTTAAACAACAACATTGCATTATTTATGTTTCAGGTTACAGGG  
EcoRI (2051)  
2001 GAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTAAAATACAGCATAGCAAACTTTAACTCCAATCAAGCCTC  
AlwNI (2141)  
2101 TACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCAATGTGCATTAGCTGTTTGCAGCCTCACCTTTTCATGGAGTTT  
SapI (2233) SspI (2290)  
2201 AAGATATAGTGTATTTTCCAAGGTTTGAACCTAGCTCTTCATTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCTTTTATGAAAAATTCAGAA  
Swal (2304) EcoO109I (2365)  
2301 AATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGG

2401 ACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGACGCAGT  
DrallI (2483)  
125 • D Q E E A V F H V C N

EagI (2503)  
2501 TGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGTCTCGCCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGAC  
113 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 V V

2601 CTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCGGCACCACCTGGTCTGGACCGGCTGATGAACAGG  
80 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 F L

SgrAI (2719) XmaI (2746) BsrBI (2782) BssHII (27 98)  
2701 GTCACGTCGTCCCGACCACACCGCGAAGTCGTCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGAGTCCAGAACTCGACCGTCCGGCGACGTCGC  
46 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 D R

SfiI (2834) AseI (2894)  
2801 GCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGTGAGTATTATACTATGCGGATATACTATGCGGATGATTAAT  
13 A T L V P V A S T L K A M

SacI (2951)  
2901 TGTCAAACACGCGTGGATGGCGTCTCCAGCTTATCTGACGGTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCCATTTG

SpeI (3049)  
3001 CGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGAAAT

SnaBI (3177)  
3101 CCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGTACTGCCAAGTAG

NdeI (3282)  
3201 GAAAGTCCATAAGGTCTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCAATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATG  
3301 TACTGCCAAGTGGGCGAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTACTATGGGAACATACGTCAATTATTGACGTC

Pacl (3468)  
PstI (3461)  
SdaI (3460) BspLU11I (3478)  
3401 AATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCA  
3501 AAAGGCCAGGAACCGTAAAAAGCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG  
3601 CGAAACCCGACAGGACTATAAAGATACCAGGCTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCGACCCTGCCGCTTACCGGATACCTGTCCG  
3701 CCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCAAGCTGGGCTGTGTGCACGA

AlwNI (3889)  
3801 ACCCCCCGTTCCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT  
3901 AACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG  
4001 CTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTCGAAAGCAGCA  
4101 GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTC

Pacl (4208) Swal (4217)  
4201 ATGGCTAGTTAATTAACATTTAAATCA