

pSELECT-zeo-GFP::Sh

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

Catalog code: psetz-zgfpsh

For research use only

Version 20K30-MM

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 promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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 mRNA³.

• **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 transcription⁴.

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 H₂O. 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

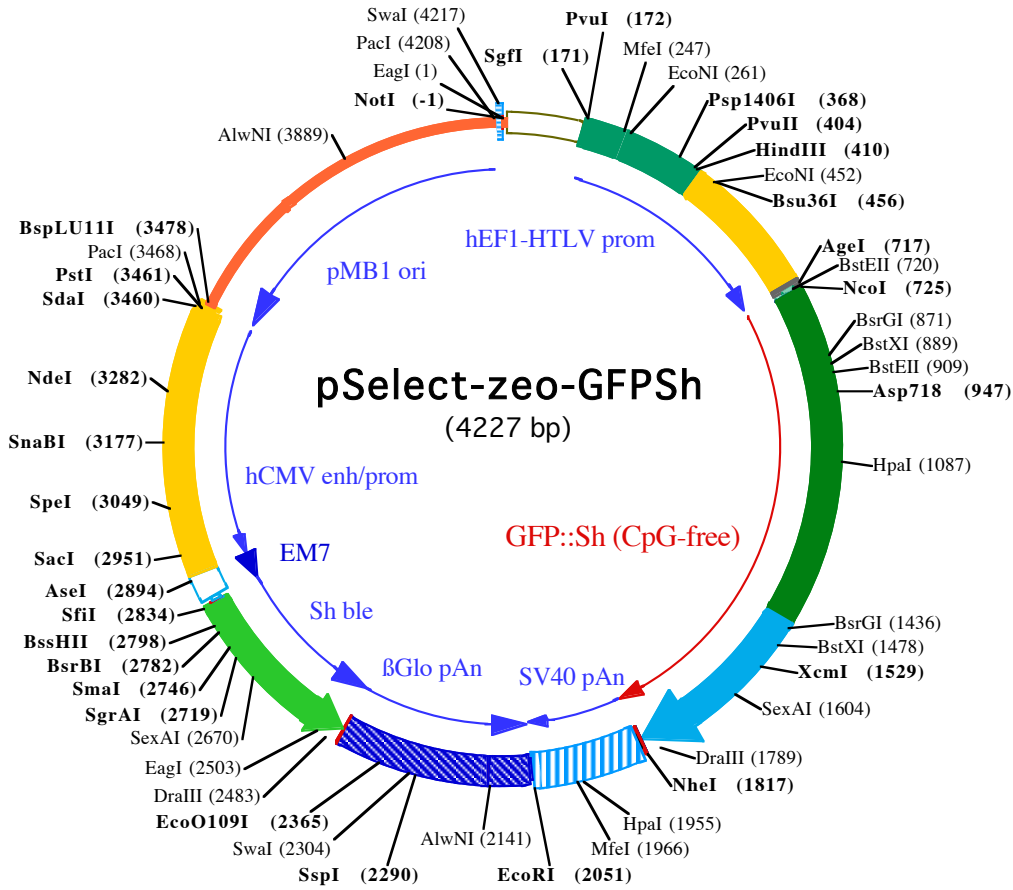
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EagI (1)
NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA

PvuII (172)
SgfI (171)
101 AAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTCCCGTCAGTGGCA

MfeI (247) EcoNI (261)
201 GAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGTCCGCAATTGAACGGTGCCTAGAGAAGTGGCGGGGTAAGTGGAAAGTGTATG

Psp1406I (368)
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCGTGAACGTTCTTTTTCCGACCGGTTTCCGCCAG

HindIII (410) Bsu36I (456)
PvuII (404) EcoNI (452)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTCTCTCCGCCCT

501 CCCCCTGTGGTGCCTCTGAACTGCGTCCCGCTAGTAAAGTTAAAGTCAAGTGCAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCGTTACAGATCCAAGCTGTGACC

NcoI (725)
BstEII (720)
AgeI (717)
701 GCGCCTACCTGAGATCaccggtcaCCATGGTTTCTAAGGAGAGAAGTCTTTACTGGTGTGTCCTCAATCTGGTTGAGCTGGATGGTGTGTAATG

BsrGI (871) BstXI (889)
801 GCCCAAATCTCTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCCAGTCCCTTG

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) Asp718 (947)
901 GCCAACTCTGGTACCACCCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAA

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 GTTATGTTCAAGAGAGACAATCTTTTAAAGGATGATGAAATTATAAGCAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGTTAACAAGATTG

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 AGCTGAAAGGCATTGATTTTAAAGGAAGTGAAGAACATTCTGGGTCAACAGCTGGAGTACAACATAATTCTCACAATGTTTACATTATGGCAGATAAGCA

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 GAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTTGAGGATGACTCTGTCCTCAACTGGCAGACATTACCAGCAGAACCCCTATTGGTGTAT

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 GGCCAGTCTCTCCAGATAATCACTATCTCAGCACTCAATCTGCTCTGTCCTCAAGACCTAATGAGAAAAGAGACCACATGGTCTCTCGAGTTTG

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 TGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGCCCAAGTTGACCAGTGTGTCCAGTGTCTCACAGCCAGGGATGTGGCTGGAGCTGT

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 TGAGTCTGGACTGACAGGTTGGGTTCTCCAGAGATTTTGGGAGGATGACTTTGCAGGTGTGGTGTGAGAGATGATGCACCTGTTTATCTCAGCAGTC

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

SexAI (1604)
1601 CAGGACCAGTGGTGCCTGACAACACCTGGCTGGTGTGGTGTGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGTGTCTCCCAACTTCA

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 GGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGAGAGTTTGCCTGAGAGACCAGCAGGCAACTGTGTGCACTTTGTGGC

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 AGAGGAGCAGGACTAAAGCTAGTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTAAAAAATGCTTTATTTG

358▶ E E Q D •

HpaI (1955) MfeI (1966)
1901 TGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTCAATAAACAAGTTAAACAACAACATTCATTCTTTATGTTTCAGGTTTCAGGGG

EcoRI (2051)
2001 GAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATCTAAAATACAGCATAGCAAAAATTTAACCTCCAAATCAAGCCTC

AlwNI (2141)
2101 TACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTATCGAGTTT

SspI (2290)
2201 AAGATATAGTATTTTCCCAAGTGTGAAGTGTGACTAGCTCTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATGAAAAATTCAGA

SwaI (2304) EcoO109I (2365)
2301 AATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTTATTAGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGG

DraIII (2483)
2401 ACTTAGGGAACAAAGAACCTTTAATAGAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGCTGCTGCTGCCACAAAGTGCACGAGT

127◀ • G • D Q E E A V F H V C N

EagI (2503)
2501 TGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCTGCTCGCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGAC

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

SexAI (2670)
2601 CTCCGACCCTGGCGTACAGCTGCTCAGGCGCGCACCCACCCAGGCCAGGGTGTGTCGGCACCACCTGGTCTGGACCGCTGATGAACAGG

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) SmaI (2746) BsrBI (2782) BssHII (2798)
2701 GTACGCTGCTCCGGACACACCGGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTGGTCCAGAACTGACCCGCTCCGGCGACGCTCGC

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 GCGCGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGAGTCTATTATACTATCCGATATACTATGCCGATGATTAAT

13◀ A T L V P V A S T L K A M

2901 TGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTG
SacI (2951)

3001 CGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAAT
SpeI (3049)

3101 CCCCCTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAG
SnaBI (3177)

3201 GAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATG
NdeI (3282)

3301 TACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTC

3401 AATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGCCAGCA
PacI (3468)
PstI (3461) SdaI (3460) BspLU11I (3478)

3501 AAAGGCCAGGAACCGTAAAGGCGCGGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG

3601 CGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCG

3701 CCTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGA

3801 ACCCCCCGTTACGCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAAACCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT
AlwNI (3889)

3901 AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAAGTATTTGGTATCTGCG

4001 CTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACACCGCTGGTAGCGGTGTTTTTTGTTTGAAGCAGCA

4101 GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGTC

4201 ATGGCTAGTTAATTAACATTAAATCA
PacI (4208) SmaI (4217)