

# pSELECT-zeo-GFP::Bsr

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

Catalog code: psetz-zgfpbsr

For research use only

Version 20K30-MMv02

## PRODUCT INFORMATION

### Content:

- 20 µg of pSELECT-zeo-GFP::Bsr 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 for 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::Bsr 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::Bsr 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 *Bsr* gene conferring blasticidin S resistance. Both genes have been modified and contain no CpG motifs, whereas their wildtype counterparts contain 60 and 50 CpG motifs respectively. This GFP::Bsr 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

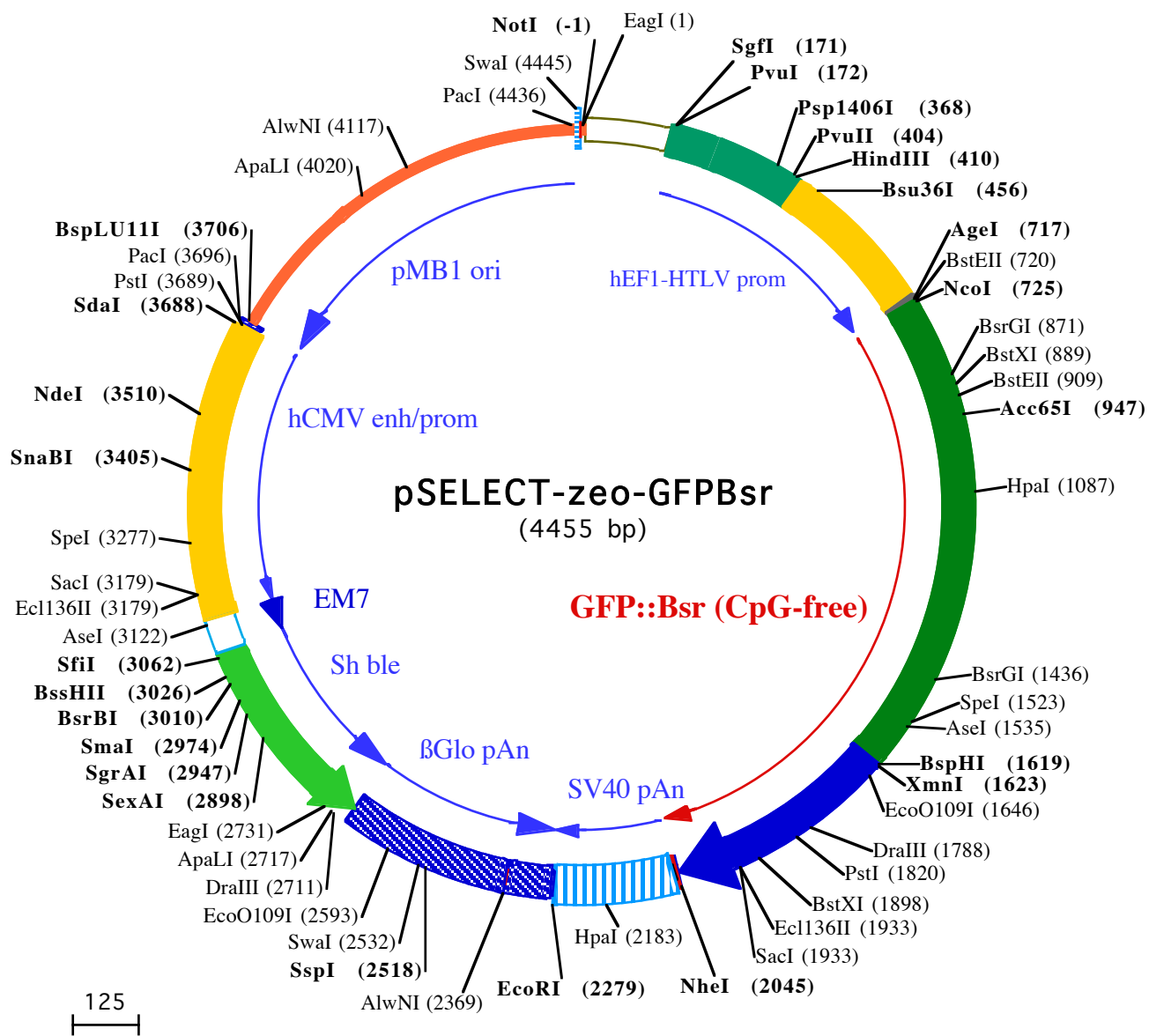
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InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



EagI (1)  
NotI (-1)  
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA

PvuII (172)  
SgfI (171)  
101 AAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTCCCGTCAGTGGCA

201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTCCGCAATTGAACGGTGCTAGAGAAGTGGCGGGGTAAACTGGGAAAGTGATG

Psp1406I (368)  
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (410)  
PvuII (404) Bsu36I (456)  
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCT

501 CCGCCTGTGGTGCCTCTGAACTCGTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACC

NeoI (725)  
BstEII (720)  
AgeI (717)  
701 GCGCCTACCTGAGATCACCGgtcaCCATGGTTCTAAGGAGAAGAACTCTTACTGGTGTGTCCTCAATCTGGTTGAGCTGGATGGTGTGTAATG

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 GCCACAAATCTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCCAGTGCCTTG

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 GCCAACTCTGGTGACCACCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAA

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 GGTATGTTCAAGGAGGACAATCTTCTTTAAGGATGATGAAATTATAAGACAAGGCGAGAAGTGAAGTTGAAGGTGATACACTGGTTAACAGAAATTG

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 AGCTGAAAGCATTGATTTAAAGGAAGTGAAGAACTTCTGGTCCACAGCTGGAGTACAACATAATTTCTCACAATGTTTACATTATGGCAGATAAGCA

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 GAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTGGCAGACATTACCAGCAGAACCCTTATTGGTAT

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 GGCCAGTCTCTCCAGATAATCACTATCTCAGCACTCAATGCTCTGTCCAAAGACCTAATGAGAAAAGACCCACATGGTCTCTGGAGTTTG

192 G P V 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)  
1401 TGACGACAGCAGGAATTAAGTCTGGGAATGGATGAGCTGTACAAGGgtaagtcactgactgtctatgcctgggaaagggtgggcaggagatggggcagtcg

225 V T A A G I T L G M D E L Y K G K S L T V Y A W E R V G R R W G S A

SpeI (1523) AseI (1535)  
1501 aggaaaagtggcactatgaaccACTAGTTGACAATTAATCATAAGCATAGTATAATACAACCTACTATAGcaattgtactaaccttcttctcttct

258 G K V A L • T H • F D N • S • A • Y N T T H Y S N C T N L L L F

XmnI (1623)  
BspHI (1619) EcoO109I (1646)  
1599 ctctcctgacagGAGGAGCCATCATGAAGACCTTCAACATCTCTCAGCAGGACCTTGAGCTGGTGAAGTTGCCACTGAGAAAATCACCATGCTCTATG

291 P L L T G G A I M K T F N I S Q Q D L E L V E V A T E K I T M L Y

DraIII (1788)  
1698 AGGACAACAAGCACCATGTTGGTCTGCCATCAGGACCAAGACAGGAGAAATCATCTCTGTGTCCACATTGAAGCCTACATTGGCAGGGTCACTGTGTG

324 E D N K H H V G A A I R T K T G E I I S A V H I E A Y I G R V T V C

PstI (1820)  
1798 TGCAGAGGCCATTGCCATTGGGTCTGCAGTCTCCAATGGGCGAGAAGACTTTGACACCATTGTGGCTGTGAGGCACCCCTACAGTGTGAGGTGGACAGG

357 A E A I A I G S A V S N G Q K D F D T I V A V R H P Y S D E V D R

BstXI (1898) SacI (1933)  
Ecl136II (1933)  
1898 TCCATCAGAGTGGTGTCCCTGTGGCATGTGCAGGGAGCTCATCTCAGACTATGCCCTGATTGCTTTGTTCTGATTGAGATGAATGGCAAGCTGGTCA

391 S I R V V S P C G M C R E L I S D Y A P D C F V L I E M N G K L V

NheI (2045)  
1998 AGACAACATTGAGGAGCTGATCCACTGAATACACCAGAACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACACCACAA

424 K T T I E E L I P L K Y T R N

HpaI (2183)  
2098 CTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACA

EcoRI (2279)  
2198 TTGCATTCATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACTCTACAAATGTGGTATGGAATCTAAAATACAGCA

AlwNI (2369)  
2298 TAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTGGCAATGTGCATTAGCT

2398 GTTTCAGCCTCACCTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTTGAAGTACTGCTTTCATTTCTTATGTTTTAAATGCACTGACCT

SspI (2518) SwaI (2532) EcoO109I (2593)  
2498 CCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGC

2598 CCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAATTGGACAGCAAGAAGCGAGCTTCTAGCTTATCCTCAG

127 G •

ApaLI (2717) DraIII (2711) EagI (2731)  
2698 TCCTGCTCCTCTGCCACAAAGTGACGAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCC

123 D Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G

2798 CGGAGGCGTCCCGAAGTTCGTGGACACGACTCCGACCACTCGGCGTACAGCTGCTCAGGCGCGCACCCACACCCAGGCCAGGGTGTGTCGGCAC

90 S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V

SexAI (2898) SgrAI (2947) SmaI (2974)  
2898 CACTGGTCTTGACCGCGCTGATGAACAGGGTACGCTGCTCCCGACCACACCGGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTGC

574 V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D

2998 **BsrBI (3010)** **BssHII (3026)** **SfiI (3062)**  
 GTCCAGAACTCGACCGCTCCGGCGACGTCCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGAGTCGTATTAT  
 234 T W F E V A G A V D R A T L V P V A S T L K A M

3098 **AseI (3122)** **SacI (3179)** **Ecl136II (3179)**  
 ACTATGCCGATATACTATGCCGATGATTAATTGTCAA**AACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGA**

3198 **SpeI (3277)**  
 CCTCCACCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTACTAGTCAAAAACAACTCCC

3298 **SpeI (3277)**  
 ATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTA**CTGCCAAAACCGCATCATCATGGTAATAGCG**

3398 **SnaBI (3405)**  
 ATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCA**TGTA**CTG**GGCATAATGCCAGGGGGCCATTTACCGTCATTGACGTCAATAG**

3498 **NdeI (3510)**  
 GGGGCGTACTTGGCATATGATACACTT**GATGTA**CTG**CCAAAGTGGCAGTTTACCGTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGCGT**

3598 **PstI (3689)** **SdaI (3688)** **PacI (3696)**  
 TACTATGGGAACATACGTCATTATTGACGTCAATGGGGGGGGT**CGTTGGCGGTCAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCTGCAGGT**

3698 **BspLU11I (3706)**  
 TAATT**AAGAACATGTGAGCAAAGGCCAGCAAAGCCAGGAACCGTAAAAAGGCCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCA**

3798 **BspLU11I (3706)**  
 TCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGACTATAAAGATACAGGCGTTTCCCGCTGGAAAGCTCCCTCGTGCCTCTCCTGTT

3898 **BspLU11I (3706)**  
 CCGACCTGCCGTTACCGGATACCTGTCCGCTTTTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG

3998 **ApaLI (4020)**  
 TCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTT**CAGCCCAGCCGCTGCGCCTTATCCGGTAACTATCGTCTT**GAGTCCAA**CCCGTAAGACA**

4098 **AlwNI (4117)**  
 CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGC

4198 **AlwNI (4117)**  
 TACTACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGATTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTG

4298 **AlwNI (4117)**  
 GTAGCGGTGGTTTTTTTGGTTTTCGCAAGCAGCAGATTACGCGCAGAAAAAAGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTG

4398 **PacI (4436)** **SwaI (4445)**  
 GAACGAAA**ACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAA**CATTTAAATCA****