pSELECT-CGFP-zeo

Plasmid for the expression of GFP-C-terminal tagged proteins

Catalog code: psetz-cgfp

For research use only

Version 20L01-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-CGFP-zeo** plasmid provided as lyophilized DNA
- 1 ml of ZeocinÊ (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 ZeocinÊ at 4 ÁC or at -20 ÁC. 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 plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human ß-globin polyA terminates the transcription of the selectable marker.

pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. The GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm). pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest.

C-terminal tag: the tag is cloned downstream of a multiple cloning site and followed by a Stop codon.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (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.

• **MCS:** The multiple cloning site contains the following restriction sites: 5' - Age I, Sal I, Eco47III, BamH I, Nco I - 3'

Each restriction site is compatible with many other enzymes.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

• **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.*

• ***Sh ble* gene** confers zeocin resistance. 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*.

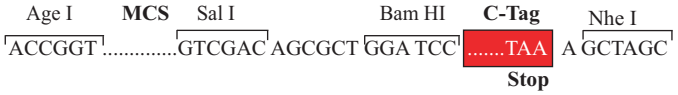
• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

CLONING STRATEGY

For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Age I/Bam HI restriction site combination for cloning into plasmids with the C-Tag. For the plasmids with C-Tag, check whether the start codon of your gene of interest is in the correct reading frame with the C-Tag.

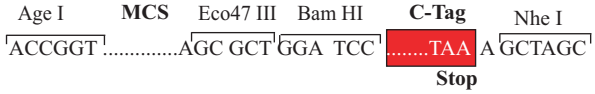
Note: The Bam HI restriction site is compatible with Bgl II.

If it is not possible to use the Age I restriction site, it is possible to another restriction site such as Sal I, upstream of Bam HI.



If it is not possible to use the Bam HI restriction site, it is possible to another restriction site such as Nco I.

Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is upstream of the Bam HI site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT
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InvivoGen Europe: +33 (0) 5-62-71-69-39
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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

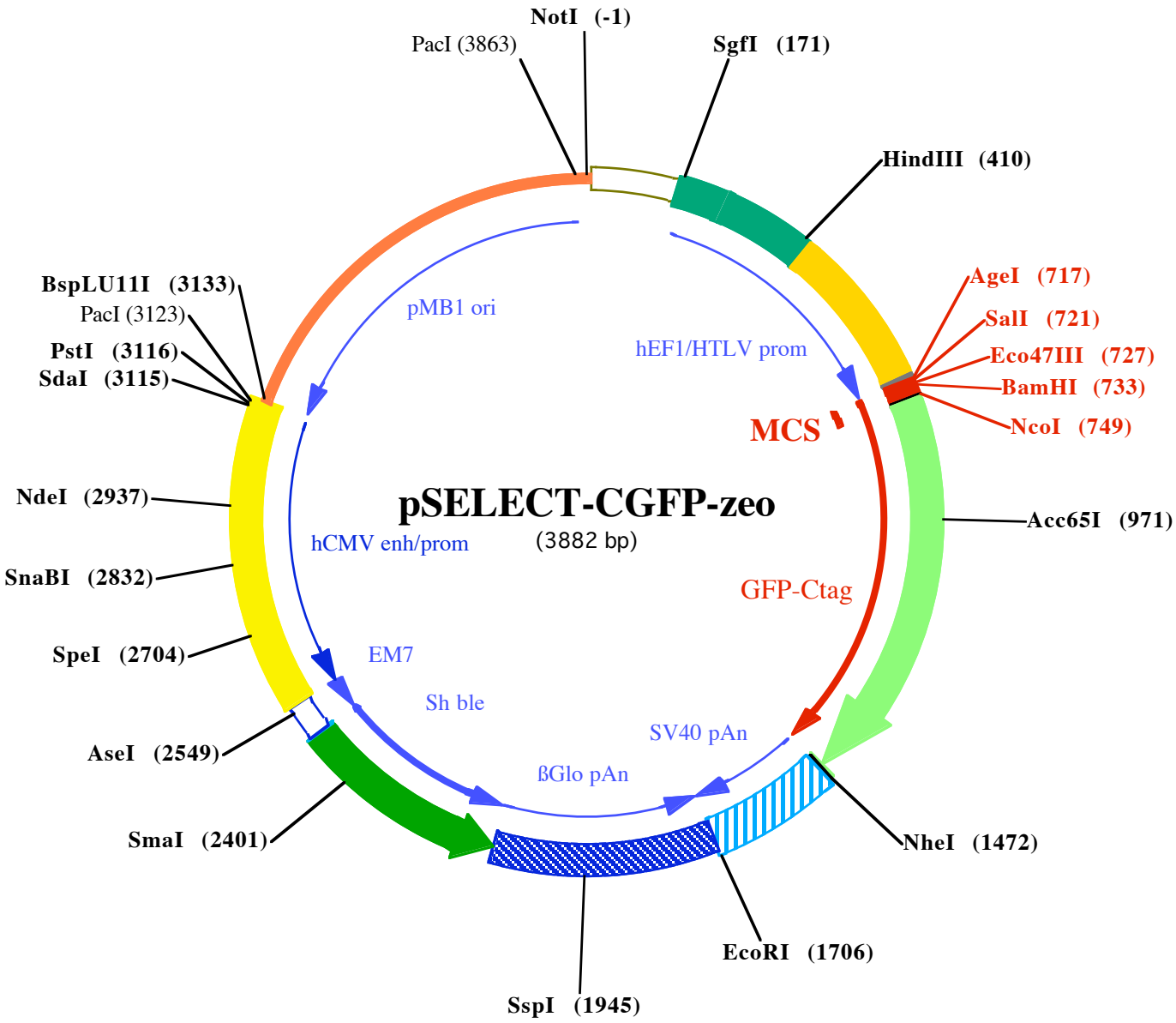
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

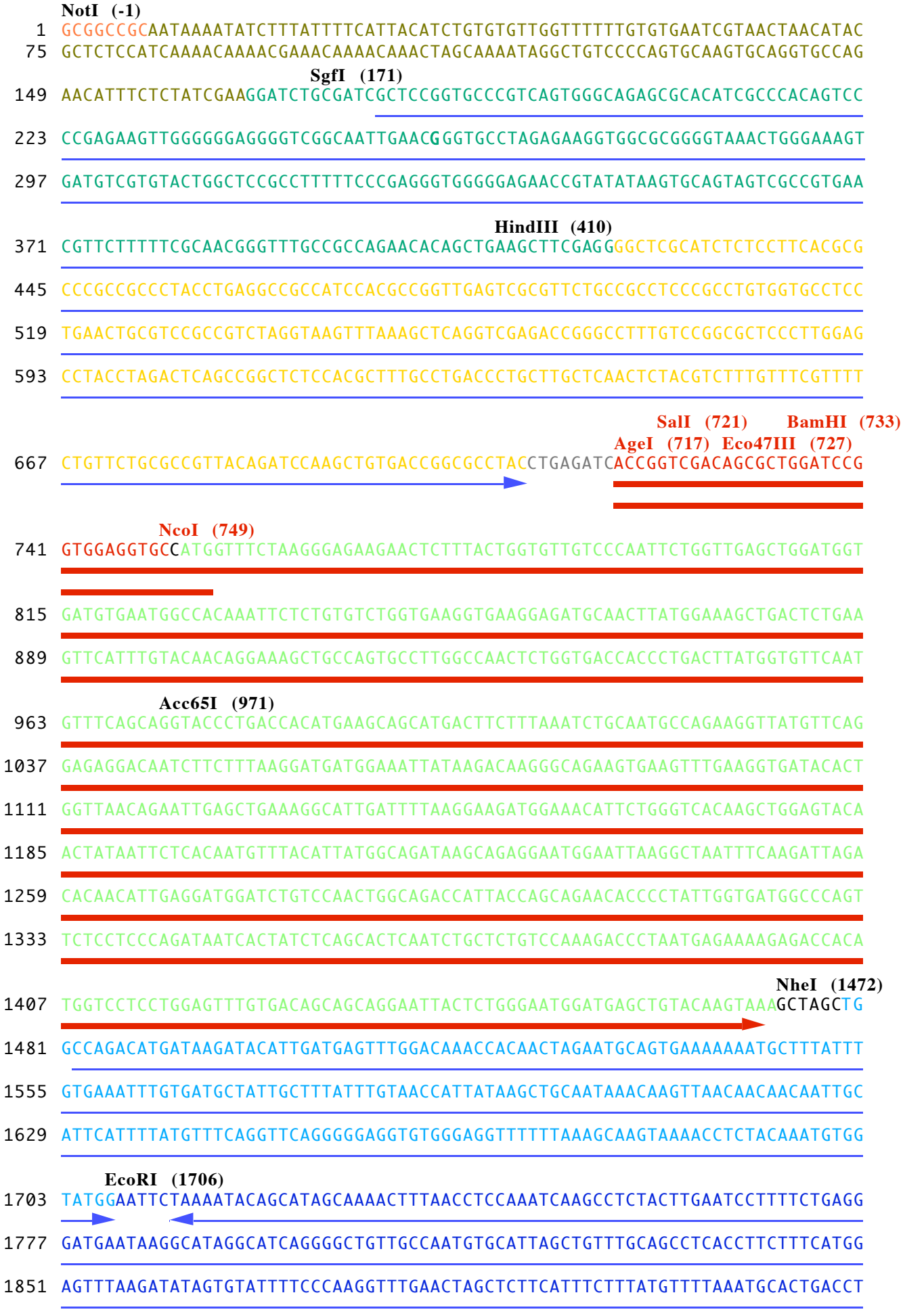
1. Kim, D.W. *et al.* (1990). Gene 2: 217-223.
2. Takebe, Y. *et al.* (1988). Mol. Cell Biol. 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). Mol. Cell Biol. 10: 4248-4258.
4. Yu J & Russell JE. (2001). Mol Cell Biol, 21(17):5879-88.

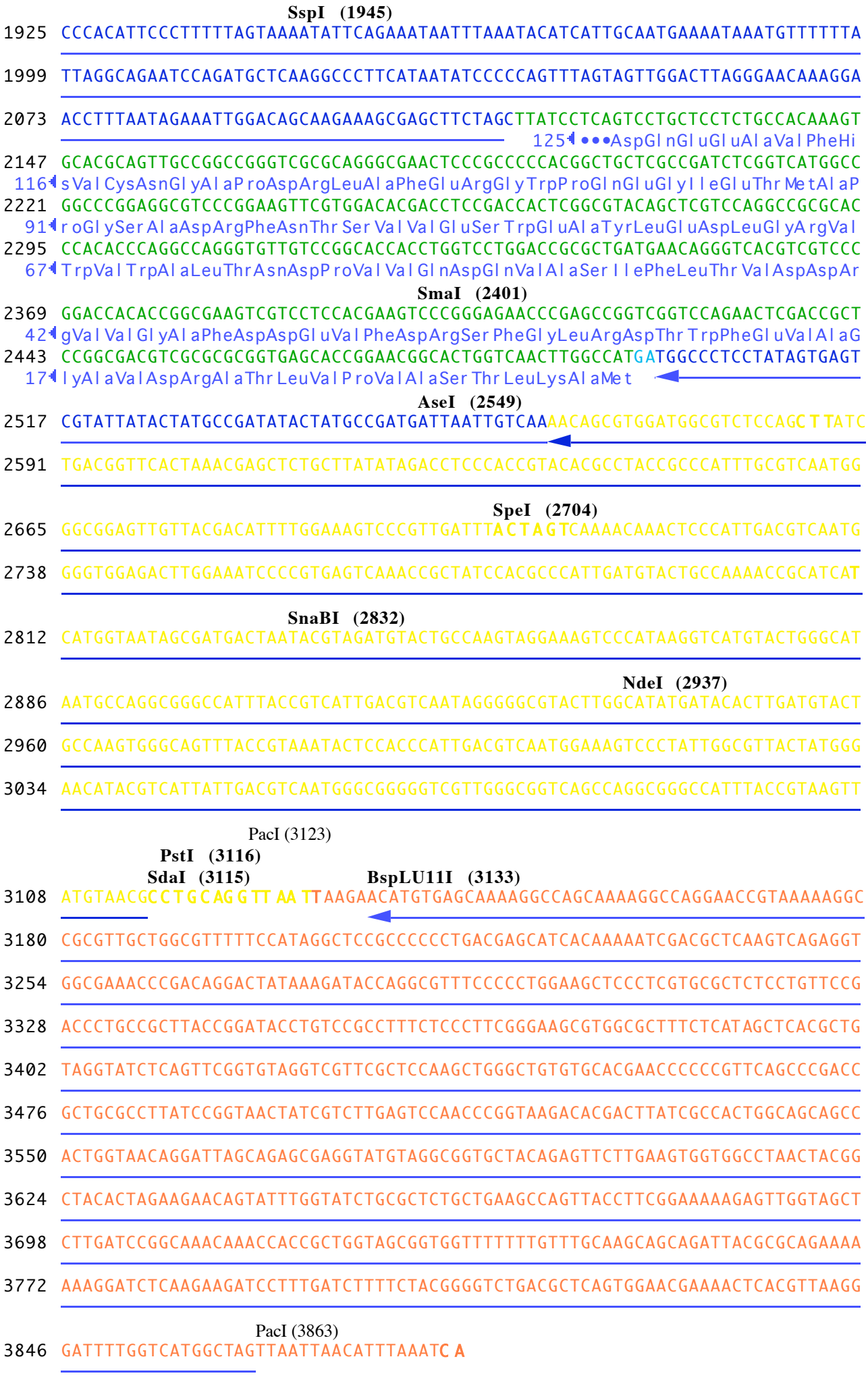
RELATED PRODUCTS

Product	Catalog Code
pSELECT-NGFP-blasti	psetb-ngfp
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-NGFP-zeo	psetz-ngfp
pSELECT-NHA-blasti	psetb-nha
pSELECT-CHA-blasti	psetb-cha
pSELECT-NHA-zeo	psetz-nha
pSELECT-CHA-zeo	psetz-cha
pSELECT-CHis-blasti	psetb-chis
pSELECT-NHis-zeo	psetz-nhis
pSELECT-CHis-zeo	psetz-chis


NotI (-1)
PacI (3863)
SgfI (171)
HindIII (410)
AgeI (717)
SalI (721)
Eco47III (727)
BamHI (733)
NcoI (749)
Acc65I (971)
NheI (1472)
EcoRI (1706)
SspI (1945)
SmaI (2401)
AseI (2549)
SpeI (2704)
SnaBI (2832)
NdeI (2937)
BspLU11I (3133)
PacI (3123)
PstI (3116)
SdaI (3115)
pMB1 ori
hEF1/HTLV prom
MCS
pSELECT-CGFP-zeo
(3882 bp)
hCMV enh/prom
GFP-Ctag
EM7
Sh ble
SV40 pAn
ßGlo pAn

InvivoGen


NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG
SgfI (171)
149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC
223 CCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGT
297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA
HindIII (410)
371 CGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCG
445 CCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCC
519 TGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG
593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTT
SalI (721) BamHI (733)
AgeI (717) Eco47III (727)
667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCGACAGCGCTGGATCCG
NcoI (749)
741 GTGGAGGTGCCATGGTTTCTAAGGGAGAAGAACTCTTTACTGGTGTTGTCCCAATTCTGGTTGAGCTGGATGGT
815 GATGTGAATGGCCACAAATTCTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGGAAAGCTGACTCTGAA
889 GTTCATTTGTACAACAGGAAAGCTGCCAGTGCCTTGGCCAACTCTGGTGACCACCCTGACTTATGGTGTTCAAT
Acc65I (971)
963 GTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTCAG
1037 GAGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACT
1111 GGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGGAAACATTCTGGGTCACAAGCTGGAGTACA
1185 ACTATAATTCTCACAATGTTTACATTATGGCAGATAAGCAGAGGAATGGAATTAAGGCTAATTTCAAGATTAGA
1259 CACAACATTGAGGATGGATCTGTCCAACTGGCAGACCATTACCAGCAGAACACCCCTATTGGTGATGGCCCAGT
1333 TCTCCTCCCAGATAATCACTATCTCAGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACA
NheI (1472)
1407 TGGTCCTCCTGGAGTTTGTGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAAGCTAGCTG
1481 GCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTT
1555 GTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGC
1629 ATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGG
EcoRI (1706)
1703 TATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGG
1777 GATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGG
1851 AGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCT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