pSELECT-CHis-blasti

Plasmid for the expression of polyhistidine (His)-C-terminal tagged proteins

Catalog code: psetb-chis

https://www.invivogen.com/pselect-his-tag

For research use only

Version 20I11-MM

PRODUCT INFORMATION

Contents

- 20 µg of **pSELECT-CHis-blasti** plasmid provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store blasticidin at 4°C or -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 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. pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest. The 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, , Eco47 III, Nco I, BamH 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

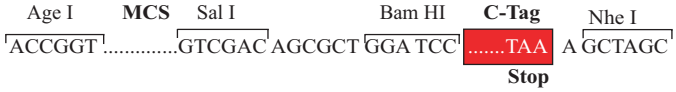
- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Blasti:** Resistance to Blasticidin S is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* 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.

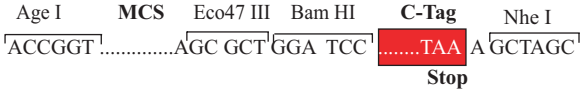
Note: The Bam HI restriction site is compatible with Bgl II.

If it is not possible to use the Age I restriction site, it is possible to use 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 use 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 Nco I site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT

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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 other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

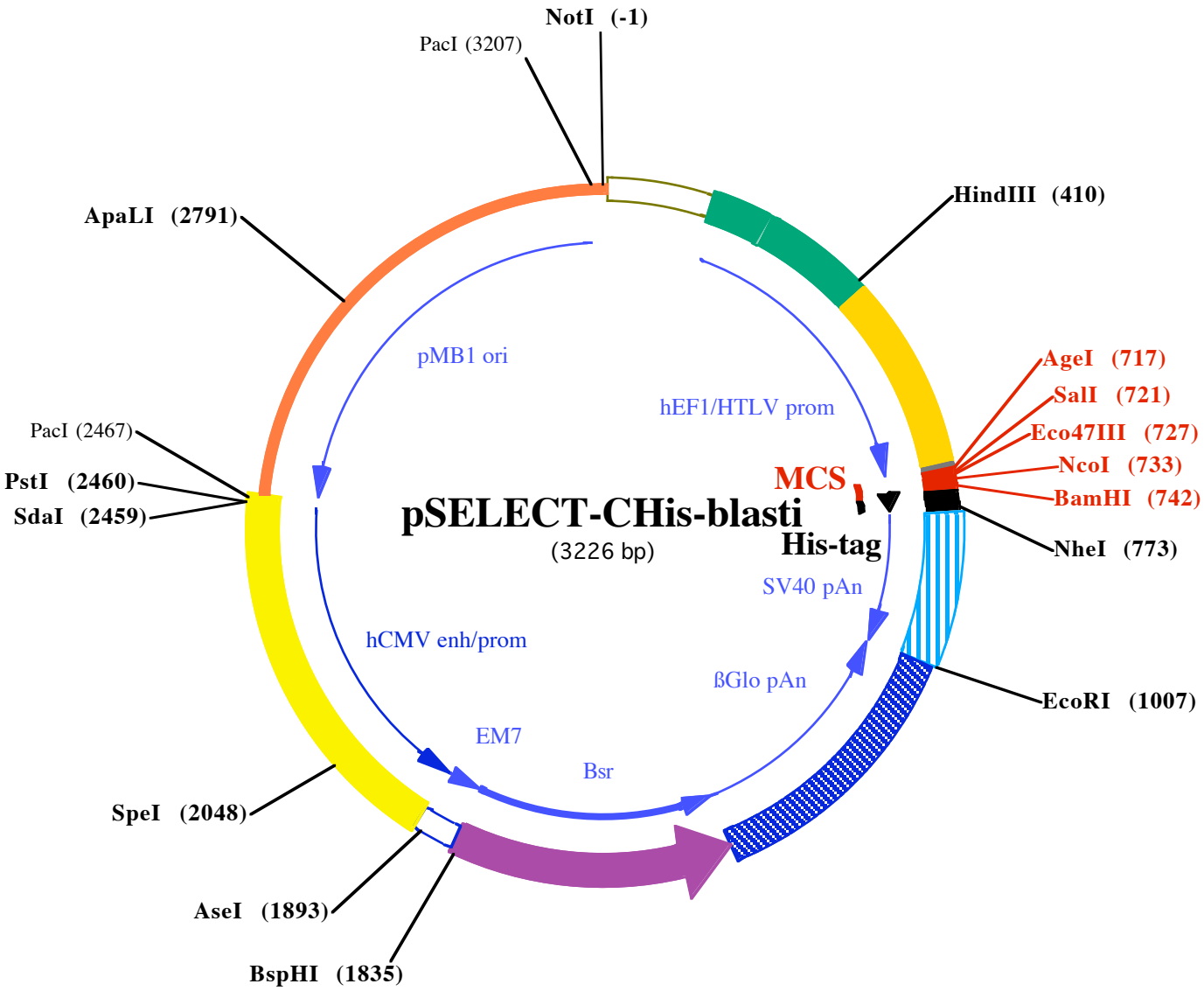
Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

References:

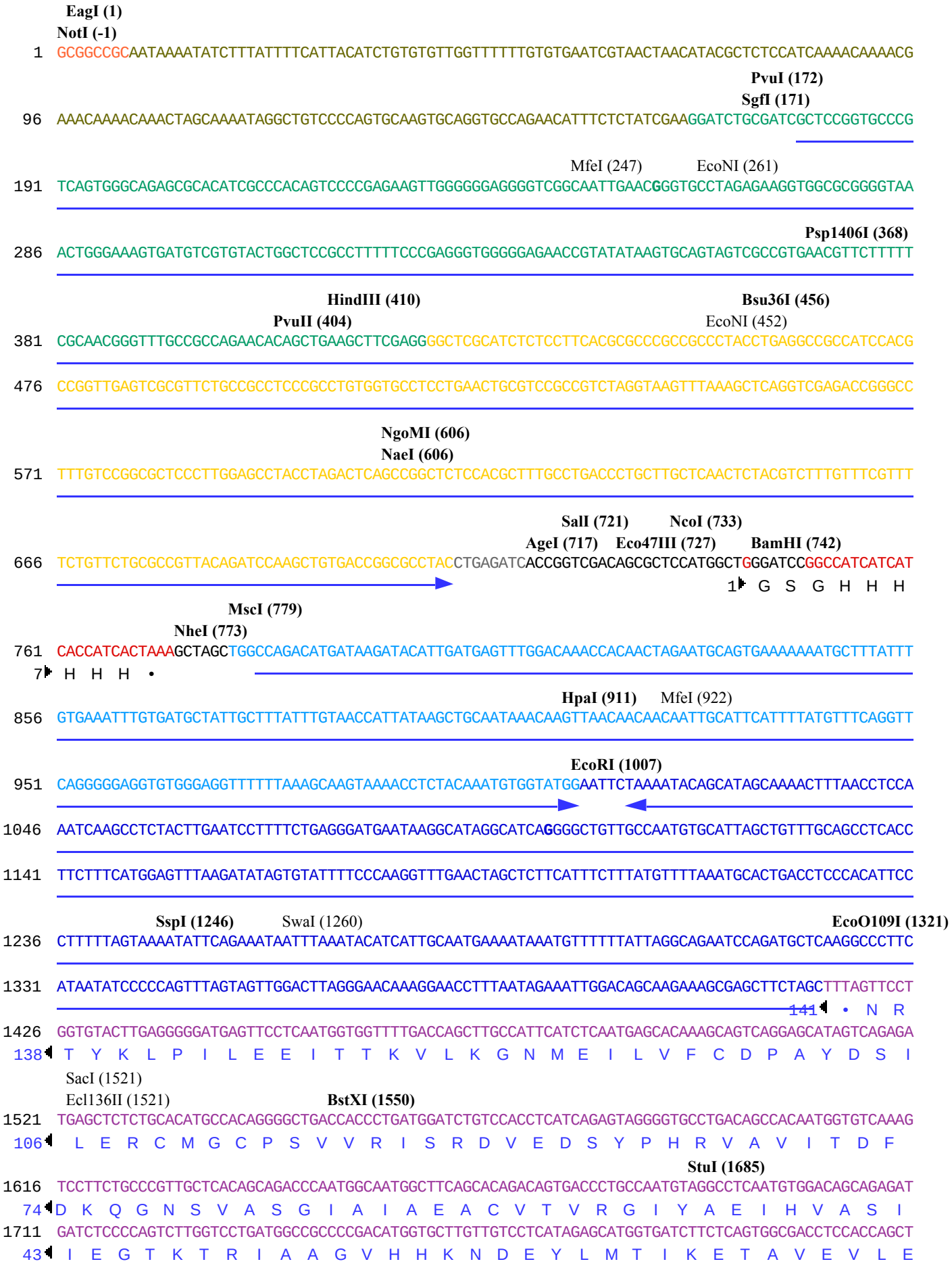
1. Kim, D.W. et al., 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 2: 217-223. **2. Takebe, Y. et al., 1988. S**R alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol. Cell Biol. 1: 466-472. **3. Carswell, S., & Alwine, J.C., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences.Mol. Cell Biol. 10: 4248-4258. **4. Yu J & Russell JE., 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.

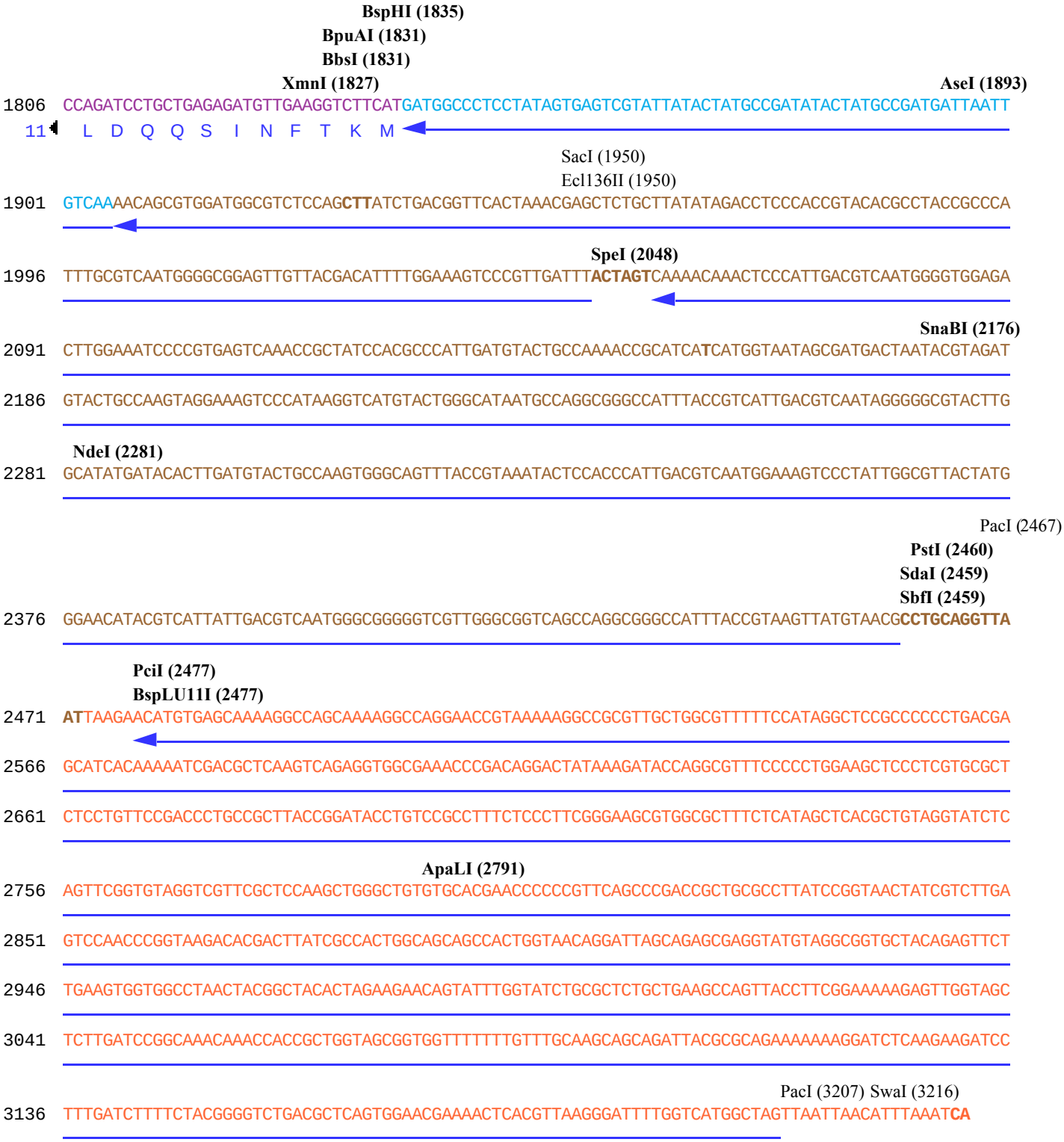
RELATED PRODUCTS

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


NotI (-1)
PacI (3207)
HindIII (410)
ApaLI (2791)
pMB1 ori
hEF1/HTLV prom
AgeI (717)
SalI (721)
Eco47III (727)
NcoI (733)
BamHI (742)
NheI (773)
PacI (2467)
PstI (2460)
SdaI (2459)
MCS
pSELECT-CHis-blasti
(3226 bp)
His-tag
SV40 pAn
hCMV enh/prom
ßGlo pAn
EcoRI (1007)
EM7
Bsr
SpeI (2048)
AseI (1893)
BspHI (1835)

InvivoGen


EagI (1)
NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
PvuI (172)
SgfI (171)
96 AAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCG
MfeI (247)
EcoNI (261)
191 TCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAA
Psp1406I (368)
286 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTT
HindIII (410)
PvuII (404)
Bsu36I (456)
EcoNI (452)
381 CGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACG
476 CCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCC
NgoMI (606)
NaeI (606)
571 TTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
SalI (721)
NcoI (733)
AgeI (717)
Eco47III (727)
BamHI (742)
666 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCGACAGCGCTCCATGGCTGGGATCCGGCCATCATCAT
1 G S G H H H
MscI (779)
NheI (773)
761 CACCATCACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTT
7 H H H •
HpaI (911)
MfeI (922)
856 GTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTT
EcoRI (1007)
951 CAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCA
1046 AATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACC
1141 TTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCC
SspI (1246)
SwaI (1260)
EcoO109I (1321)
1236 CTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC
1331 ATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCT
141 • N R
1426 GGTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGA
138 T Y K L P I L E E I T T K V L K G N M E I L V F C D P A Y D S I
SacI (1521)
Ecl136II (1521)
BstXI (1550)
1521 TGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAG
106 L E R C M G C P S V V R I S R D V E D S Y P H R V A V I T D F
StuI (1685)
1616 TCCTTCTGCCCGTTGCTCACAGCAGACCCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGAT
74 D K Q G N S V A S G I A I A E A C V T V R G I Y A E I H V A S I
1711 GATCTCCCCAGTCTTGGTCCTGATGGCCGCCCCGACATGGTGCTTGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCT
43 I E G T K T R I A A G V H H K N D E Y L M T I K E T A V E V L E


BspHI (1835)
BpuAI (1831)
BbsI (1831)
XmnI (1827)
AseI (1893)
1806 CCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATT
11 L D Q Q S I N F T K M
SacI (1950)
Ecl136II (1950)
1901 GTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCA
SpeI (2048)
1996 TTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGA
SnaBI (2176)
2091 CTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGAT
2186 GTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTG
NdeI (2281)
2281 GCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATG
PacI (2467)
PstI (2460)
SdaI (2459)
SbfI (2459)
2376 GGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTA
PciI (2477)
BspLU11I (2477)
2471 ATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGA
2566 GCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT
2661 CTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTC
ApaLI (2791)
2756 AGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA
2851 GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT
2946 TGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC
3041 TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCC
PacI (3207) SwaI (3216)
3136 TTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA