pSELECT-NHA-zeo

Plasmid for the expression of human influenza hemagglutinin (HA)-N-terminal tagged proteins

Catalog # psetz-nha

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-NHA-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 for 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. pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest.

N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS).

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.*

• ***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*.

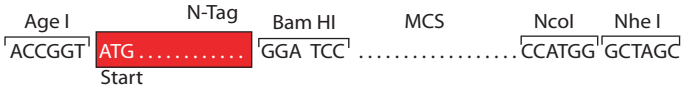
• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

CLONING STRATEGY

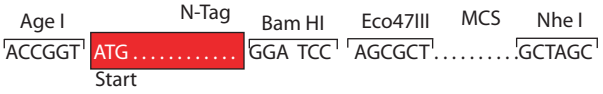
For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use BamHI/NheI restriction site combination for cloning into plasmids with the N-Tag. For the plasmids with N-Tag, check whether your gene of interest and the start codon of the N-tag are in the correct reading frame.

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

If it is not possible to use the Nhe I restriction site, it is possible to use another restriction site such as NcoI.



Alternatively, blunt end cloning can be achieved using the Eco47III site, which is downstream of the BamHI site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT
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E-mail: info@invivogen.com

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

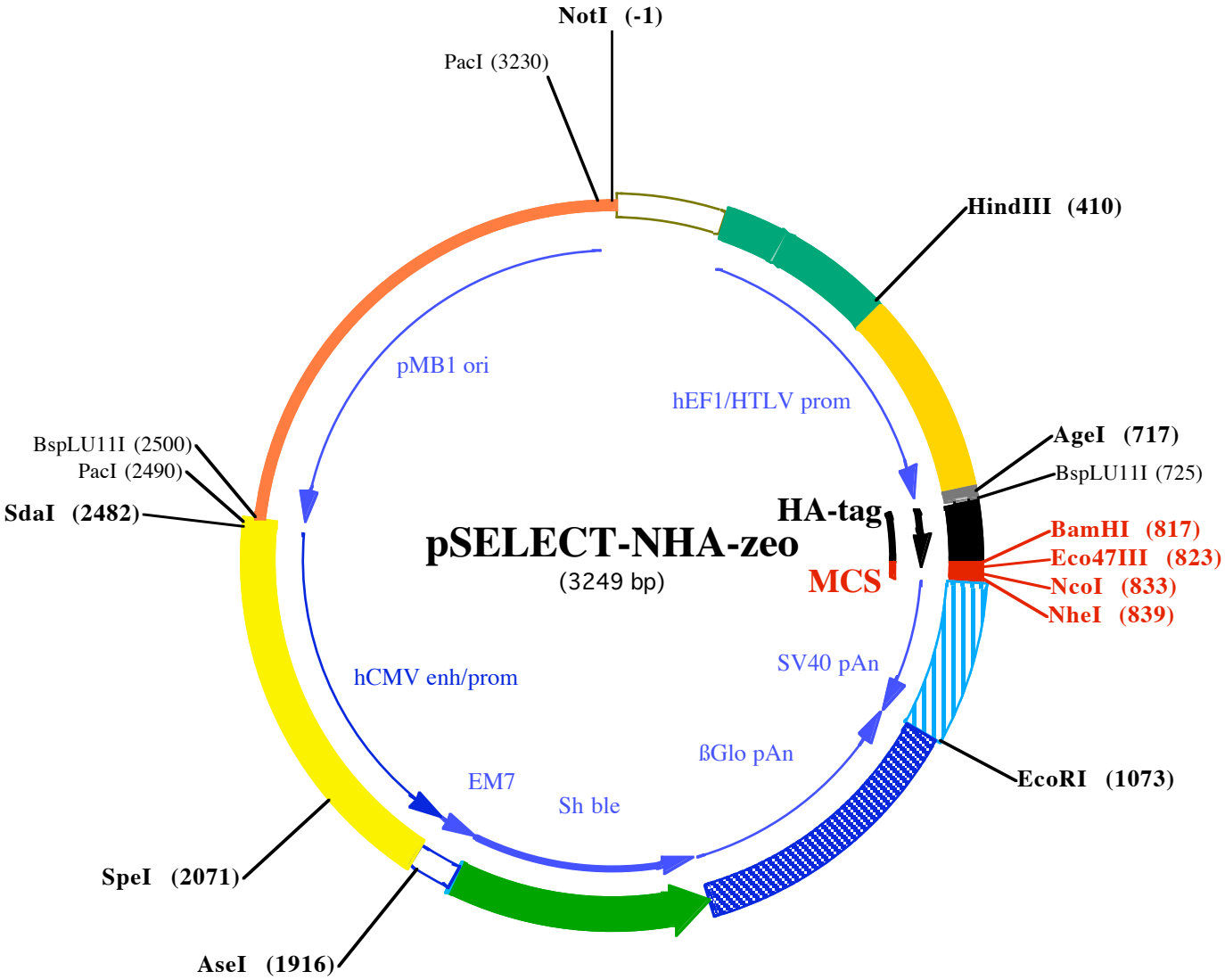
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

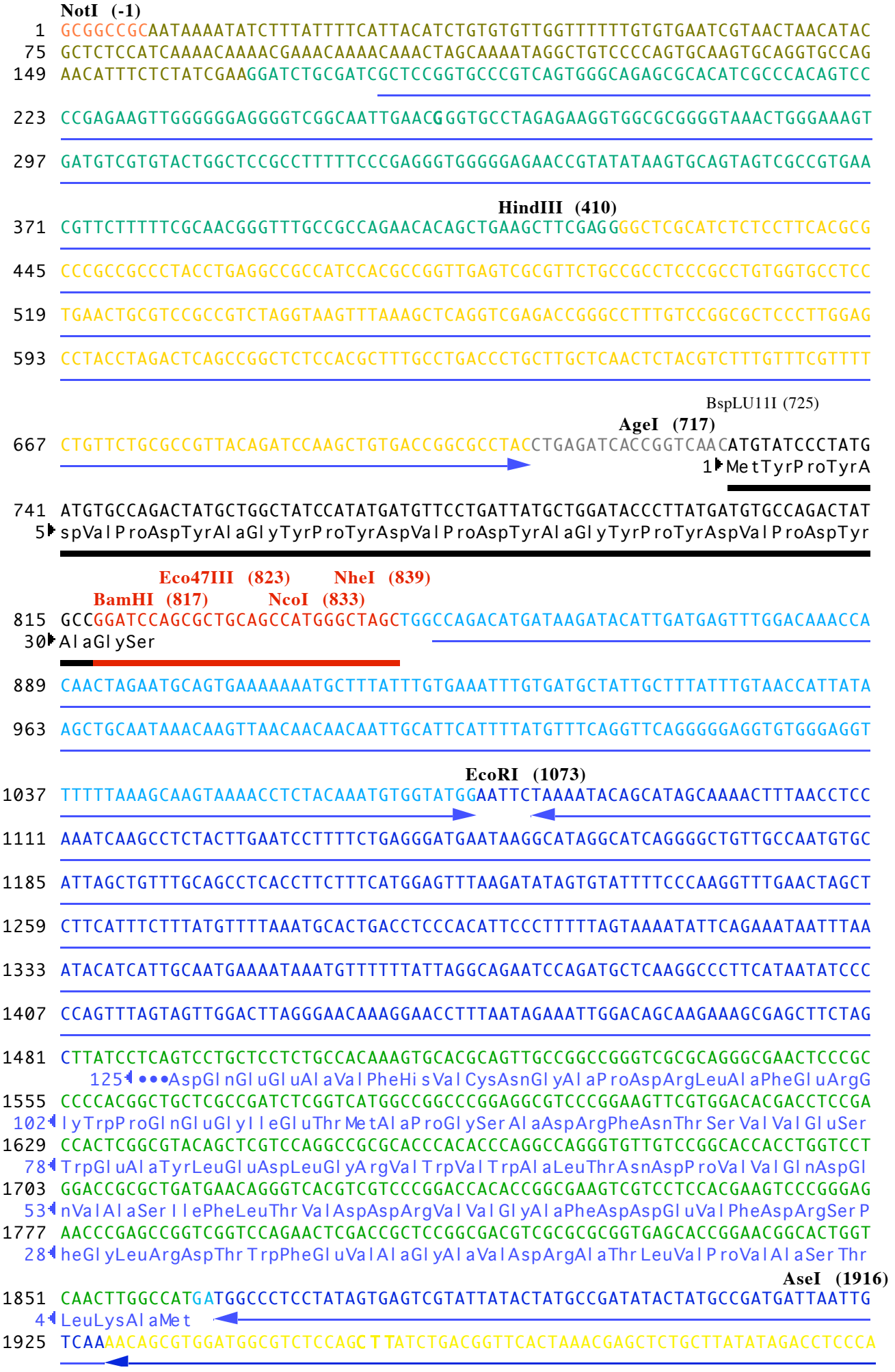
References:

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-CGFP-zeo	psetz-cgfp
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-blasti	psetb-chis


NotI (-1)
PacI (3230)
HindIII (410)
pMB1 ori
hEF1/HTLV prom
BspLU11I (2500)
PacI (2490)
SdaI (2482)
AgeI (717)
BspLU11I (725)
HA-tag
pSELECT-NHA-zeo
(3249 bp)
MCS
BamHI (817)
Eco47III (823)
NcoI (833)
NheI (839)
hCMV enh/prom
SV40 pAn
ßGlo pAn
EM7
Sh ble
EcoRI (1073)
SpeI (2071)
AseI (1916)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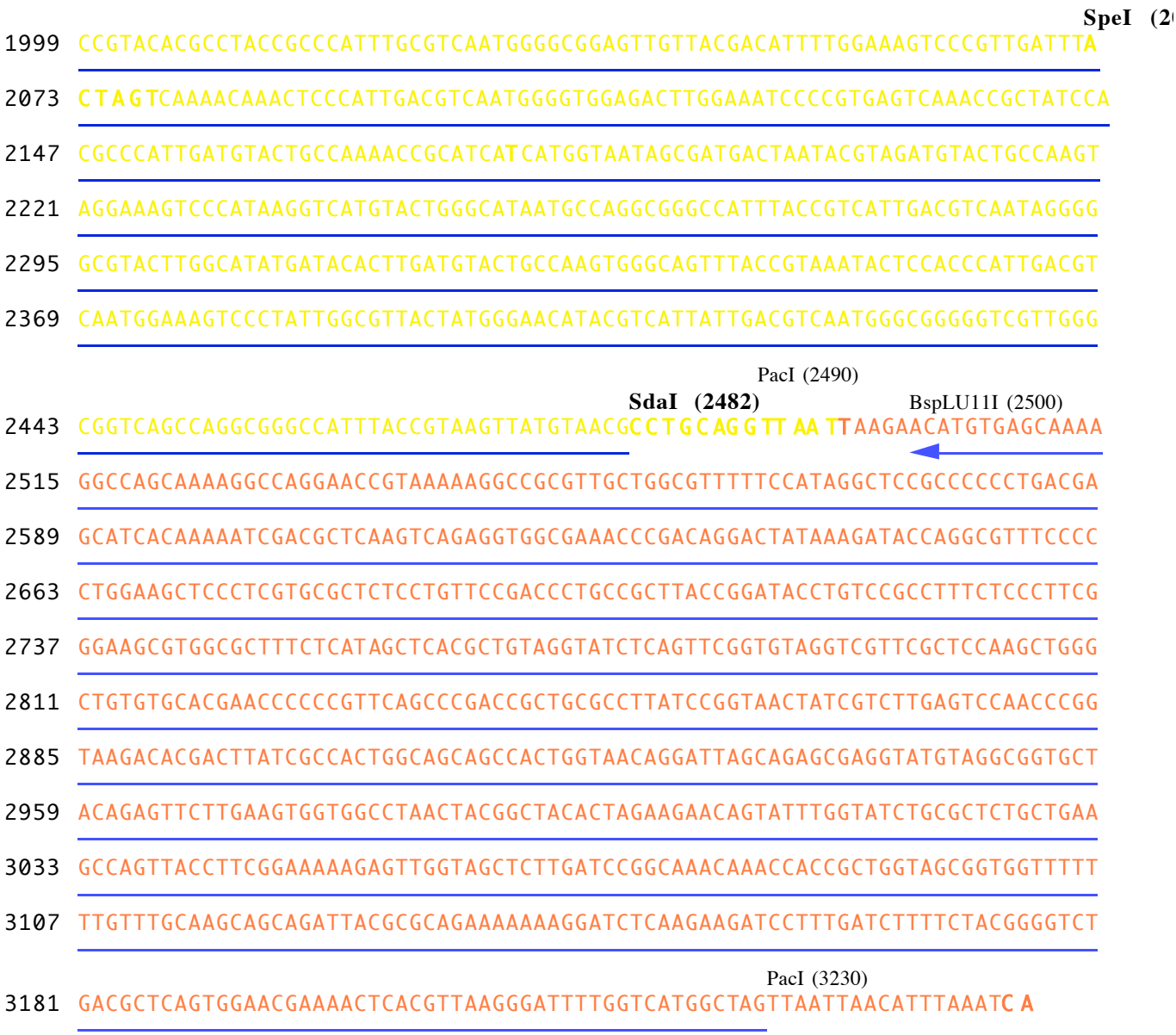
AseI (1916)


SpeI (2
1999 CCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTA
2073 CTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCA
2147 CGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT
2221 AGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG
2295 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGT
2369 CAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGG
PacI (2490)
SdaI (2482)
BspLU11I (2500)
2443 CGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAA
2515 GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGA
2589 GCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC
2663 CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCG
2737 GGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGG
2811 CTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG
2885 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
2959 ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA
3033 GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTT
3107 TTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCT
PacI (3230)
3181 GACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA