pSELECT-zeo-LacZnls

A plasmid encoding a CpG-free LacZ gene with SV40 nuclear localization signal

Catalog code: psetz-lacznls

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-zeo-LacZnls** 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 LacZnls 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 casssette 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[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.

• **LacZnls CpG-free:** The lacZ reporter gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. The synthetic lacZ gene engineered by InvivoGen is entirely free of CpG motifs. LacZnls contains a nuclear localization signal of SV40 large T that allows the targeting of the chimeric protein to the nucleus.

• **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.*

• **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*.

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

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_2O. 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
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InvivoGen Hong Kong: +852 3622-3480
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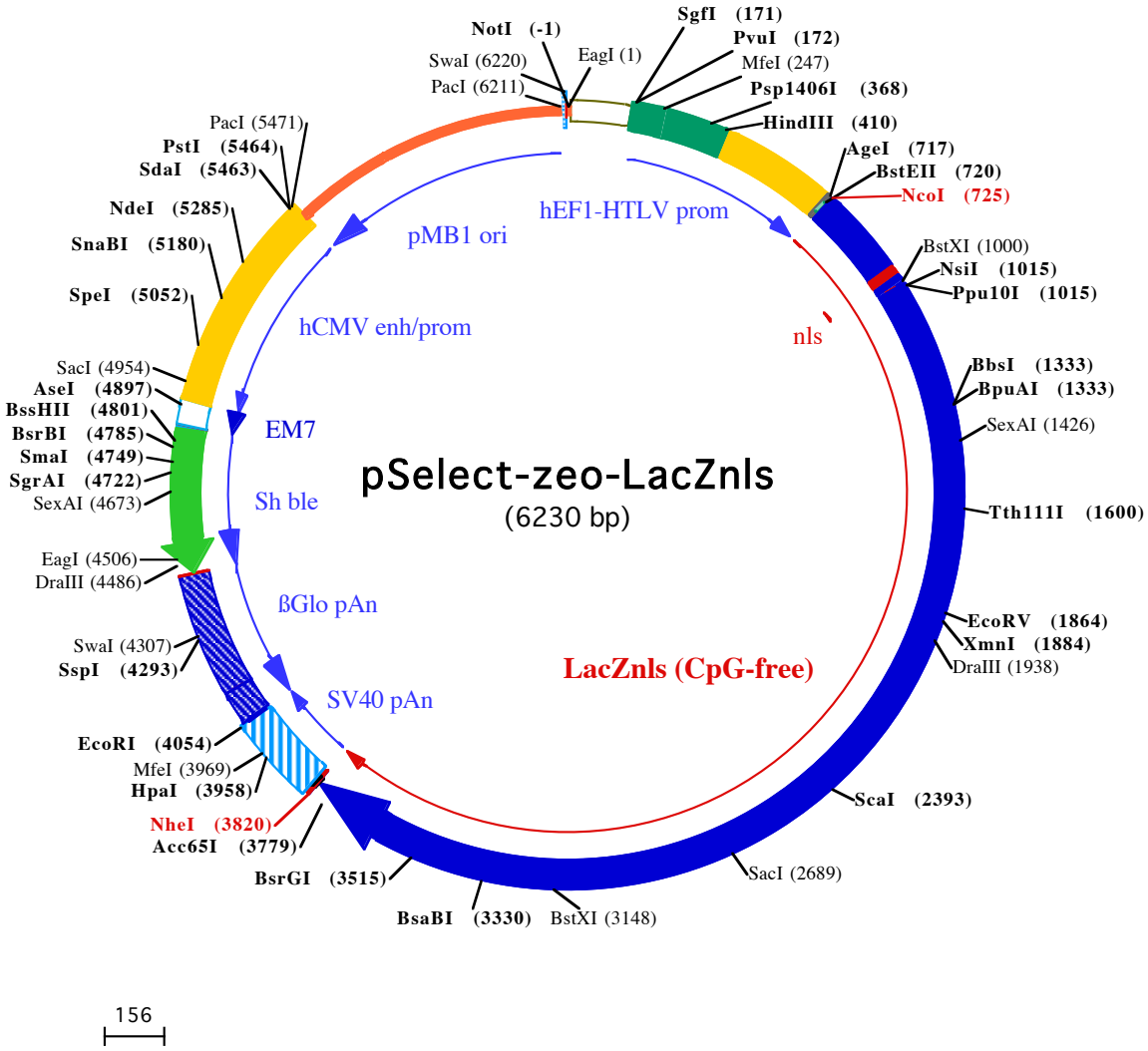
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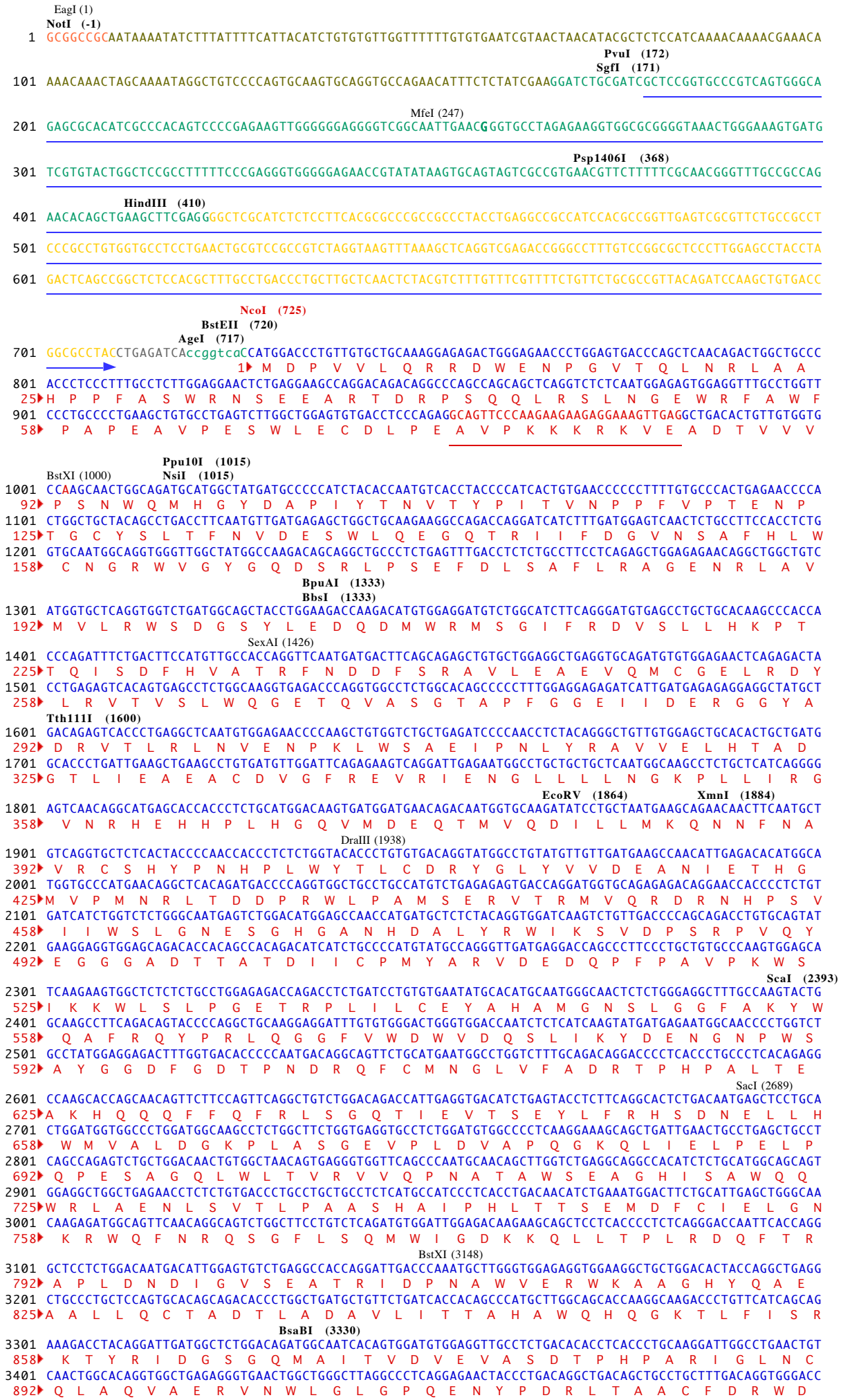
NotI (-1)
SwaI (6220)
PacI (6211)
EagI (1)
SgfI (171)
PvuI (172)
MfeI (247)
Psp1406I (368)
HindIII (410)
AgeI (717)
BstEII (720)
NcoI (725)
BstXI (1000)
NsiI (1015)
Ppu10I (1015)
BbsI (1333)
BpuAI (1333)
SexAI (1426)
Tth111I (1600)
EcoRV (1864)
XmnI (1884)
DraIII (1938)
ScaI (2393)
SacI (2689)
BstXI (3148)
BsaBI (3330)
BsrGI (3515)
Acc65I (3779)
NheI (3820)
HpaI (3958)
MfeI (3969)
EcoRI (4054)
SspI (4293)
SwaI (4307)
DraIII (4486)
EagI (4506)
SexAI (4673)
SgrAI (4722)
SmaI (4749)
BsrBI (4785)
BssHII (4801)
AseI (4897)
SacI (4954)
SpeI (5052)
SnaBI (5180)
NdeI (5285)
SdaI (5463)
PstI (5464)
PacI (5471)
pMB1 ori
hEF1-HTLV prom
nls
hCMV enh/prom
EM7
Sh ble
ßGlo pAn
SV40 pAn
LacZnls (CpG-free)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NotI (-1)
PvuI (172)
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MfeI (247)
Psp1406I (368)
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BstEII (720)
AgeI (717)
Ppu10I (1015)
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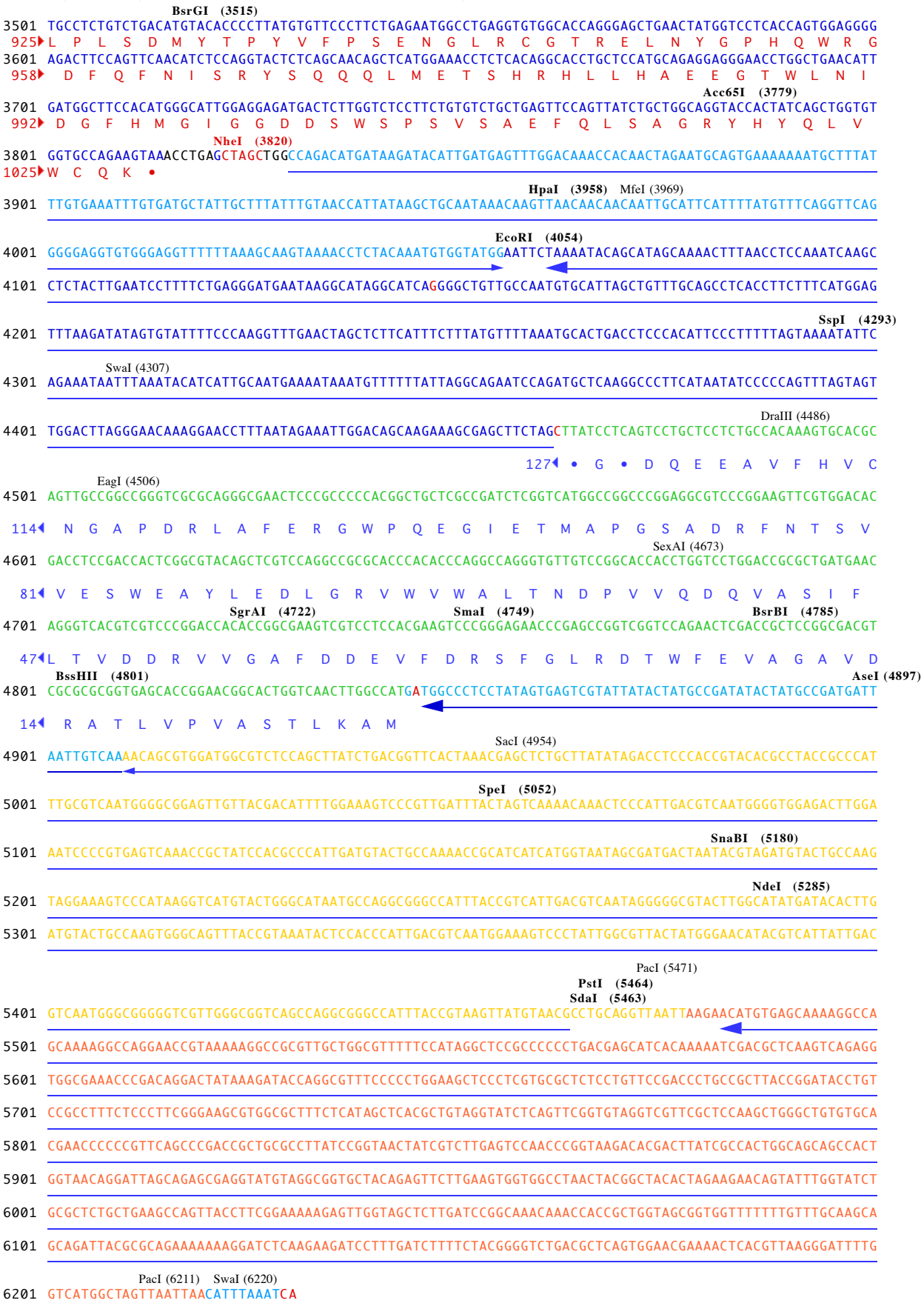
BsrGI (3515)
3501 TGCCTCTGTCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCCTCACCAGTGGAGGGG
925 L P L S D M Y T P Y V F P S E N G L R C G T R E L N Y G P H Q W R G
3601 AGACTTCCAGTTCAACATCTCCAGGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTGGCTGAACATT
958 D F Q F N I S R Y S Q Q Q L M E T S H R H L L H A E E G T W L N I
Acc65I (3779)
3701 GATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCCAGTTATCTGCTGGCAGGTACCACTATCAGCTGGTGT
992 D G F H M G I G G D D S W S P S V S A E F Q L S A G R Y H Y Q L V
NheI (3820)
3801 GGTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTAT
1025 W C Q K •
HpaI (3958) MfeI (3969)
3901 TTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAG
EcoRI (4054)
4001 GGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGC
4101 CTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAG
SspI (4293)
4201 TTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTC
SwaI (4307)
4301 AGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGT
DraIII (4486)
4401 TGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGC
127 • G • D Q E E A V F H V C
EagI (4506)
4501 AGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACAC
114 N 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
SexAI (4673)
4601 GACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAAC
81 V 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
SgrAI (4722) SmaI (4749) BsrBI (4785)
4701 AGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGT
47 L 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
BssHII (4801) AseI (4897)
4801 CGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATT
14 R A T L V P V A S T L K A M
SacI (4954)
4901 AATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCAT
SpeI (5052)
5001 TTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGA
SnaBI (5180)
5101 AATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAG
NdeI (5285)
5201 TAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTG
5301 ATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGAC
PacI (5471)
PstI (5464)
SdaI (5463)
5401 GTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCA
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6001 GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCA
6101 GCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTG
PacI (6211) SwaI (6220)
6201 GTCATGGCTAGTTAATTAACATTTAAATCA