pSELECT-zeo-LucSh

A plasmid encoding a synthetic CpG-free firefly Luc-Zeocin resistance fusion gene

Catalog code: psetz-lucsh

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-zeo-LucSh** 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 LucSh 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.

• **LucSh:** Synthetic LucSh fusion gene (LucSh-ΔCpG): InvivoGen has engineered a fusion between the firefly luciferase gene and the *Sh ble* gene conferring Zeocin™ resistance. Both genes have been modified and contain no CpG, whereas their wildtype counterparts contain 95 and 50 CpG motifs respectively. This fusion exhibits a higher luciferase activity and enables a better and faster selection of Zeocin™ resistant clones.

• **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.

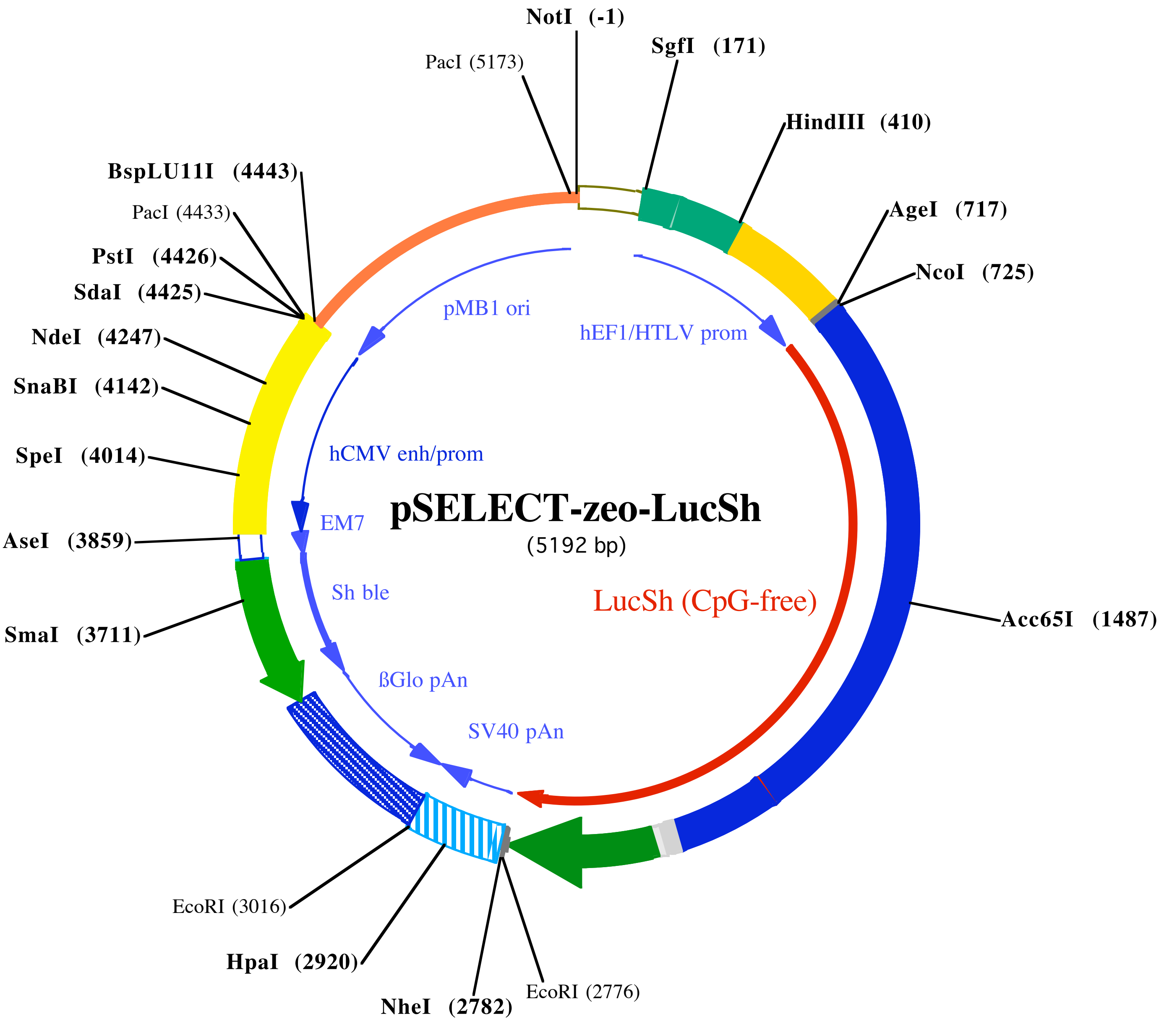
Plasmid amplification and cloning

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
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com



NotI (-1)
SgfI (171)
HindIII (410)
AgeI (717)
NcoI (725)
Acc65I (1487)
EcoRI (2776)
NheI (2782)
HpaI (2920)
EcoRI (3016)
SmaI (3711)
AseI (3859)
SpeI (4014)
SnaBI (4142)
NdeI (4247)
SdaI (4425)
PstI (4426)
PacI (4433)
BspLU11I (4443)
PacI (5173)
pMB1 ori
hEF1/HTLV prom
hCMV enh/prom
EM7
Sh ble
ßGlo pAn
SV40 pAn
LucSh (CpG-free)
pSELECT-zeo-LucSh
(5192 bp)

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       NotI  (-1)
   1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACT
  68 AACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCA
                                             SgfI  (171)
 135 AGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAG

 202 AGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAG

 269 AAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

 336 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAA

              HindIII  (410)
 403 CACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCA

 470 TCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTC

 537 TAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGAC

 604 TCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGT

                                                                 NcoI  (725)
                                                         AgeI  (717)
 671 TCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGGAGGATG
                                                                 1▸ M  E  D
 738 CCAAGAATATTAAGAAAGGCCCTGCCCCATTCTACCCTCTGGAAGATGGCACTGCTGGTGAGCAACT
   4▸A  K  N  I  K  K  G  P  A  P  F  Y  P  L  E  D  G  T  A  G  E  Q  L
 805 GCACAAGGCCATGAAGAGGTATGCCCTGGTCCCTGGCACCATTGCCTTCACTGATGCTCACATTGAG
  26▸ H  K  A  M  K  R  Y  A  L  V  P  G  T  I  A  F  T  D  A  H  I  E
 872 GTGGACATCACCTATGCTGAATACTTTGAGATGTCTGTGAGGCTGGCAGAAGCCATGAAAAGATATG
  49▸ V  D  I  T  Y  A  E  Y  F  E  M  S  V  R  L  A  E  A  M  K  R  Y
 939 GACTGAACACCAACCACAGGATTGTGGTGTGCTCTGAGAACTCTCTCCAGTTCTTCATGCCTGTGTT
  71▸G  L  N  T  N  H  R  I  V  V  C  S  E  N  S  L  Q  F  F  M  P  V  L
1006 AGGAGCCCTGTTCATTGGAGTGGCTGTGGCCCCTGCCAATGACATCTACAATGAGAGAGAGCTCCTG
  93▸ G  A  L  F  I  G  V  A  V  A  P  A  N  D  I  Y  N  E  R  E  L  L
1073 AACAGCATGGGCATCAGCCAGCCAACTGTGGTCTTTGTGAGCAAGAAGGGCCTGCAAAAGATCCTGA
 116▸ N  S  M  G  I  S  Q  P  T  V  V  F  V  S  K  K  G  L  Q  K  I  L
1140 ATGTGCAGAAGAAGCTGCCCATCATCCAGAAGATCATCATCATGGACAGCAAGACTGACTACCAGGG
 138▸N  V  Q  K  K  L  P  I  I  Q  K  I  I  I  M  D  S  K  T  D  Y  Q  G
1207 CTTCCAGAGCATGTATACCTTTGTGACCAGCCACTTACCCCCTGGCTTCAATGAGTATGACTTTGTG
 160▸ F  Q  S  M  Y  T  F  V  T  S  H  L  P  P  G  F  N  E  Y  D  F  V
1274 CCTGAGAGCTTTGACAGGGACAAGACCATTGCTCTGATTATGAACAGCTCTGGCTCCACTGGACTGC
 183▸ P  E  S  F  D  R  D  K  T  I  A  L  I  M  N  S  S  G  S  T  G  L
1341 CCAAAGGTGTGGCTCTGCCCCACAGAACTGCTTGTGTGAGATTCAGCCATGCCAGAGACCCCATCTT
 205▸P  K  G  V  A  L  P  H  R  T  A  C  V  R  F  S  H  A  R  D  P  I  F
1408 TGGCAACCAGATCATCCCTGACACTGCCATCCTGTCTGTGGTTCCATTCCATCATGGCTTTGGCATG
 227▸ G  N  Q  I  I  P  D  T  A  I  L  S  V  V  P  F  H  H  G  F  G  M
                    Acc65I  (1487)
1475 TTCACAACACTGGGGTACCTGATCTGTGGCTTCAGAGTGGTGCTGATGTATAGGTTTGAGGAGGAGC
 250▸ F  T  T  L  G  Y  L  I  C  G  F  R  V  V  L  M  Y  R  F  E  E  E
1542 TGTTTCTGAGGAGCCTACAAGACTACAAGATCCAGTCTGCCCTGCTGGTGCCCACTCTGTTCAGCTT
 272▸L  F  L  R  S  L  Q  D  Y  K  I  Q  S  A  L  L  V  P  T  L  F  S  F
1609 CTTTGCCAAGAGCACCCTCATTGACAAGTATGACCTGAGCAACCTGCATGAGATTGCCTCTGGAGGA
 294▸ F  A  K  S  T  L  I  D  K  Y  D  L  S  N  L  H  E  I  A  S  G  G
1676 GCACCCCTGAGCAAGGAGGTGGGTGAGGCTGTGGCAAAGAGGTTCCATCTCCCAGGAATCAGACAGG
 317▸ A  P  L  S  K  E  V  G  E  A  V  A  K  R  F  H  L  P  G  I  R  Q
1743 GCTATGGCCTGACTGAGACCACCTCTGCCATCCTCATCACCCCTGAAGGAGATGACAAGCCTGGTGC
 339▸G  Y  G  L  T  E  T  T  S  A  I  L  I  T  P  E  G  D  D  K  P  G  A
1810 TGTGGGCAAGGTGGTTCCCTTTTTTGAGGCCAAGGTGGTGGACCTGGACACTGGCAAGACCCTGGGA
 361▸ V  G  K  V  V  P  F  F  E  A  K  V  V  D  L  D  T  G  K  T  L  G
1877 GTGAACCAGAGGGGTGAGCTGTGTGTGAGGGGTCCCATGATCATGTCTGGCTATGTGAACAACCCTG
 384▸ V  N  Q  R  G  E  L  C  V  R  G  P  M  I  M  S  G  Y  V  N  N  P
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1944 AGGCCACCAATGCCCTGATTGACAAGGATGGCTGGCTGCACTCTGGTGACATTGCCTACTGGGATGA
 406▸E  A  T  N  A  L  I  D  K  D  G  W  L  H  S  G  D  I  A  Y  W  D  E
2011 GGATGAGCACTTTTTCATTGTGGACAGGCTGAAGAGCCTCATCAAGTACAAAGGCTACCAAGTGGCA
 428▸  D  E  H  F  F  I  V  D  R  L  K  S  L  I  K  Y  K  G  Y  Q  V  A
2078 CCTGCTGAGCTAGAGAGCATCCTGCTCCAGCACCCCAACATCTTTGATGCTGGTGTGGCTGGCCTGC
 451▸ P  A  E  L  E  S  I  L  L  Q  H  P  N  I  F  D  A  G  V  A  G  L
2145 CTGATGATGATGCTGGAGAGCTGCCTGCTGCTGTTGTGGTTCTGGAGCATGGAAAGACCATGACTGA
 473▸P  D  D  D  A  G  E  L  P  A  A  V  V  V  L  E  H  G  K  T  M  T  E
2212 GAAGGAGATTGTGGACTATGTGGCCAGTCAGGTGACCACTGCCAAGAAGCTGAGGGGAGGTGTGGTG
 495▸  K  E  I  V  D  Y  V  A  S  Q  V  T  T  A  K  K  L  R  G  G  V  V
2279 TTTGTGGATGAGGTGCCAAAGGGTCTGACTGGCAAGCTGGATGCCAGAAAGATCAGAGAGATCCTGA
 518▸ F  V  D  E  V  P  K  G  L  T  G  K  L  D  A  R  K  I  R  E  I  L
2346 TCAAGGCCAAGAAGGGTGGCAAACAATTGATCTCTGGAGCCAATGGAGTCATGGCCAAGTTGACCAG
 540▸I  K  A  K  K  G  G  K  Q  L  I  S  G  A  N  G  V  M  A  K  L  T  S
2413 TGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGG
 562▸  A  V  P  V  L  T  A  R  D  V  A  G  A  V  E  F  W  T  D  R  L  G
2480 TTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTCATCT
 585▸ F  S  R  D  F  V  E  D  D  F  A  G  V  V  R  D  D  V  T  L  F  I
2547 CAGCAGTCCAGGACCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGA
 607▸S  A  V  Q  D  Q  V  V  P  D  N  T  L  A  W  V  W  V  R  G  L  D  E
2614 GCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACA
 629▸  L  Y  A  E  W  S  E  V  V  S  T  N  F  R  D  A  S  G  P  A  M  T
2681 GAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACT
 652▸ E  I  G  E  Q  P  W  G  R  E  F  A  L  R  D  P  A  G  N  C  V  H
                                                  NheI (2782)
                                            EcoRI (2776)
2748 TTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTCGCTAGCTGGCCAGACATGATAAGATACATTGA
 674▸F  V  A  E  E  Q  D  •
2815 TGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCT

                                                            HpaI (2920)
2882 ATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTA

2949 TGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAT

      EcoRI (3016)
3016 GGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCT

3083 GAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACC

3150 TTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATG

3217 TTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCA

3284 TTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCC

3351 CAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGC

3418 TTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAG
             125◂ •  D  Q  E  E  A  V  F  H  V  C  N  G  A  P  D  R  L
3485 GGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAG
 107◂ A  F  E  R  G  W  P  Q  E  G  I  E  T  M  A  P  G  S  A  D  R  F
3552 TTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCA
  84◂N  T  S  V  V  E  S  W  E  A  Y  L  E  D  L  G  R  V  W  V  W  A  L
3619 GGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCAC
  62◂  T  N  D  P  V  V  Q  D  Q  V  A  S  I  F  L  T  V  D  D  R  V  V
                                    SmaI (3711)
3686 ACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCT
  40◂ G  A  F  D  D  E  V  F  D  R  S  F  G  L  R  D  T  W  F  E  V  A
3753 CCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTAT
  17◂G  A  V  D  R  A  T  L  V  P  V  A  S  T  L  K  A  M
                                                     AseI (3859)
3820 AGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGC
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3887 GTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCT

SpeI (4014)

3954 ACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAG

4020 TCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTAT

SnaBI (4142)

4087 CCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGT

4154 ACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTC

NdeI (4247)

4221 ATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTAC

4288 CGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCA

4355 TTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGT

PacI (4433)

PstI (4426)

SdaI (4425)

BspLU11I (4443)

4422 AACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAA

4487 GGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCA

4554 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG

4621 TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGT

4688 GGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGC

4755 TGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA

4822 ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA

4889 TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTT

4956 GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAC

5023 AAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATC

5090 TCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGG

PacI (5173)

5157 ATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA