STOP

Before using this product, please read the Limited Use License statement below:

Important Limited Use License information for pSELECT-NLucia-zeo

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pSELECT-NLucia-zeo

Plasmid for the expression of Lucia-N-terminal tagged proteins

Catalog # psetz-nlucia

For research use only

Version 20L01-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-NLucia-zeo** 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 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 Lucia-Tag is a family of expression plasmids designed to generate tagged proteins in mammalian cells with Lucia luciferase in order to detect and quantify the tagged protein of interest by bioluminescence assay. Lucia luciferase is a novel secreted luciferase with strong bioluminescent activity. The luciferase activity of Lucia luciferase can be detected and quantified directly in the culture medium of transfected cells using InvivoGen's QUANTI-Luc™ detection reagent.

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-1a (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' - BamH I, Eco47III, Nco I, NheI - 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

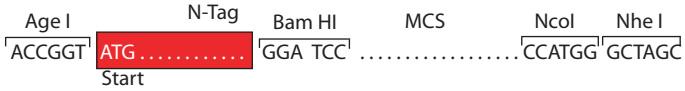
- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene 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

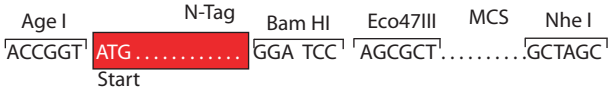
For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Bam HI/Nhe I restriction site combination for cloning into plasmids with the N-Tag. For the plasmids with N-Tag, check whether your gene of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



Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is downstream of the Bam HI 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.

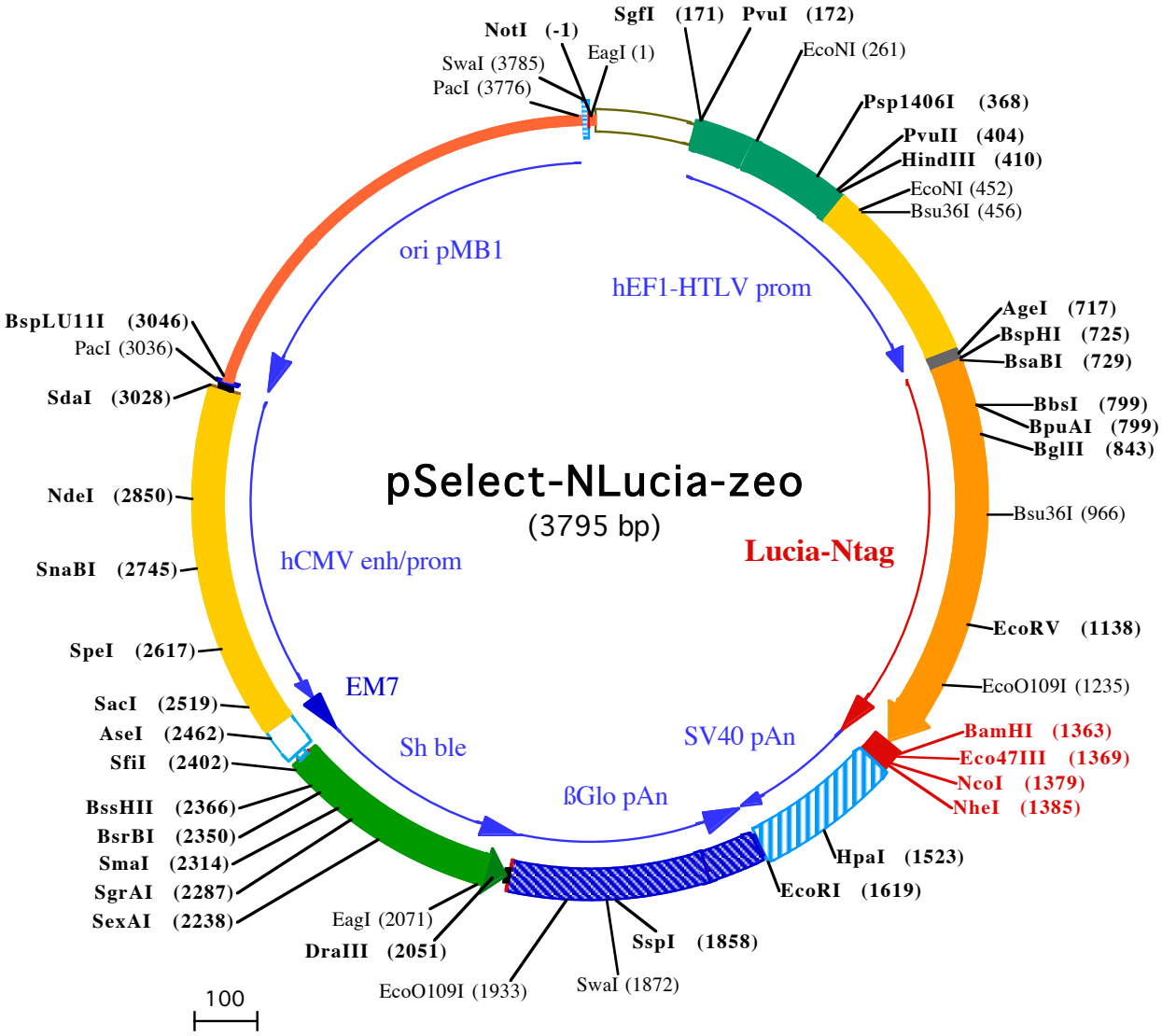
Zeocin™ usage
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

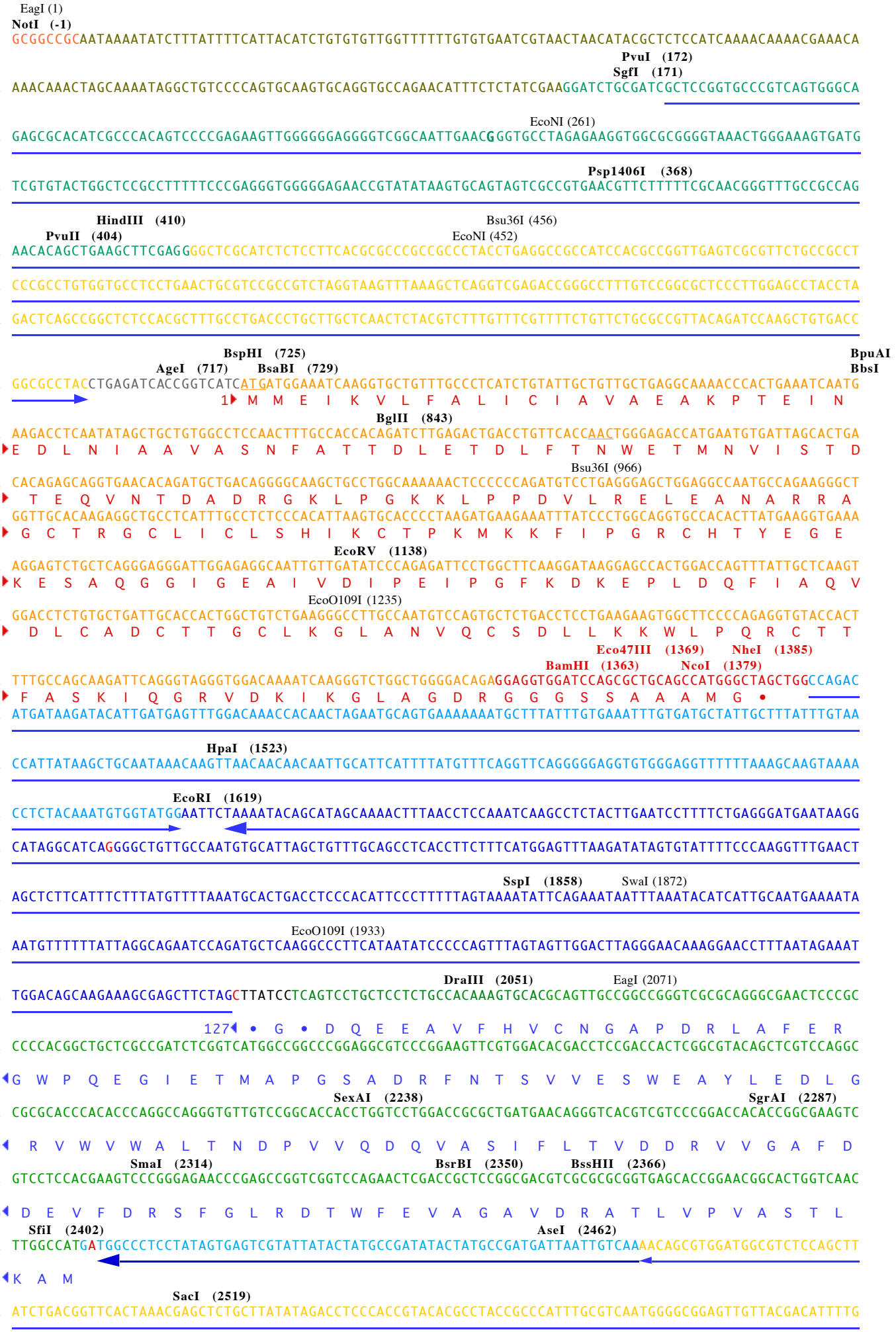
References:

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-NLucia-blasti	psetb-nlucia
QUANTI-Luc™	rep-qlc1
ChemiComp GT116	gt116-11


pSelect-NLucia-zeo
(3795 bp)
ori pMB1
hEF1-HTLV prom
hCMV enh/prom
EM7
Sh ble
ßGlo pAn
SV40 pAn
Lucia-Ntag
NotI (-1)
SwaI (3785)
PacI (3776)
EagI (1)
SgfI (171)
PvuI (172)
EcoNI (261)
Psp1406I (368)
PvuII (404)
HindIII (410)
EcoNI (452)
Bsu36I (456)
AgeI (717)
BspHI (725)
BsaBI (729)
BbsI (799)
BpuAI (799)
BglII (843)
Bsu36I (966)
EcoRV (1138)
EcoO109I (1235)
BamHI (1363)
Eco47III (1369)
NcoI (1379)
NheI (1385)
HpaI (1523)
EcoRI (1619)
SspI (1858)
SwaI (1872)
EagI (2071)
DraIII (2051)
EcoO109I (1933)
BssHII (2366)
BsrBI (2350)
SmaI (2314)
SgrAI (2287)
SexAI (2238)
SfiI (2402)
AseI (2462)
SacI (2519)
SpeI (2617)
SnaBI (2745)
NdeI (2850)
SdaI (3028)
PacI (3036)
BspLU11I (3046)
100


EagI (1)
NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA
PvuI (172)
SgfI (171)
101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
EcoNI (261)
201 GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATG
Psp1406I (368)
301 TCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG
HindIII (410)
PvuII (404)
Bsu36I (456)
EcoNI (452)
401 AACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCT
501 CCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTA
601 GACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC
BspHI (725)
BpuAI
AgeI (717)
BsaBI (729)
BbsI
701 GGCGCCTACCTGAGATCACCGGTCATCATGATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATG
1 M M E I K V L F A L I C I A V A E A K P T E I N
BglII (843)
801 AAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACCAACTGGGAGACCATGAATGTGATTAGCACTGA
25 E D L N I A A V A S N F A T T D L E T D L F T N W E T M N V I S T D
Bsu36I (966)
901 CACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCT
58 T E Q V N T D A D R G K L P G K K L P P D V L R E L E A N A R R A
1001 GGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAA
92 G C T R G C L I C L S H I K C T P K M K K F I P G R C H T Y E G E
EcoRV (1138)
1101 AGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGT
125 K E S A Q G G I G E A I V D I P E I P G F K D K E P L D Q F I A Q V
EcoO109I (1235)
1201 GGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGAAGAAGTGGCTTCCCCAGAGGTGTACCACT
158 D L C A D C T T G C L K G L A N V Q C S D L L K K W L P Q R C T T
Eco47III (1369)
NheI (1385)
BamHI (1363)
NcoI (1379)
1301 TTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAGGAGGTGGATCCAGCGCTGCAGCCATGGGCTAGCTGGCCAGAC
192 F A S K I Q G R V D K I K G L A G D R G G G S S A A A M G •
1401 ATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA
HpaI (1523)
1501 CCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAA
EcoRI (1619)
1601 CCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGG
1701 CATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACT
SspI (1858)
SwaI (1872)
1801 AGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATA
EcoO109I (1933)
1901 AATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAAT
DraIII (2051)
EagI (2071)
2001 TGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGC
127 • G • D Q E E A V F H V C N G A P D R L A F E R
2101 CCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGC
102 G W P Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D L G
SexAI (2238)
SgrAI (2287)
2201 CGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTC
69 R V W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A F D
SmaI (2314)
BsrBI (2350)
BssHII (2366)
2301 GTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAAC
36 D E V F D R S F G L R D T W F E V A G A V D R A T L V P V A S T L
SfiI (2402)
AseI (2462)
2401 TTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTT
2 K A M
SacI (2519)
2501 ATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTG


SpeI (2617)
2601 GAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATT
SnaBI (2745)
2701 GATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAA
NdeI (2850)
2801 TGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACT
2901 CCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGG
PacI (3036)
SdaI (3028)
BspLU11I (3046)
3001 CGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGC
3101 TGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC
3201 GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCT
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3601 GTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAG
PacI (3776) SwaI (3785)
3701 ATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA