

# pSELECT-zeo-LacZ::Sh

A plasmid encoding a CpG-free LacZ-Zeocin resistance fusion gene

Catalog code: psetz-laczh

For research use only

Version 20K30-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pSELECT-zeo-LacZ::Sh 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 LacZ::Sh 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 cassette 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<sup>1</sup> 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<sup>2</sup>. 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.

• **LacZ::Sh CpG-free:** InvivoGen has engineered a fusion gene between a humanized LacZ gene and the *Sh ble* gene conferring Zeocin™ resistance. Both genes have been modified and contain no CpG motifs. The lacZ 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 CpG-free lacZ gene is ten times more active than the wild-type gene in mammalian cells. This LacZ::Sh fusion gene 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<sup>3</sup>.

• **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*.

• **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>4</sup>.

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<sub>2</sub>O. 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

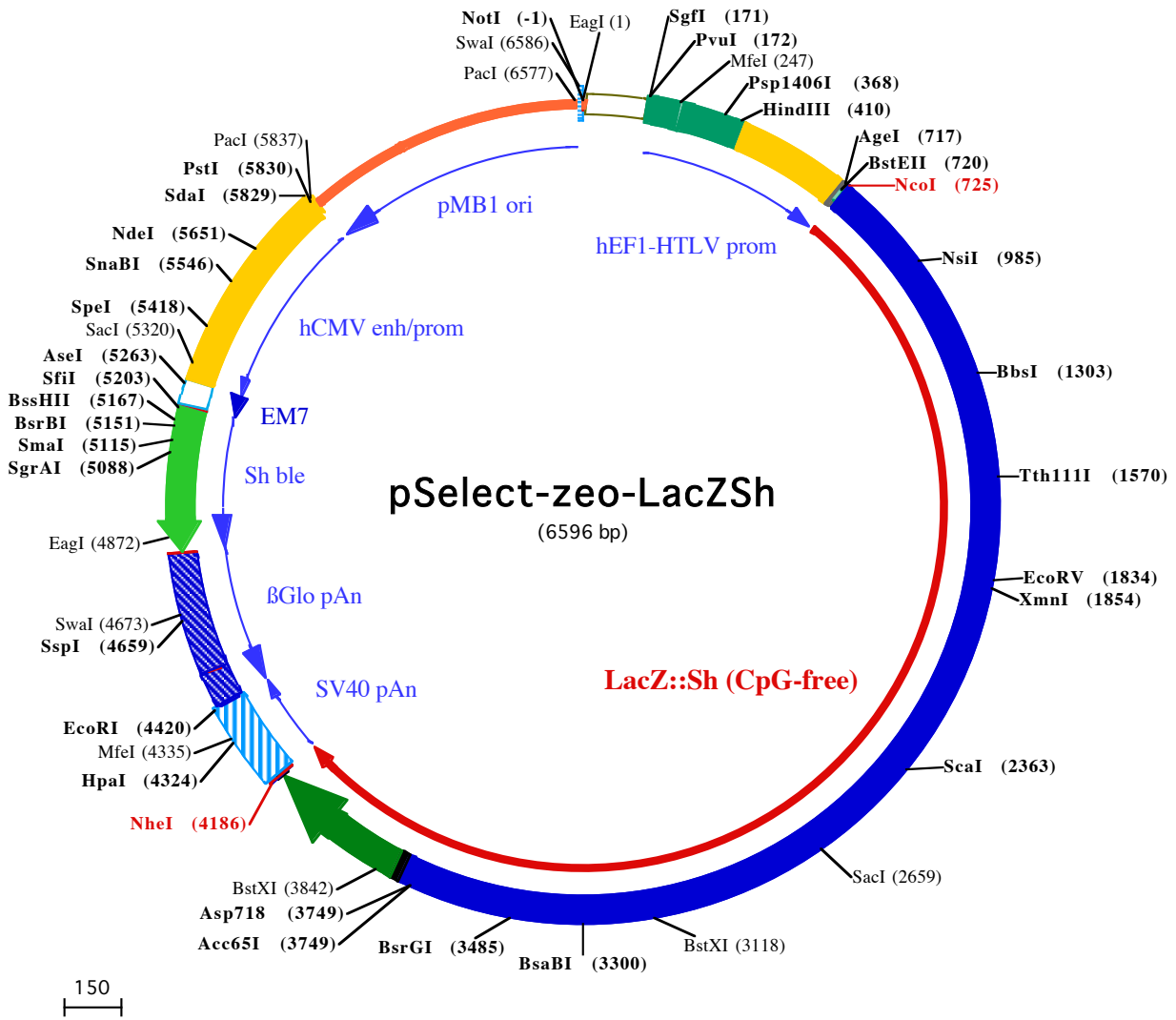
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EagI (1)  
NotI (-1)  
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATGTAACATAACATACGCTCTCCATCAAAAACAAACGAAACA  
PvuI (172)  
SgfI (171)  
101 AAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCTGCGATCGCTCCGGTCCCGTCACTGGGCA  
MfeI (247)  
201 GAGCGACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCCGGGGTAACTGGAAAGTGTATG  
Psp1406I (368)  
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGAACGGGTTTCCGCCAG  
HindIII (410)  
401 AACACAGCTGAAGCTTCAGGGCTCGCATCTCTCTTACCGCGCCCGCCCTACCTGAGCGCCCATCCACGCCGTTGAGTCCGCTTCTGCCCT  
501 CCGCCTGTGGTCCCTCTGAAGTCCGCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGCCCTTTGTCCGGCGCTCCCTTGGAGCTACCTA  
601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCGTTACAGATCCAAGCTGTGACC  
NcoI (725)  
BstEII (720)  
AgeI (717)  
701 GCGCCTACCTGAGATCaccgggtcaCATGGACCTGTTGTGCTCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCC  
1 M D P V V L Q R R D W E N P G V T Q L N R L A A  
801 ACCCTCCCTTTGCCCTTTGGAGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCCAGCAGCTCAGGTTCTCAATGGAGAGTGGAGGTTGCCCTGGT  
25 H P P F A S W R N S E E A R T C R P S Q Q L R S L N G E W R F A W F  
NsiI (985)  
901 CCCTGCCCTGAAGCTGTGCTGAGTCTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTGGTGGCCAGCAACTGGCAGATGCATGGCTATGAT  
58 P A P E A V P E S W L E C D L P E A D T V V V P S N W Q M H G Y D  
1001 GCCCATCTACCAATGTACCTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCTGGCTGCTACAGCCTGACCTCAATGTTG  
92 A P I Y T N V T Y P I T V N P P F V P T E N P T G C Y S L T F N V  
1101 ATGAGAGCTGGCTCAAGAAGCCAGCAGGATCATCTTTGATGGAGTCAACTGCTCCACCTTGGTGAATGGCAGGTGGTGGCTATGGCCA  
125 D E S W L Q E G Q T R I I F D G V N S A F H L W C N G R W V G Y G Q  
1201 AGACAGCAGGCTGCCCTCTGAGTTGACCTCTGCTCCCTCAGAGCTGGAGAGAAGCAGGCTGGCTGTGATGGTGTGCTGAGGTGGTGTGATGGCAGCTAC  
158 D S R L P S E F D L S A F L R A G E N R L A V M V L R W S D G S Y  
BbsI (1303)  
1301 CTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCTGTGCACAAGCCACCACCCAGATTCTGACTTCCATGTTGCCACCA  
192 L E D Q D M W R M S G I F R D V S L L H K P T T Q I S D F H V A T  
1401 GGTTCATGATGCTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGAGTGTGAGGAGAAGTCAAGAGCTGAGAGCTGAGAGTCAAGTCAAGTCTGCGCAAG  
225 R F N D D F S R A V L E A E V Q M C G E L R D Y L R V T V S L W Q G  
Tth1111 (1570)  
1501 TGAGACCCAGGTGGCCTCTGGCACAGCCCCCTTTGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAG  
258 E T Q V A S G T A P F G G E I I D E R G G Y A D R V T L R L N V E  
1601 AACCCCAAGCTGTGCTGCTGAGATCCCAACCTCTACAGGCTGTTGTGGAGCTGCACACTGCTGATGGCACCTGATTGAAGCTGAAGCCTGTGATG  
292 N P K L W S A E I P N L Y R A V V E L H T A D G T L I E A E A C D  
1701 TTGGATCAGAGAAGTCAAGTGGCCTGCTGCTCAATGGCAAGCCTGCTGCTCATCAGGGAGTCAACAGGCTGAGCACCACCTCTGCA  
325 V G F R E V R I E N G L L L N G K P L L I R G V N R H E H H P L H  
EcoRV (1834) XmnI (1854)  
1801 TGGACAAGTGGATGAACAGACAATGGTCAAGATCTGCTAATGAAGCAGAACAACCTTCAATGCTGTCAGGTGCTCTCACTACCCCAACCCCT  
358 G Q V M D E Q T M V Q D I L L M K Q N N F N A V R C S H Y P N H P  
1901 CTTGGTACACCTGTGTGACAGTATGGCTGTATGTTGATGAAGCCAACTGATGAGACACATGGCATGGTGGCCATGAACAGGCTACAGATGACC  
392 L W Y T L C D R Y G L Y V D E A N I E T H G M V P M N R L T D D  
2001 CCAGGTGGCTGCCTGCTGAGAGAGTACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGATGATCATCTGGTCTCTGGCAATGAGTCTGG  
425 P R W L P A M S E R V T R M V Q R D R N H P S V I I W S L G N E S G  
2101 ACATGGAGCCAACTGATGCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCAATGAAGAGGTGGAGCAGACACACAGCCACA  
458 H G A N H D A L Y R W I K S V D P S R P V Q Y E G G G A D T T A T  
2201 GACATCATCTGCCCATGATGCCAGGGTGTGAGGACAGCCCTTCCCTGCTGTGCCAAGTGGAGCATCAAGAAAGTGGCTCTCTGCTGAGAGAGA  
492 D I I C P M Y A R V D E D Q P F P A V P K W S I K K W L S L P G E  
ScaI (2363)  
2301 CCAGACCTGATCTGTGTAATGACATGCAATGGGCAACTCTGGGAGGCTTTGCCAAGTACTGGCAAGCCTTCAGACAGTACCCCAAGCTGCA  
525 T R P L I L C E Y A H A M G N S L G G F A K Y W Q A F R Q Y P R L Q  
2401 AGGAGGATTTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCTGGTCTGCCTATGGAGGAGACTTTGGTACACCCCC  
558 G G F V W D W V D Q S L I K Y D E N G N P W S A Y G G D F G D T P  
2501 AATGACAGGCAGTTCTGCATGAATGGCCTGGTCTTTCAGACAGGACCCCTCACCTGCCCTCACAGAGGCCAAGCACCAGCAAGTCTTCCAGTTCA  
592 N D R Q F C M N G L V F A D R T P H P A L T E A K H Q Q Q F  
SaeI (2659)  
2601 GGCTGTCTGCAGACACCATTGAGGTGACATCTGAGTACCTCTCAGGCACTGACAATGAGTCTGCACTGGATGGTGGCCTGGATGGCAAGCTCT  
625 R L S G Q T I E V T S E Y L F R H S D N E L L H W M V A L D G K P L  
2701 GGCTTCTGGTGGTGGCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACTGCTGAGCTGCCTCAGCCAGAGTCTGCTGGACAATGTGGCTA  
658 A S G E V P L D V A P Q G K Q L I E L P E L P Q P E S A G Q L W L  
2801 ACAGTGAAGGTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGAGCCACATCTGTCATGGCAGCAGTGGAGGCTGGCTGAGAACCTCTGTGACCC  
692 T V R V V Q P N A T A W S E A G H I S A W Q Q W R L A E N L S V T  
2901 TGCTGCTGCTCATGCAATCCCTCACCTGACAACATCTGAAATGACTTCTGCAATGAGCTGGGCAACAGAGATGGCAGTTCAACAGGCACTGCTGG  
725 L P A A S H A I P H L T T S E M D F C I E L G N K R W Q Q S G  
3001 CTTCTGCTCAGATGTGGATGGAGACAAGCAGCTCCTCACCCCTCTCAGGACCAATTACCAGGCTCCTCTGGACAATGACATGGAGTGTCT  
758 F L S Q M W I G D K K Q L L T P L R D Q F T R A P L D N D I G V S  
BstXI (3118)  
3101 GAGGCCACCAGGATTGACCCAAATGCTTGGTGGAGAGGTGGAAGGCTGCTGGACACTACCAGGCTGAGGCTGCCTGCTCCAGTGACAGCAGACACC  
792 E A T R I D P N A W V E R W K A A G H Y Q A E A A L L Q C T A D T  
3201 TGGCTGATGCTGTCTGATCACCACGCCATGCTGGCAGCACAAGGCAAGCCCTGTTTCATCAGCAGAAAGACCTACAGGATGATGGCTGGACA  
825 L A D A V L I T T A H A W Q H Q G K T L F I S R K T Y R I D G S G Q  
BsaBI (3300)  
3301 GATGGCAATCACAGTGGATGTGGAGTTCCTCTGACACACCTCACCTGCAAGGATTGGCTGAACTGTCAACTGGCACAGGTGGCTGAGAGGGTGAAC  
858 M A I T V D V E V A S D T P H P A R I G L N C Q L A Q V A E R V N  
BsrGI (3485)  
3401 TGGCTGGGCTTAGGCCCTCAGGAGAATACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTGGGACCTGCCTCTGTGATGATGTACACCCCTTATG  
892 W L G L G P Q E N Y P D R L T A A C F D R W D L P L S D M Y T P Y  
3501 TGTTCCTTCTGAGAATGGCTGAGGTGGCACCAGGAGCTGAACATGGTCTCACCAGTGGAGGGGAGACTTCCAGTTCAACATCTCCAGGTACTC  
925 V F P S E N G L R C G T R E L N Y G P H Q W R G D F Q F N I S R Y S  
3601 TCAGCAACAGCTCAAGAACTCTCACAGCCTGCTCAGAGGAGGAACTGGCTGAACATGATGGCTTCCACATGGCATTGGAGAGAT  
958 Q Q Q L M E T S H R H L L H A E E G T W L N I D G F H M G I G G D

Asp718 (3749)  
Acc65I (3749)

3701 GACTCTTGGTCTCCTTCTGTGCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCACATATCAGCTGGTGGTGGCCAGAAAGCTCATCTCTGGAGCCAATG  
992▶ D S W S P S V S A E F Q L S A G R Y H Y Q L V W C Q K L I S G A N

BstXI (3842)

3801 GAGTCATGGCCAAGTTGACCAAGTGTGCCAGTGTCCAGCTCACAGCCAGGGATGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTCTCCAGAGA  
1025▶ G V M A K L T S A V P V L T A R D V A G A V E F W T D R L G F S R D  
3901 TTTTGTGGAGGATGACTTTGACAGTGTGGTCCAGAGATGATGTCACCTGTTTCATCTCAGCAGTCCAGGACCAGGTTGGTGCCTGACAACCCCTGGCTTG  
1058▶ F V E D D F A G V V R D D V T L F I S A V Q D Q V V P D N T L A W  
4001 GTGTGGTGGAGGACTGGATGAGCTGTATGCTGAGTGAGTGGAGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAG  
1092▶ V W V R G L D E L Y A E W S E V V S T N F R D A S G P A M T E I G

NheI (4186)

4101 AGCAGCCCTGGGGAGAGAGTTTGCCTGAGAGCCAGCAGGCAACTGTGTGCACTTTTGGCAGAGGAGCAGGACTGAGGATAAGCTAGCTGGCCAGA  
1125▶ E Q P W G R E F A L R D P A G N C V H F V A E E Q D •  
4201 CATGATAAGATACATTGATGAGTTTGGACAACCACAAC TAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTA

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HpaI (4324) MfeI (4335)

4301 ACCATTATAAGCTGCAATAAACAAGTTAAACAACAACATTCATTCTTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAA

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EcoRI (4420)

4401 ACCTCTACAATGTGGTATGAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAG  
4501 GCATAGGCATCAGGGGCTGTGCCAATGTGCATTAGCTGTTTGACGCTCACCTCTTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTTGAAC

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SspI (4659) SwaI (4673)

4601 TAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATAGTAAAATATTCAGAAAATAATTTAAATACATCATTGCAATGAAAAT  
4701 AAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAGGAACCTTTAATAGAAA

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EagI (4872)

4801 TTGGACAGCAAGAAAGCAGCTTCTAGCTTATCCTCAGTCTGCTCCTGCCCACAAAGTGACGAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCG  
4901 CCCCACGGCTGCTCGCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGG  
103▶ 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  
5001 CCGCGCACCCACCCAGGCCAGGGTGTTCGGCACCACCTGGTCTGGACCGCTGATGAACAGGGTACAGTCTCCCGACCAACCCGCGGAAGT  
69▶ G 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

SgrAI (5088)

5101 CGTCTCCACGAAGTCCGGGAGAACCCGAGCCGGTGGTCCAGAACTGACCGCTCCGGCGACGTCGCGCGGTGAGCACCGGAACGGCACTGGTCAA  
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 (5203) AseI (5263)

5201 CTTGGCCATGATGGCCCTCTATAGTGAAGTCTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCT  
3▶ K A M

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SacI (5320)

5301 TATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACAGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTT

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SpeI (5418)

5401 GGAAAGTCCCGTTGATTTACTAGTCAAACAAACTCCCAATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCAGCCCAT

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SnaBI (5546)

5501 TGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATA

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NdeI (5651)

5601 ATGCCAGCGGGCCATTTACCGTCAATTGACGTCAATAGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCGAGTTTACCGTAAATAC  
5701 TCCACCAATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATATTGACGTCAATGGGCGGGGTCTTGGCGGTGACGCCAG

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PacI (5837)  
PstI (5830)  
SdaI (5829)

5801 GCGGGCCATTACCGTAAGTTATGTAACGCCCTGCAGGTTAATAAGAATGTGAGCAAAAAGGCCAGCAAAAAGGCCAGGAACCGTAAAAAGCCGCTTG  
5901 CTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGGAAACCCGACAGGACTATAAAGATACCGG  
6001 CGTTTTCCCTGGAAAGTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCTTCGGGAAGCGTGGCGTTTC  
6101 TCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCTTA  
6201 TCCGGTAACTATCGTCTTGTAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCG  
6301 GTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAG  
6401 AGTTGGTAGCTCTTGTCCGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAA  
6501 GATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAATCA

PacI (6577) SwaI (6586)