

# 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 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.

• **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<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

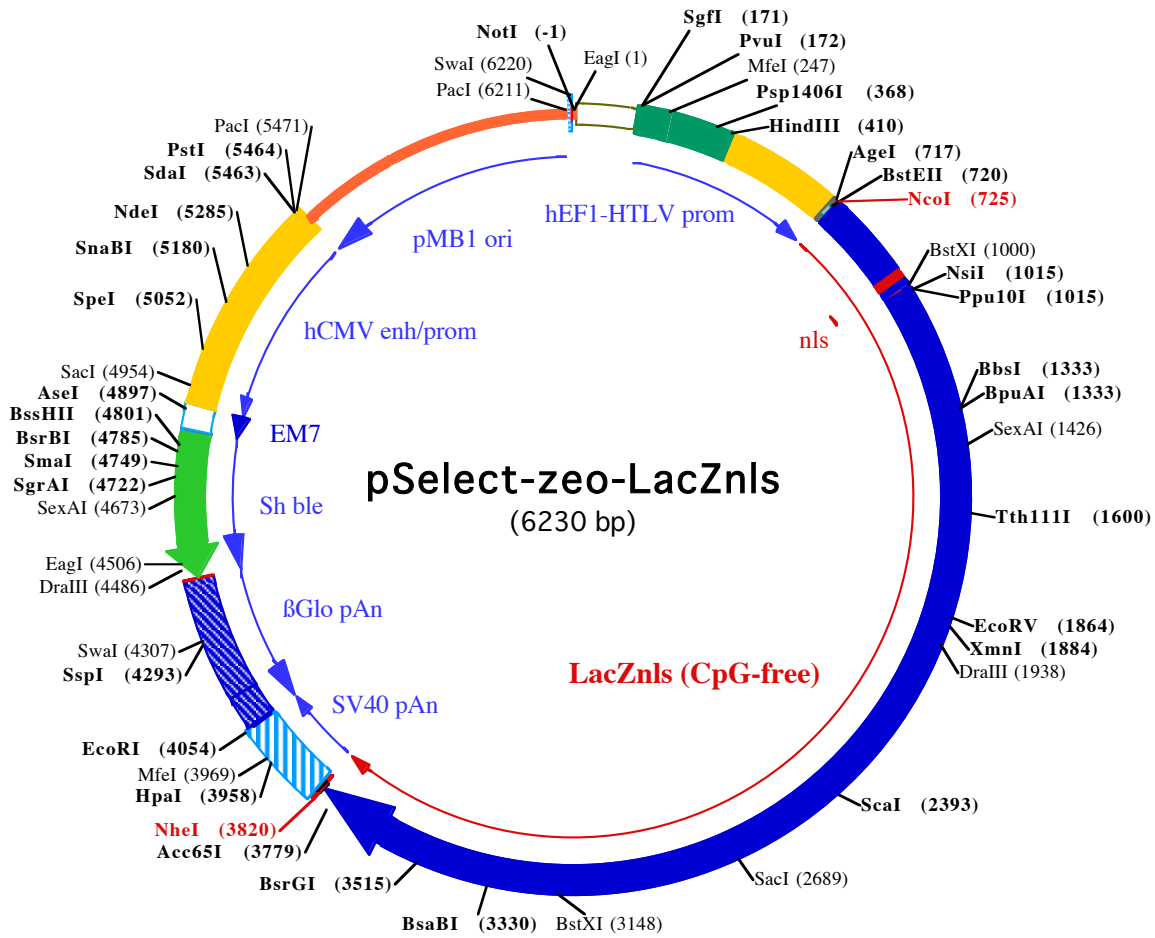
InvivoGen USA (Toll-Free): 888-457-5873

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](mailto:info@invivogen.com)



EagI (1)  
NotI (-1)  
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA  
PvuI (172)  
SgfI (171)  
101 AAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCTGCGATCGCTCCGGTCCCGTCAGTGGCA  
MfeI (247)  
201 GAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGTGGCCGGGGTAACTGGGAAAGTGTG  
Psp1406I (368)  
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGTTGGGGGAGAACCGTATATAAGTGCAGTAGTCCCGTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAG  
HindIII (410)  
401 AACACAGCTGAAGCTTCAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCCGCTTCTGCCCT  
501 CCGCCTGTGGTCCCTCTGAAGTCCGCTCCGCGTCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGCCCTTTGTCCGGGCTCCCTTGGAGCTACCTA  
601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCCCTGCTTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC  
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 ACCCTCCCTTTGCCCTTTGGGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGT  
25 H P P F A S W R N S E E A R T D R P S Q Q L R S L N G E W R F A W F  
901 CCCTGCCCTGAAGCTGTGCTGAGTCTGGTGGAGTGTGACCTCCAGAGGCAGTCCCAAGAAGAAGAGAAAGTTGAGGCTGACACTGTTGTGGT  
58 P A P E A V P E S W L E C D L P E A V P K K K R K V E A D T V V V  
Ppu10I (1015)  
BstXI (1000)  
NsiI (1015)  
1001 CCAAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTACCTACCCCATCTGTGAACCCCTTTTGTGCCACTGAGAACC  
92 P S N W Q M H G Y D A P I Y T N V T Y P I T V N P P F V P T E N P  
1101 CTGGTCTACAGCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGCCAGCCAGGATCATCTTTGATGGAGTCAACTTGCCTCCACCTCTG  
125 T G C Y S L T F N S E E A R T D R P S Q Q L R S L N G E W R F A W F  
1201 GTCAATGGCAGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTACCTCTCTGCTTCTCAGAGCTGGAGAGAACAGGCTGGCTGT  
158 C N G R W V G Y G Q D S R L P S E F D L S A F L R A G E N R L A V  
BpuAI (1333)  
BbsI (1333)  
1301 ATGGTGCTCAGGTGGTCTGATGGCAGTACCTGGAAGCAAGACATGTGGAGATGTCTGGCATCTTCAGGGATGTGAGCCTGTGCACAAGCCACCA  
192 M V L R W S D G S Y L E D Q D M W R M S G I F R D V S L L H K P T  
SexAI (1426)  
1401 CCCGATTTCTGACTTCCATGTTGCCACAGGTTCAATGATGACTTACAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCAGAGACTA  
225 T Q I S D F H V A T R F N D D F S R A V L E A E V Q M C G E L R D Y  
1501 CCTGAGACTCAGCTGAGCTTGGCAAGGTGAGACCCAGTGGCTTCCGACAGCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCT  
258 L R V T V S L W Q G E T Q V A S G T A P F G G E I I D E R G G Y A  
Tth111I (1600)  
1601 GACAGAGTCAACCCTGAGGCTCAATGTGGAGAACCACCAAGCTGTGGTCTGCTGAGATCCCCAACCTTACAGGGCTGTTGTGGAGTGCACACTGCTGAT  
292 D R V T L R L N V E N P K L W S A E I P N L Y R A V V E L H T A D  
1701 GCACCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTGAGAGAAGTCAAGATTGAGAATGGCCTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGG  
325 G T L I E A E A C D V G F R E V R I E N G L L L N G K P L I R G  
EcoRV (1864) XmnI (1884)  
1801 AGTCAACAGGCATGAGCACCCTCTGCATGGACAAGTGTGATGAACAGACAATGGTCAAGATATCTGCTAATGAAGCAGAACTTCAATGCT  
358 V N R H E H H P L H G Q V M D E Q T M V Q D I L L M K Q N N F N A  
DraIII (1938)  
1901 GTCAGGTCTCTACTACCCCAACCCTCTGTTACACCTGTGTGACAGGTATGGCTGTATGTTGTTGATGAAGCCAACATTGAGACACATGGCA  
392 V R C S H Y P N H P L W Y T L C D R Y G L Y V V D E A N I E T H G  
2001 TGGTGCCCATGAACAGCTCACAGATGACCCAGGTTGGCTGCCTGCCATGTCTGAGAGAGTACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGT  
425 M V P M N R L T D D P R W L P A M S E R V T R M V Q R D R N H P S V  
2101 GATCATGGTCTCTGGGCAATGAGTCTGGACATGGAGCAACCATGATGCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCGAT  
458 I I W S L G N E S G H G A N H D A L Y R W I K S V D P S R P V Q Y  
2201 GAAGGAGTGGAGCAGACCCACAGCCACAGACATCATCTGCCATGATGCCAGGTTGATGAGGACCAGCCCTCCCTGCTGTGCCAAGTGGAGCA  
492 E G G G A D T T A T D I I C P M Y A R V D E D Q P F P A V P K W S  
ScaI (2393)  
2301 TCAAGAAGTGGCTCTCTGCTGGAGAGACCAGACCTCTGATCTGTGTAATGCACATGCAATGGCAACTCTCTGGAGGCTTTGCCAAGTACTG  
525 I K K W L S L P G E T R P L I L C E Y A H A M G N S L G G F A K Y W  
2401 GCAAGCCTCAGCAGTACCCAGGCTGCAAGGAGTTTGTGGACTGGTGGACCAATCTCTCATCAAGATGATGAGAATGATGAGAATGATGAGAATGATGAGA  
558 Q A F R Q Y P R L Q G G F V W D W V D Q S L I K Y D E N G N P W S  
2501 GCCTATGGAGGAGACTTTGGTACACCCCAATGACAGGAGTCTGATGAATGGCCTGGTCTTTGACAGCAGGACCCTCACCTGCCCTCACAGAGG  
592 A Y G G D F G D T P N D R Q F C M N G L V F A D R T P H P A L T E  
SacI (2689)  
2601 CCAAGCACCAGCAACAGTCTTCCAGTTCAGGCTGCTGGACAGACCATGAGGTGACATCTGAGTACCTTTCAGGCACTCTGACAATGAGCTCTGCA  
625 A K H Q Q F F Q F R L S G Q T I E V T S E Y L F R H S D N E L L H  
2701 CTGGATGGTGGCCTGGATGGCAAGCTCTGGCTTCTGGTGGTGCCTCTGGATGTGGCCCTCAAGGAAGCAGATGATTGAAGTCTGAGCTGCCT  
658 W M V A L D G K P L A S G E V P L D V A P Q G K Q L I E L P E L P  
2801 CAGCCAGAGTCTGTCGCAACTGTGGCTAACAGTGGGTTGGTTCAGCCCAATGCAACAGCTTGGTCTGAGGCAGGACCATCTGCTGATGGCAGCAGT  
692 Q P E S A G Q L W L T V R V V Q P N A T A W S E A G H I S A W Q Q  
2901 GGAGGCTGGTGAACCTCTCTGTGACCCCTGCTGCTCATGCCATCCCTCACCTGACAACATCTGAATGGACTTCTGCATTGAGCTGGGCAA  
725 W R L A E N L S V T L P A A S H A I P H L T S E M D F C I E L G N  
3001 CAAGAGATGGCAGTTCAACAGGCACTGCTGCTGCTGATGATGGGATTGGAGCAAGAAGCAGCTCCACCCCTCTCAGGACCAATTACCAGG  
758 K R W Q F N R Q S G F L S Q M W I G D K K Q L L T P L R D Q F T R  
BstXI (3148)  
3101 GCTCCTCTGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGTGGAGAGTGGAAAGCTGCTGGACACTACCAGGCTGAGG  
792 A P L D N D I G V S E A T R I D P N A W V E R W K A A G H Y Q A E  
3201 CTGCCCTGCTCAGTGACAGCAGACCCCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGCACCAGGCAAGCCCTGTTTCATCAGCAG  
825 A A L L Q C T A D T L A D A V L I T T A H A W Q H Q G K T L F I S R  
BsaBI (3330)  
3301 AAAGACCTACAGGATGATGGCTCTGGACAGATGGCAATCAGTGGATGTGGAGGTTGCCCTGACACACCTCACCTGCAAGGATGGCCTGAAGTGT  
858 K T Y R I D G S G Q M A I T V D V E V A S D T P H P A R I G L N C  
3401 CAACTGGCAGGTTGGCTGAGAGGTTGAAGTGGCTGGCTTGGCCCTGAGGAGCAACTACCTGACAGGCTGACAGCTGCTGTTGACAGGTTGGAGC  
892 Q L A Q V A E R V N W L G L G P Q E N Y P D R L T A A C F D R W D

**BsrGI (3515)**  
3501 TGCCTCTGTCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGGCCACCAGGAGCTGAACTATGGTCTCACCAGTGGAGGGG  
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 AGACTTCAGTTCAACATCTCCAGGTACTCTCAGCAACAGCTCATGGAACTCTCACAGGCACCTGCTCCATGCAGAGGGGAACTGGCTGAACATT  
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 GATGGCTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCACTATCAGTGGTGT  
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 GGTGCCAGAAGTAACTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACCACTAGAATGCAGTGAAAAAATGCTTTAT  
1025▶ W C Q K •

**HpaI (3958) MfeI (3969)**  
3901 TTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTGAATAAACAAGTTAACAAACAATTGCATTCAATTTTATGTTTCAGTTCCAG

**EcoRI (4054)**  
4001 GGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGAATTCTAAAATACAGCATAGCAAACTTTAACTCCAATCAAGC  
4101 CTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGGCAATGTCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAG

**SspI (4293)**  
4201 TTTAAGATATAGTGATTTTTCCCAAGTTTGAAGTACTGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTAGTAAAAATTC

**SwaI (4307)**  
4301 AGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGT

**DraIII (4486)**  
4401 TGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTGCCACAAAGTGACCGC  
1274 • G • D Q E E A V F H V C

**EagI (4506)**  
4501 AGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCC GCCCCACGGCTGCTCGCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACAC  
1144 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 GACCTCCGACCCTGGCGTACAGCTCGTCCAGGCGCGCACCCACCCAGGCCAGGGTGTGTCGGCACCACCTGGTCTGGACCGCGTGTGAAAC  
814 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 AGGGTCACGTCGTCGCCGACCACACCGGCGAAGTGTCTCCACGAAGTCCCGGGGAGAACCCGAGCCGGTCCGAGAACTGACCCGCTCCGGCGACGT  
474 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 CGCGCGCGGTGAGCACCAGGAAAGGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGTGATTATACTATGCCGATATACTATGCCGATGATT  
14 R A T L V P V A S T L K A M

**SacI (4954)**  
4901 AATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCAT

**SpeI (5052)**  
5001 TTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTACTAGTCAAAAACAACTCCCACTTACGCTCAATGGGGTGGAGACTTGGAA

**SnaBI (5180)**  
5101 AATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAG

**NdeI (5285)**  
5201 TAGGAAAGTCCATAAGGTACTGTACTGGGCATAATGCCAGGCGGGCCATTACCCTGATTGACGTCAATAGGGGCGTACTTGGCATATGATACACTTG  
5301 ATGTACTGCCAAGTGGGCGAGTTTACCCTAAATACTCCACCCATTGACGTCAATGAAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGAC

**PacI (5471) PstI (5464) SdaI (5463)**  
5401 GTCAATGGCGGGGGTCTGTTGGCGGTGACCCAGGCGGGCCATTACCCTAAGTTATGTAACCGCTGCAGGTTAATTAAGAATGTGAGCAAAAGGCCA  
5501 GCAAAAGCCAGGAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG  
5601 TGGCGAAACCCGACAGGACTATAAAGATACAGGCGTTTTCCCTGGAAGTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGTTACCGGATACCTGT  
5701 CCGCCTTCTCCCTTCGGAAGCGTGGCGCTTTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCA  
5801 CGAACCCCCGTTGAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACT  
5901 GGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCT  
6001 GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGATTGGTAGCTTTGATCCGGCAAAACACCAGCTGGTAGCGGTGGTTTTTTGTTTGAAGCA  
6101 GCAGATTACGCGCAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTG  
6201 GTCATGGCTAGTTAATTAACATTTAAATCA

**PacI (6211) SwaI (6220)**