

# pSELECT-GFPzeo-LacZ

A plasmid selectable with Zeocin™ co-expressing LacZ and GFP reporter genes

Catalog # psetgz-lacz

For research use only

Version 20L01-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pSELECT-GFPzeo-LacZ 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. 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 β-globin polyA terminates the transcription of the selectable marker.

pSELECT-LacZ plasmids can be used as control vectors or for cloning of an open reading frame, as the LacZ 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.

## PLASMID FEATURES

### First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (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**: The *E. coli 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.
- **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.
- **EC2K** is a bacterial promoter that enables the constitutive expression of the Zeocin® resistance gene in *E. coli*. The EC2K promoter is located within an intron between the GFP and Zeocin™ resistance genes.
- **GFP::zeo** is a fusion gene that encodes a red-shifted variant of the jellyfish GFP and resistance to the Zeocin™. This GFP hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>4</sup>.

## 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 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.

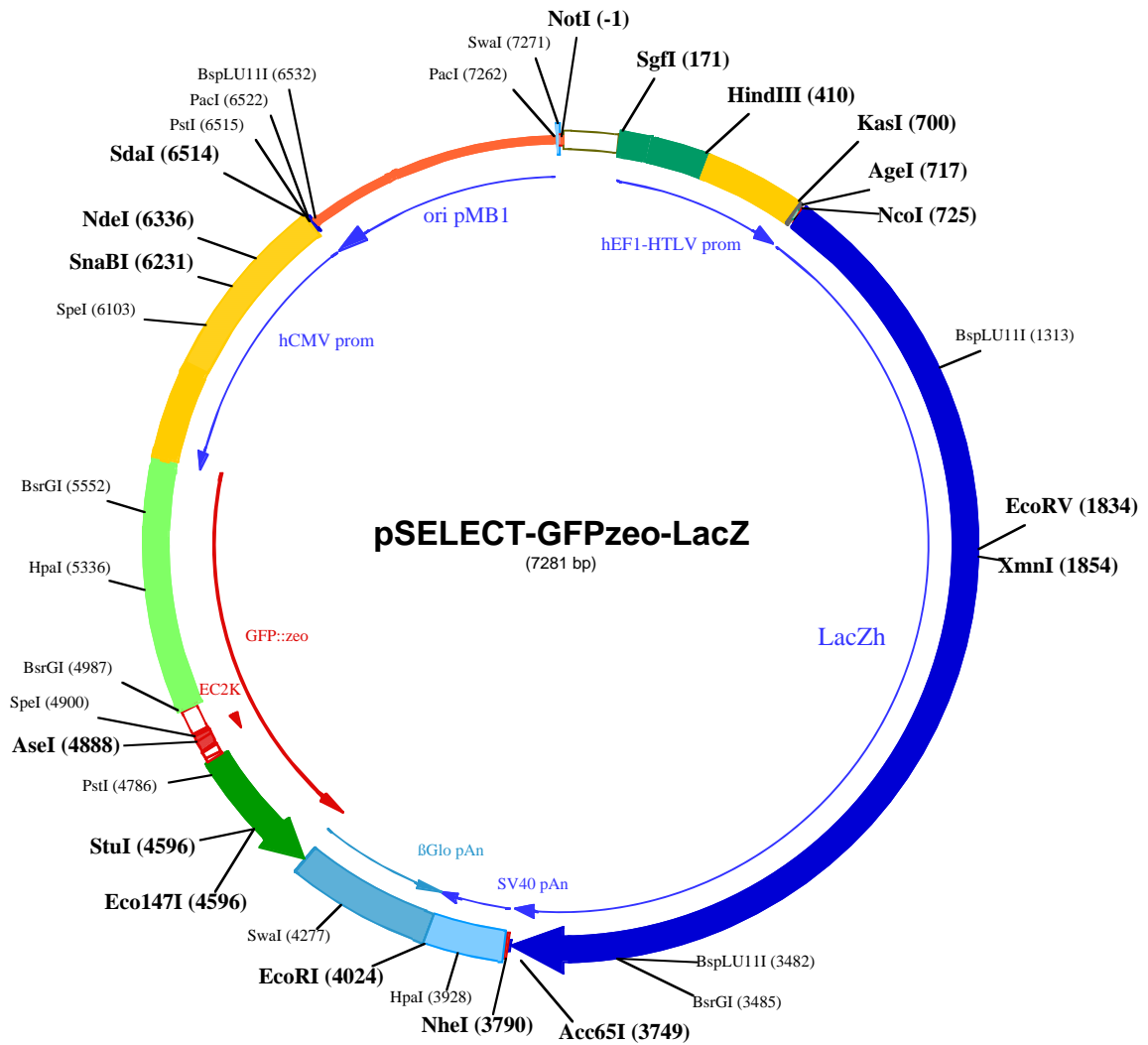
### References:

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.

### TECHNICAL SUPPORT

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150

**NotI (-1)**

1 CGCGCCGCAATAAAAAATATCTTTTATTTTTCATTACATCTGTGTGTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACA

**SgfI (171)**

101 AAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCCGATCGCTCCGGTCCCGTCACTGAGTGGCA

201 GAGCGACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGGTCCGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGGAAAGTGATG

301 TCGTGTACTGGCTCCGCTTTTTCGCCAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCCGCGTGAACGTTCTTTTTCGCAACCGGTTTTCGCCGAC

**HindIII (410)**

401 AACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTACCGCGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCCGCTTCTGCCGCTT

501 CCCGCCGTGGTGCCTCCGAACTCGTCCGCGCTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTA

601 GACTCAGCGCGCTCTCCACGCTTTGCGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTGTTTTCTGTTTCTGCGCGTTACAGATCCAAGCTGTGACC

**NcoI (725)**

**KasI (700) AgeI (717)**

701 GGCGCCTACCTGAGATCAccggtaacCATGAGCCTGTGTGCTGCAAAGGAGAGACTGGGAGAACCTGGAGTGACCCAGCTCAACAGACTGGCTGCC

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 ACCCTCCCTTTGCCTCTTGAGGAAGCTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGCCTGGTT

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 CCCTGCCCTGAAAGCTGTGCCTGAGTCTGTGGCTGGAGTGTGACTCCAGAGGCTGACACTGTGTGTGTCGCCAGCAACTGGCAGATGCATGGCTATGAT

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 GCCCCATCTACACCAATGTACCTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCACTGGCTGCTACAGCCTGACCTTCAATGTTG

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 ATGAGAGCTGGCTGCAAGAAGGCCAGCAGGATCATCTTTGAGTGAAGTCAACTCTGCCTTCCACCTCTGGTGAATGGCAGGTGGGTGGCTATGGCCA

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 AGACAGCAGGCTGCCCTCTGAGTTTGAACCTCTGCGCTTCTCAGAGCTGGAGAGAACAGGCTGGCTGTCATGTTGCTCAGGTGGTCTGATGGCAGCTAC

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

**BspLU111 (1313)**

1301 CTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCCACCACCCAGATTTCTGACTTCCATGTTGCCACCA

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 GGTTCATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCAGAGACTACCTGAGAGTCAAGTGAAGCTTGGCAAGG

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

1501 TGAGACCCAGGTGGCTCTGGCACAGCCCCCTTTGAGGAGAGATCATTTGATGAGAGAGGAGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAG

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 AACCCCAAGCTGTGGTCTGAGTGAATCCCAACCTCTACAGGCTGTTGTGGAGCTGCACACTGCTGATGGCACCCCTGATTGAAGCTGAAGCCTGTGATG

292 N P K L W S A E I P N L Y R A V T L E H L T A D G T L I E A E A C C D

1701 TTGGATTCAGAGAAGTCAAGGATGAGAATGCCTGCTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGAGTCAACAGGATGAGCACCCCTCTGCA

325 V G F R E V R I E N G L L 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 TGGACAAGTGGATGGAACAGACCAATGGTGAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTGTCAAGTGTCTCACTACCCCAACCCACCT

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 CTCTGGTACACCCTGTGTGACAGGTATGGCCTGTATGTTGTTGATGAAGCCAACATTTGAGACACATGGCATGGTGGCCATGAACAGGCTCACAGATGACC

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 CCAGGTGGCTGCTGCCATGTCTGAGAGAGTGAACAGGATGGTGCAGAGAGACAGGAACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGG

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 ACATGGAGCCAACCATGATGCTCTCTACAGTGGATCAAGTCTGTGACCCAGCAGACCTGTGCAGTATGAAGGAGTGGAGCAGACACCCACAGCCACA

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 GACATCATCTGCCCATGTATGCCAGGTTGATGAGGACAGCCCTTCCCTGCTGTGCCAAGTGGAGCATCAAGAAGTGGCTCTCTCTGCTGGAGAGA

492 D I I C P M Y A R V D E D Q P F P F A V P K W S I K K W L S L P G E

2301 CCAGCTCTGATCTGTGTAATGACATGCAATGGCAACTCTCTGAGGAGCTTGGCAAGTACTGGCAAGCCTTCAAGAGTCAACAGGATGAGTGGTGGCCCTGGT

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 AGGAGGATTTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCTGGTCTGCCTATGGAGGAGACTTTGGTGAACCCCC

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 AATGACAGGAGTTCGATGAATGGCCTGGTCTTTGACAGAGGACCCCTCACCTGCCCTCACAGAGGCCAAGCACCAGCAACAGTTCCTCCAGTTCA

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 F Q F

2601 GGCTGTCTGGACAGACCATGTAGGTTGACTCTGAGTACCTCTTCCAGGACTCTGACCAATGAGCTCCTGAGTGGTGGCCCTGGTGGCCCTGGTGGCCCTCT

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 GGCTTCTGGTGGTGGCTCTGGATGTGGCCCTCAAGGAAAGCAGTGTGAAGTGCCTGAGTGCCTCAGCAGAGTCTGCTGGACCACTGTGGCTA

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 ACATGAGGTTGGTTCAGCCAAATGCAACAGCTTTGGTCTGAGGACAGCCATCTCTGATGGCAGTGGAGGCTGGTGGAGAACCTCTCTGTGACCC

692 T V R V V Q P N A T A W S E A G H I S A W Q W R L A E N L S V T

2901 TGCTGTCTGCTCTCATGCCATCCCTCACCTGACCAACTCTGAAATGGACTTCTGCAATGAGCTGGGCAACAAGAGATGGCAGTTCAACAGGAGTCTGG

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 F N R Q S G

3001 CTCTCTGCTCAGATGTGGATGGAGACAAGAAGCAGCTCTCACCCCTCTCAGGACCAATTCACCAGGCTCTCTGGACAATGACATTTGGATGTCT

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

3101 GAGGCCACAGGATTCAGCCAAATGCTTTGGTGGAGAGGTGGAAGGCTGCTGGACACTACCAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACACC

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 TGGCTGATGCTGTCTGATNACCAAGCCATGCTTGGCAGCACCAAGGACAGACCTGTTTCAATCAGCAGAAAGACCTACAGGATGATGGCTCTGGACA

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

3301 GATGGCAATCACAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCCTGAACTGTCAACTGGCAAGTGGCTGAGAGGGTGAAC  
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)

BspLU11I (3482)

3401 TGGCTGGGCTTAGGCCCTCAGGAGAACTACCTGACAGGCTGACAGCTGCCTGCTTGTACAGGTGGGACCTGCCTCTGTCTGACATGTACACCCCTTATG  
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 TGTTCCTTCTGAGAATGGCCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCTCACCAGTGGAGGGGAGACTTCCAGTTCAACATCTCCAGTACTC  
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 TCAGCAACAGCTCATGGAAACCTCTCACAGCAGCTGCTCCATGCAGAGGAGGAACTGGCTGAAACATTGATGGCTTCCACATGGGCATTGGAGGAGAT  
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

Acc65I (3749)

NheI (3790)

3701 GACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCAGTTATCTGTCTGGCAGGTACCCTATCAGCTGTGTGGTCCAGAAGTAAACCTGAGCTAGCTGGC  
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 • -

3801 CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT

HpaI (3928)

3901 TGTAACCAATTATAAGCTGCAATAAACAGTTAACCAACAACAAATTGCATTCATTTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAG

EcoRI (4024)

4001 TAAAACCTCTACAAATGTGGTATGGAATTTCTAAAATACAGCATAGCAAAAACCTTAAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA

4101 TAAGGCATAGGCATCAGGGGCTGTGGCCAAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTTCATGGAGTTTAAGATATAGTGTATTTTTCCCAAGTTTT

SwaI (4277)

4201 GAACTAGCTCTTCAATTTCTTTATGTTTAAATGCACTGACCTCCACATTCCTTTTGTAGTAAATATTCAGAAATAATTTAAATACATCATTTGCAATGA

4301 AAATAAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGAACCTTTAATA

4401 GAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTCTCTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAAATTCCTTT

StuI (4:

Eco147

4501 CCCCAAGGTTGTTTCAACCAATTTCTGTCTGCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGC

4601 CTCTGACCCAGACCCCAAGCAAGGGTGTGTGTCAGGGACAACCTGGTCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATC

PstI (4786)

4701 ATCTTCAACAAAGTCTCTGGAGAACTCTAATCTGTCTGAGTCCAGAACTCTACAGCCCTGCAACATCCCTTGTCTGTGAGGACTGGGACTGCAGAAGTGAGT

AseI (4888)

4801 TTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaatgctATAGTGTGTAATTATACTATGCTTATGATTAATTTGTCAA

SpeI (4900)

BsrGI (4987)

4901 ACTAGTgggttcatagtgcacttttctgactgccccatctcctgccccacctttcccagcatagacagt cagt gacttacCCTTGTCAGCTCATC

5001 CATTCCCAGAGTAATTCCTGCTGTCTGTCACAAAACCTCCAGGAGGACCATGTGGTCTCTTTCTTCATTAGGGTCTTTGGACAGAGCAGATTTGAGTGTCTGAA

5101 TAGTGATTAATCTGGGAGGAGAACTGGGCCATCACAATAGGGGTGTTCTGCTGTTAATGGTCTGCCAGTTGGACAGATCCATCCTCAATGTTGTCTCTAA

5201 TCTTGAAATTAGCCTTAATTCATTCCTCTGCTTATCTGCCATAATGTAACATTGTGAGAATTATAGTTGTAAGTACTCCAGCTTGTGACCCAGAATGTTTTCC

HpaI (5336)

5301 ATCTTCTTTAAAATCAATGCCTTTCAGCTCAATCTGTTTAACAGCTGTATCACCTTCAAACCTTCACCTTCTGCCCTTGTCTTATAATTTCCATCATCTTTA

5401 AAGAAGATTGTCTCTCCTGAACATAAACCCTTCTGGCAATTGAGATTTAAAGAAGTCATGCTGCTTTCATGTTGGTTCAGGGTATCTGCTGAAACATTTGAACAC

BsrGI (5552)

5501 CATAAGTCAGGGTGGTCACCAGAGTTGGCCAAAGCACTGGCAGCTTTCCTGTTGTACAAAATGAACTTCAGAGTCAGCTTTCATTAAGTTGCATCTCCTTC

5601 ACCTTACCAGACACAGAGAATTTGTGGCAATTCACATCACCATCCAGCTCAACCCAGAAATGGGACAACACCAGTAAAGAGTTCTTCTCCCTGTGCTCATG

5701 GTGGCTTGGATCTGTAAACGGCGCAGAAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGG

5801 TAGGCTCCAAGGAGCGCCGGACAAAGGCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTTCAGGAGGCACCACAGGCGGGAGGC

5901 GGCAGAACGCGACTCAACCGGCGTGGATGGCGGCTCAGGTAGGGCGGCGGGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTGATCTGACGGTTCCAC

6001 TAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCCTTGA

SpeI (6103)

6101 TTTACTAGTCAAAACAAACTCCCATTGACGTCATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCAITGATGTACTGCCAAA

**SnaBI (6231)**

6201 ACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTCCCAAGTAGGAAAGTCCCATAGGTCATGTAAGTCCATAATGCCAGGCGGGCCA

**NdeI (6336)**

6301 TTTACCGTCATTGACGTCATAGGGGGCGTACTTGGCATATGATACACTTGTATGTAAGTCCCAAGTGGGCGAGTTTACCGTAAATACTCCACCCATTGACGT

6401 CAATGGAAAGTCCCTATTGGCGTTACTTATGGGAACATACGTCATTATTGACGTCATGGGCGGGGTCGTTGGGCGGTCCAGCCAGGCGGCCATTTACCG

PacI (6522)

PstI (6515)

**SdaI (6514)**

BspLU11I (6532)

6501 TAAGTTATGTAAACGCTGCAGCTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCCGCTGCTGGCGTTTTTCCAT

6601 AGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAA

6701 GCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTG

6801 TAGGTATCTCAGTTTCGGTGTAGGTCGTTTCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGT

6901 CTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT

7001 TGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG

7101 ATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAGCAGAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTT

PacI (7262) SmaI (7271)

7201 TCTACGGGGTCTGACGCTCAGTGGAAACGAAAACACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACAATTTAAATCA