

pSELECT-hygro-LacZ

A LacZ-expression plasmid selectable with Hygromycin

Catalog code: pseth-lacZ

<https://www.invivogen.com/pselect-hygro>

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-hygro-LacZ plasmid provided as lyophilized DNA
- 1 ml of Hygromycin B Gold at 100 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store Hygromycin B Gold at 4°C or -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.

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 water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

PLASMID FEATURES

First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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 mRNA³.
- **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*.
- **Hygro**: Resistance to Hygromycin B is conferred by the *hph* gene from *E. coli* which encodes a phosphotransferase. The *hph* 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 transcription⁴.

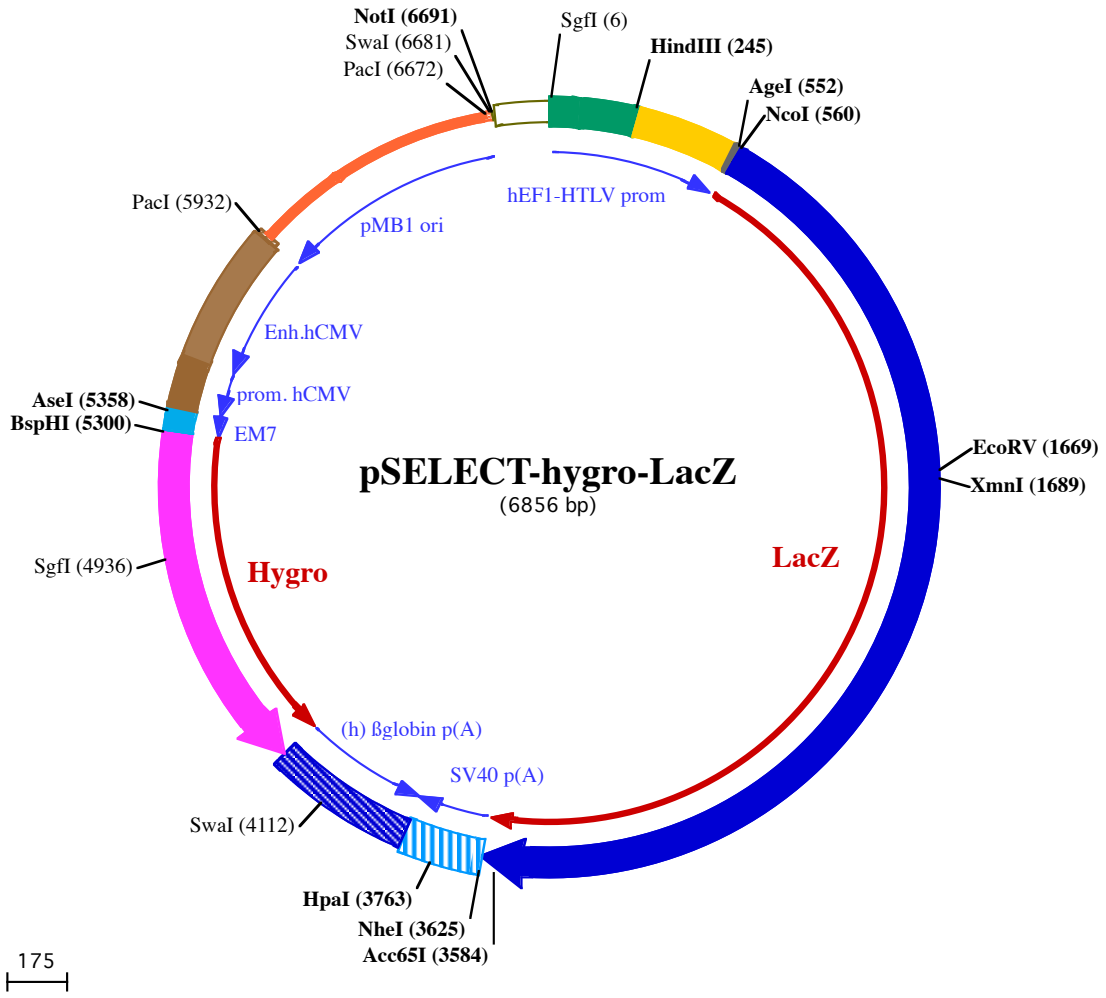
References

1. Kim D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223. 2. Takebe Y. *et al.*, 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol.* 1: 466-472. 3. Carswell S. & Alwine J.C., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258. 4. Yu J. & Russell J.E., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. *Mol Cell Biol*, 21(17):5879-88.

TECHNICAL SUPPORT

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SgfI (6)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTATGTCGTGACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (245)

201 GTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGTGAAGCTTCGAGGGCTCGCATCTCTCTTACCGCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCTCTCTGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGCTTTGTTTGTGTTT

AgeI (552) NcoI (560)

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTACCATTGGACCCTGTTGTGCTGCAAAGGAGAGACTGGGAGAA
13 P G V T Q L N R L A A H P P F A S W R N S E E A R T D R P S Q Q L
601 CCCTGGAGTGACCAGCTCAACAGACTGGCTGCCACCTCCCTTTGCCTCTTGGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTC
701 AGGTCCTCAATGGAGAGTGGAGGTTTCCCTGGTCCCTGCCCTGAAGCTGTGCCTGAGTCTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTG
47 R S L N G E W R F A W F P A P E A V P E S W L E C D L P E A D T V
801 TGGTGCCAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTACCTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAA
80 V V 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
901 CCCACTGGCTGTACAGCCTGACCTTCAATGTTGATGAGAGTGGCTGCAAGAAGCCAGACAGGATCATCTTTGATGGAGTCAACTCTGCCTCCAC
113 P T G C Y S L T F N V D E S W L Q E G Q T R I I F D G V N S A F H
1001 CTCTGGTCAATGGCAGTGGGTTGGCTATGGCAAGACAGCAGGCTGCCCTGAGTTTACCTCTCTGCCTTCTCAGAGCTGGAGAGAACAGGCTGG
147 L W 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
1101 CTGTCATGGTGTCTCAGTGGTCTGATGGCAGCTACCTGGAAGCAAGACTGTGGAGGATCTGGCATCTTCAGGATGTGAGCCTGCTGCACAAGCC
180 A V 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
1201 CACCACCCAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGGAGAACTCAGA
213 T 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
1301 GACTACCTGAGAGTCACAGTGAAGCTTGGCAAGGTGAGACCCAGGTGGCCTTGGCAGACCCCTTGGAGGAGAGATCATTGATGAGAGAGGAGGCT
247 D Y 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
1401 ATGCTGACAGAGTCACTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGCTGTTGTGGAGTGCACACTGC
280 Y A 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 L H T A
1501 TGATGGCACCCCTGATTGAAGCTGAAGCCTGTGATGTTGATTACAGAGAAGTCAAGATTGAGAATGGCCTGCTGCTCAATGGCAAGCCTCTGCTCATC
313 D G T L I E A E A C D V G F R E V R I E N G L L L L N G K P L L I

EcoRV (1669)

XmnI (1689)

1601 AGGGGAGTCAACAGGCATGAGCACCACCTCTGCATGGACAAGTATGGATGAACAGACAATGGTGAAGATATCCTGCTAATGAAGCAGAACAATTCA
347 R G V N R H E 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
1701 ATGCTGTCAAGTGTCTCACTACCCCAACCACCTCTCTGGTACACCTGTGTGACAGGATGGCCTGTATGTTGTTGATGAAGCCAACATTGAGACACA
380 N A 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
1801 TGGCATGTTGCCATGACAGGCTCACAGATGACCCAGGTGGCTGCCTGCCATGTCTGAGAGAGTACCAGGATGGTGCAGAGACAGGAACCAACCC
413 G 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
1901 TCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGC
447 S V 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
2001 AGTATGAAGGAGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCCCAAGTG
480 Q Y 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
2101 GAGCATCAAGAAGTGGCTCTCTGCCTGGAGAGACCAGACCTCTGATCCTGTGTAATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAG
513 S 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
2201 TACTGGCAAGCCTTCCAGCAGTACCCAGGCTCAAGGATTTGTTGGGATGGGTTGGCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCT
547 Y W 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
2301 GGTCTGCCTATGGAGGAGACTTTGGTACACCCCAATGACAGGCAGTCTGCATGAATGGCCTGGTCTTTGCAGACAGGACCCCTCACCTGCCCTCAC
580 W S 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
2401 AGAGGCCAAGCACCAGCAACAGTTCTTCCAGTTCAGGCTGTCTGGACAGACATTGAGGTGACATCTGAGTACCTTTCAGGCACTCTGACAAATGAGCTC
613 E A K H Q 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
2501 CTGCACTGGATGGCTGGCCTGGATGGCAAGCCTCTGGCTTCTGGTGGAGTGGCTGGATGGGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGC
647 L H 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 G I E L P E
2601 TGCCCTCAGCCAGAGTCTGCTGGACAACGTGGCTAACAGTGGGTTGAGGTTGAGCCCAATGCAACAGCTGGTCTGAGGACAGCCACATCTCTGCATGGCA
680 L P 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
2701 GCAGTGGAGGCTGGCTGAGAACCCTCTGTGACCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTG
713 Q W R L A E N L S V T L P A A S H A I P H L T T S E M D F C I E L
2801 GGCACAAGAGATGGCAGTTCAACAGGCAGTCTGGCTTCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGACCAATTCA
747 G N 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
2901 CCAGGGCTCTCTGGACAATGACATTGGAGTGTCTGAGGCCACAGGATTGACCCAAATGCTGGTGGAGAGGTGGAAGGCTGCTGGACACTACCAGGC
780 T R 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
3001 TGAGGCTGCCCTGCTCCAGTGCACAGCAGACCCCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGACCAAGGCAAGACCCTGTTCACT
813 E 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
3101 AGCAGAAAGCCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCCTGA
847 S R 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

3201 ACTGTCAACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAGAAGTACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTG
880▶ N C 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
3301 GGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCTCACCACTGG
913▶ D 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
3401 AGGGGAGACTTCCAGTTCAACATCTCCAGTACTCTCAGCAACAGCTCATGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGACCTGGCTGA
947▶ R G 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

Acc65I (3584)

3501 ACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCCTATCAGCT
980▶ N I 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

NheI (3625)

3601 GGTGTGGTGCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGC
1013▶ V W C Q K •

HpaI (3763)

3701 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACAATTGCATTCATTTTATGTTTCAGG
TTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAAT
CAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCTCACCTTCTTTCA
TGGAGTTAAGATATAGTGATTTTCCCAAGTTTGAAGTACTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATAGTAAA

SwaI (4112)

4101 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTA
GTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCGAATTCTCGACTCATTCTTTGCCCTCGGACGA
342▶ • E K A R R P

4301 GTGCTGGGGCGTCGGTTTTCCACTATCGGCGAGTACTTCTACACAGCCATCGGTCCAGACGGCCGCTTCTGCGGGCGATTGTGTACGCCCCGACAGTCC
334▶ T S P R R N G S D A L V E V C G D T W V A A S R R A I Q T R G V T G
4401 CGGCTCCGGATCGGACGATTGCGTCGCATCGACCTGCGCCCAAGTGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAAT
301▶ A G S R V I A D C R G Q A W A A D D F N G D V L S Q Y L Q D L G I
4501 CGGAGCATATACGCCCCGAGCCGCGCGATCTGCAAGTCCGGATGCCTCCGCTCGAAGTAGCGCTGTGCTGCTCCATAAAGCAACACCGGCCTC
268▶ R L M Y A R L R P S G A L E P H R R E F Y R T Q Q E M C A L W P R
4601 CAGAAGAAGATGTTGGCAGCTCGTATTGGGAATCCCCGAACATCGCTCGCTCCAGTCAATGACCGCTGTTATGCGCCATTGTCCGTGAGGACATTGT
234▶ W F F I N A V E Y Q S D G F M A E S W D I V A T I R G N D T L V N N
4701 TGGAGCCGAAATCCGCGTGCACGAGGTGCCGACTTCGGGGCAGTCTCGGCCAAAGCATCAGCTCATCGAGAGCCTGCGCGACGGACGCACTGACGGT
201▶ S G F D A H V L H R V E P C D E A W L M L E D L A Q A V S A S V T
4801 GTCGTCCATCACAGTTTCCAGTGATACATGGGGATCAGCAATCGCGCATATGAAATCACGCCATGTAGTGATTGACCGATTCTTCCGGTCCGAAT
168▶ D D M V T Q W H Y V H P D A I A C I F D R W T T Y Q G I G Q P G F

SgfI (4936)

4901 GGGCCGAACCCGCTCGTCTGGTAAAGTACGGCCGAGCGATCGCATGAGCTCCGCGACGGGTTGCAGAACAGCGGGCAGTTCGGTTTCAGGCAGGT
134▶ P G F G S T Q S L D A A A I A D M L E A V P Q L V A P L E T E P L D
5001 CTTGCAACGTGACACCCCTGTGCACGGCGGGAGATGCAATAGTTCAGGCTCTGCTGAATCCCAATGTCAAGCACTTCCGGAATCGGGAGCGCGCCGA
101▶ Q L T V G Q A R R S I C Y T L S E S F E G I D L V E P I P L A A S
5101 TGCAAAGTCCGATAAACATAACGATCTTTGTAGAAACCATCGGCGCAGCTATTTACCGCAGGACATATCCACGCCCTCTACATCGAAGCTGAAAGCA
68▶ A F H R Y V Y R D K Y F G D A C S N V R L V Y G R G G V D F S F A
5201 CGAGATCTTCCGCTCCGAGAGCTGCATCAGGTCCGAGACGCTGTGCAACTTTTTCGATCAGAAACTTCCGACAGACGTCGCGGTGAGTTCAGGCTTTT
34▶ R S E E G E S L Q M L D S V S D F K E I L F K A V S T A T L E P K K

BspHI (5300)

AseI (5358)

5301 TCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCT
1▶ M

5401 GACGGTTCATAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGAAA
GTCCCGTTGATTTACTAGTCAAAACAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATG
TACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCC
AGGCGGGCCATTTACCGTCAATGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCGAGTTTACCGTAAATACTCCAC
CCATTGACGTCAATGAAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATATTGACGTCAATGGGGGGGGTCTTGGCGGTCAGCCAGGCGGG
PacI (5932)

5901 CCATTTACGTAAGTTATGTAACGCTGCAGTTAATTAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGC
GTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTT
CCCCCTGGAAGCTCCCTCGTGCCTCTCTGTCCGACCTGCGGTTACCGGATACCTGTCCGCTTTCTCCCTTCCGGAAGCGTGGCGTTTCTCATA
GCTCAGCTGTAGGTATCTCAGTTCCGTTGAGGTCGTTTCGCTCCAAGTGGGCTGTGTGCACGAACCCCGTTCCAGCCGACCGCTGCGCTTATCCGG

6301 TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
6401 ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTG
6501 GTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCC
6601 TTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACACAGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCA
6701 ATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAAACAAAACAACT
6801 AGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA

PacI (6672) SmaI (6681) NotI (6691)