STOP

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

Important Limited Use License information for pVITRO2-hygro-Lucia/SEAP

The purchase of the pVITRO2-hygro-Lucia/SEAP vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact 10515 Vista Sorrento Parkway, San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

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

InvivoGen
www.invivogen.com

pVITRO2-hygro-Lucia/SEAP

A multigenic plasmid for high levels of expression of the Lucia luciferase and SEAP reporter genes

Catalog code: pvitro2-lucsp

https://www.invivogen.com/pvitro2-luciaseap

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO2-hygro-Lucia/SEAP provided as lyophilized DNA
- 1 ml 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

pVITRO is a family of vectors with improved features. pVITRO plasmids allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels.

pVITRO2-Lucia/SEAP contains the Lucia luciferase and SEAP reporter genes. pVITRO2-Lucia/SEAP can be used as a control vector.

pVITRO2-Lucia/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (BspH I/Avr II for Lucia luciferase and Nco I/Nhe I for SEAP) that allow for convenient cloning of ORFs.

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

• **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein[1]. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[2].

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV) is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is several-fold more active than the SV40 enhancer[3].

• **pMB1 Ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **Lucia luciferase** is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase. ORF size (from the ATG to the stop codon): 634 bp

Lucia luciferase activity can be evaluated using QUANTI-Luc™, an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression[4].

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

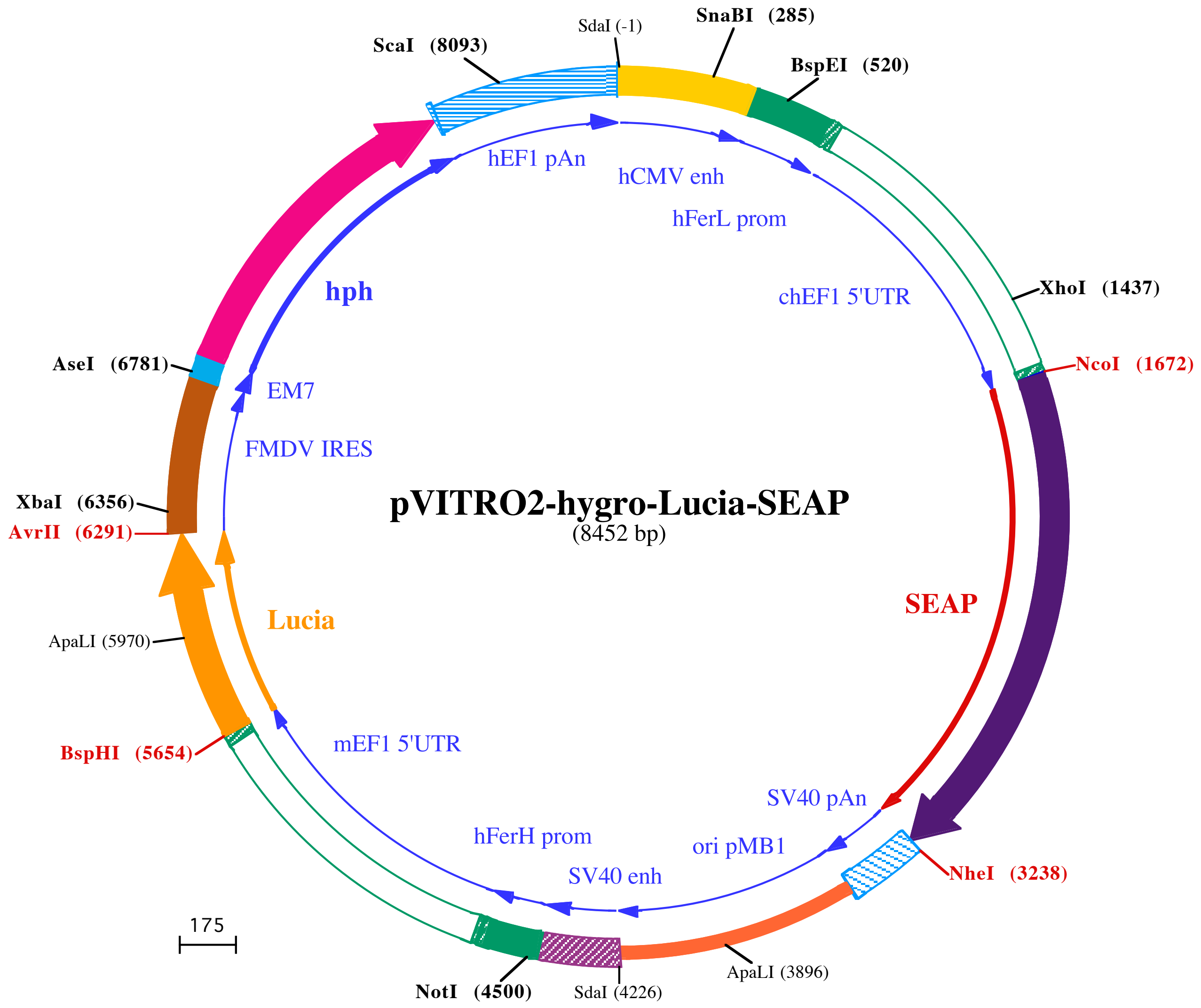
• ***hph*** gene confers resistance to Hygromycin B both in *E. coli* and mammalin cells. In bacteria, hph is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, hph is transcribed from the rat EF-1α promoter as a polycistronic mRNA and translated via the FMDV IRES.

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

• **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophometer at 405 nm. SEAP activity that can be readily assessed qualitatively and quantitatively using HEK-Blue™ Detection or QUANTI-Blue™.

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*[5]

1. Kim DW. *et al.***, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system Gene 91:217-23. **2. Dean DA.** *et al.***, 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **3. Boshart M.** *et al.***, 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41:521-30. **4. Ramesh N. et al., 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24:2697-700. **5. Carswell S. & Alwine JC. ,1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 9: 4248-58.


pVITRO2-hygro-Lucia-SEAP
(8452 bp)
SdaI (-1)
SnaBI (285)
BspEI (520)
XhoI (1437)
NcoI (1672)
NheI (3238)
ApaLI (3896)
SdaI (4226)
NotI (4500)
BspHI (5654)
ApaLI (5970)
AvrII (6291)
XbaI (6356)
AseI (6781)
ScaI (8093)
hEF1 pAn
hCMV enh
hFerL prom
chEF1 5'UTR
SEAP
SV40 pAn
ori pMB1
SV40 enh
hFerH prom
mEF1 5'UTR
Lucia
FMDV IRES
EM7
hph
175

```
SdaI (-1)
1     CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

101   CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT

                                                                                       SnaBI (285)
202   ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGC

303   TATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGA

404   GTTTGTTTTGACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGGGGG

                BspEI (520)
504   CTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCCTCCG

605   AGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGgtaag

705   tgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattcttgat

806   cccgagcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcgctgggg

907   ccgccgcgtgctaatctggtggcaccttcgcgcctgtctcgctgctttcgctaagtctctagccatttaaaatttttgataaccagctgcgacgcttttt

1008  tctggcgagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagcgcaca

1109  tgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaaactggccggcctgctctggtgcctggcctcgcgccgccgtg

1210  tatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatgga

1311  ggacgcggcgcccgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccttcctcatccgtcgcttcatgtgactccacggagtaccgg

                          XhoI (1437)
1412  gcgccgtccaggcacctcgattagttctcgagcttttggagtacgtcgtctttaggttggggggaggggttttatgcgatggagtttccccacactgagtg

1513  ggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttgcctcattctcaagcctcagac

                                                           NcoI (1672)
1614  agtggttcaaagtttttttcttccatttcagGTGTCGTGAAAACTACCCCTAAAAGCCACCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGC
                                                              1▸ M  V  L  G  P  C  M  L  L  L  L  L  L
1715  TGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAG

 14▸ L  G  L  R  L  Q  L  S  L  G  I  I  P  V  E  E  E  N  P  D  F  W  N  R  E  A  A  E  A  L  G  A  A  K
1816  AAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCA

 48▸ K  L  Q  P  A  Q  T  A  A  K  N  L  I  I  F  L  G  D  G  M  G  V  S  T  V  T  A  A  R  I  L  K  G  Q
1917  GAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACA

 81▸ K  K  D  K  L  G  P  E  I  P  L  A  M  D  R  F  P  Y  V  A  L  S  K  T  Y  N  V  D  K  H  V  P  D
2018  GTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGC

115▸ S  G  A  T  A  T  A  Y  L  C  G  V  K  G  N  F  Q  T  I  G  L  S  A  A  A  R  F  N  Q  C  N  T  T  R
2119  GGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCAC

149▸ G  N  E  V  I  S  V  M  N  R  A  K  K  A  G  K  S  V  G  V  V  T  T  T  R  V  Q  H  A  S  P  A  G  T
2220  CTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCA

182▸ Y  A  H  T  V  N  R  N  W  Y  S  D  A  D  V  P  A  S  A  R  Q  E  G  C  Q  D  I  A  T  Q  L  I  S
2321  ACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACC

216▸ N  M  D  I  D  V  I  L  G  G  G  R  K  Y  M  F  R  M  G  T  P  D  P  E  Y  P  D  D  Y  S  Q  G  G  T
2422  AGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCC

250▸ R  L  D  G  K  N  L  V  Q  E  W  L  A  K  R  Q  G  A  R  Y  V  W  N  R  T  E  L  M  Q  A  S  L  D  P
2523  GTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGG

283▸ S  V  T  H  L  M  G  L  F  E  P  G  D  M  K  Y  E  I  H  R  D  S  T  L  D  P  S  L  M  E  M  T  E
2624  CTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCA

317▸ A  A  L  R  L  L  S  R  N  P  R  G  F  F  L  F  V  E  G  G  R  I  D  H  G  H  H  E  S  R  A  Y  R  A
2725  CTGACTGAGACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGT

351▸ L  T  E  T  I  M  F  D  D  A  I  E  R  A  G  Q  L  T  S  E  E  D  T  L  S  L  V  T  A  D  H  S  H  V
2826  CTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAA

384▸ F  S  F  G  G  Y  P  L  R  G  S  S  I  F  G  L  A  P  G  K  A  R  D  R  K  A  Y  T  V  L  L  Y  G
2927  ACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGAC

418▸ N  G  P  G  Y  V  L  K  D  G  A  R  P  D  V  T  E  S  E  S  G  S  P  E  Y  R  Q  Q  S  A  V  P  L  D
```

```
3028 GAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCAT

452▸ E  E  T  H  A  G  E  D  V  A  V  F  A  R  G  P  Q  A  H  L  V  H  G  V  Q  E  Q  T  F  I  A  H  V  M
3129 GGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTC

485▸  A  F  A  A  C  L  E  P  Y  T  A  C  D  L  A  P  P  A  G  T  T  D  A  A  H  P  G  R  S  R  S  K  R
             NheI  (3238)
3230 TGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTG

519▸L  D  •
3331 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGG

3432 AGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGA

3532 GCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGT

3633 GGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGT

3734 TAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACC

                                                                           ApaLI (3896)
3835 GGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

3936 ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC

4037 GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG

                                                                                             SdaI (4226)
4138 GGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAACCTGCAGGGC

4237 CTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGC

4338 TCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCAT

                                                                             NotI  (4500)
4439 GCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGGGCGGGGTCCCGC

4539 GCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGCTGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGCAGGGCCA

4640 GACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGCTTCCGCGGGCCGCCGAGCTGGAGGTCCTGCTCCGAGCGGGCCGGGCC

4740 CCGCTGTCGTCGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGCGCGGGAGGCAGAGTGCGAGGCCTAGCGGCAACCCCGTAGCCTCGC

4841 CTCGTGTCCGGCTTGAGGCCTAGCGTGGTGTCCGCGCCGCCGCCGCGTGCTACTCCGGCCGCACTCTGGTCTTTTTTTTTTTTGTTGTTGTTGCCCTGCTG

4942 CCTTCGATTGCCGTTCAGCAATAGGGGCTAACAAAGGGAGGGTGCGGGGCTTGCTCGCCCGGAGCCCGGAGAGGTCATGGTTGGGGAGGAATGGAGGGACA

5043 GGAGTGGCGGCTGGGGCCCGCCCGCCTTCGGAGCACATGTCCGACGCCACCTGGATGGGGCGAGGCCTGGGGTTTTTCCCGAAGCAACCAGGCTGGGGTTA

5144 GCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCGGCGCCGCGTTGCCCTGCCTCCCTAACTAGGGTGAGGCCATCCCGTCCGGCAC

5245 CAGTTGCGTGCGTGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGAGCTCAAAATGGAGGACGCGGCAGCCCGGTGGAGCGGGCGGGTGAGTCACCCAC

5346 ACAAAGGAAGAGGGCCTGGTCCCTCACCGGCTGCTGCTTCCTGTGACCCCGTGGTCCTATCGGCCGCAATAGTCACCTCGGGCTTTTGAGCACGGCTAGTC

5447 GCGGCGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGGAAGTCATTTTTGGAATTTG

5548 TCCCCTTGAGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTTAAACCCTTTTTTAGGTGTTGTGAAAACCACCGCTAATTCA

          BspHI  (5654)
5649 AAGCAATCATGATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCT

       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  E  D  L  N  I  A  A
5750 GTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACCAACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGA

 32▸ 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  T  E  Q  V  N  T  D
5851 TGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCA

 65▸  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  G  C  T  R  G  C  L
                  ApaLI (5970)
5952 TTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATT

 99▸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  K  E  S  A  Q  G  G  I
6053 GGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCAC

133▸ 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  D  L  C  A  D  C  T  T
6154 TGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGG

166▸  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  F  A  S  K  I  Q  G  R
```

AvrII (6291)

6255 TGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATACCTAGGAGCAGGTTTCCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAG

200 V D K I K G L A G D R •

XbaI (6356)

6356 GTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCG

6457 TGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCCGTCATGTGTGCAACCCCAGCACGGCGACTTTACTGCGAAAC

6558 CCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGA

6659 AGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCTAT

AseI (6781)

6760 GAATACAA**CTGACTGT**TTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAACTG

1 M K K P E L

6860 ACAGCAACTTCTGTTGAGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTTGA

7 T A T S V E K F L I E K F D S V S D L M Q L S E G E E S R A F S F D

6961 TGTTGGAGGAAGAGGTTATGTTCTGAGGGTCAATTCTTGTGCTGATGGTTTTTACAAAGACAGATATGTTTACAGACACTTTGCCTCTGCTGCTCTGCCAA

40 V G G R G Y V L R V N S C A D G F Y K D R Y V Y R H F A S A A L P

7062 TTCCAGAAGTTCTGGACATTGGAGAATTTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAG

74 I P E V L D I G E F S E S L T Y C I S R R A Q G V T L Q D L P E T E

7163 CTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTGGATTTGGTCCTTTTGGTCCCCAAGGCAT

108 L P A V L Q P V A E A M D A I A A A D L S Q T S G F G P F G P Q G I

7264 TGGTCAGTACACCACTTGGAGGGATTTCATTTGTGCCATTGCTGATCCTCATGTCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTG

141 G Q Y T T W R D F I C A I A D P H V Y H W Q T V M D D T V S A S V

7365 CTCAGGCACTGGATGAACTCATGCTGTGGGCAGAAGATTGTCCTGAAGTCAGACACCTGGTCCATGCTGATTTTGGAAGCAACAATGTTCTGACAGACAAT

175 A Q A L D E L M L W A E D C P E V R H L V H A D F G S N N V L T D N

7466 GGCAGAATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTTGCAT

209 G R I T A V I D W S E A M F G D S Q Y E V A N I F F W R P W L A C M

7567 GGAACAACAAACAAGATATTTTGAAAGAAGACACCCAGAACTGGCTGGTTCCCCCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATC

242 E Q Q T R Y F E R R H P E L A G S P R L R A Y M L R I G L D Q L Y

7668 AATCTCTGGTTGATGGAAACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGTCTGGTGCTGGAACTGTTGGAAGAACTCAAATT

276 Q S L V D G N F D D A A W A Q G R C D A I V R S G A G T V G R T Q I

7769 GCAAGAAGGTCTGCTGCTGTTTGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAAACAGGAGACCCTCCACAAGACCCAGAGCCAAGGAATGAAT

310 A R R S A A V W T D G C V E V L A D S G N R R P S T R P R A K E •

7870 **ATTAGCTAG**ATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGT

7969 AGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGT

ScaI (8093)

8070 TTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAAACAACTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAAGTTAAATGAGAA

8171 ACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTT

8272 AACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTGCTTTGATTACAACACT

8373 GGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG