

STOP

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TECHNICAL SUPPORT

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pVITRO2-neo-Lucia/SEAP

An expression plasmid encoding Lucia luciferase and SEAP reporter genes

Catalog code: pvitro2-nlucsp

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

For research use only

Version 19L18-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-neo-Lucia/SEAP provided as lyophilized DNA

Storage and stability

- Product is shipped at room temperature.
- Resuspended DNA is stable for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

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 new family of vectors that allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and yield high levels of expression.

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

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

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 protein¹. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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 plasmids².

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (hCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The hCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer³.

• **pMB1 ori:** 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 quantitatively measure the activity of Lucia 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 expression⁴.

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

• **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In bacteria, *neo* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *neo* is transcribed from the human FerH composite 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 spectrophotometer 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. Eisenstein RS, and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58. 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* 141(2):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(14):2697-700. 5. Carswell S, and Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258.

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 H₂O. 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.

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent <i>E. coli</i> cells	gt116-11
G418	Selection antibiotic	ant-gn-1

TECHNICAL SUPPORT

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

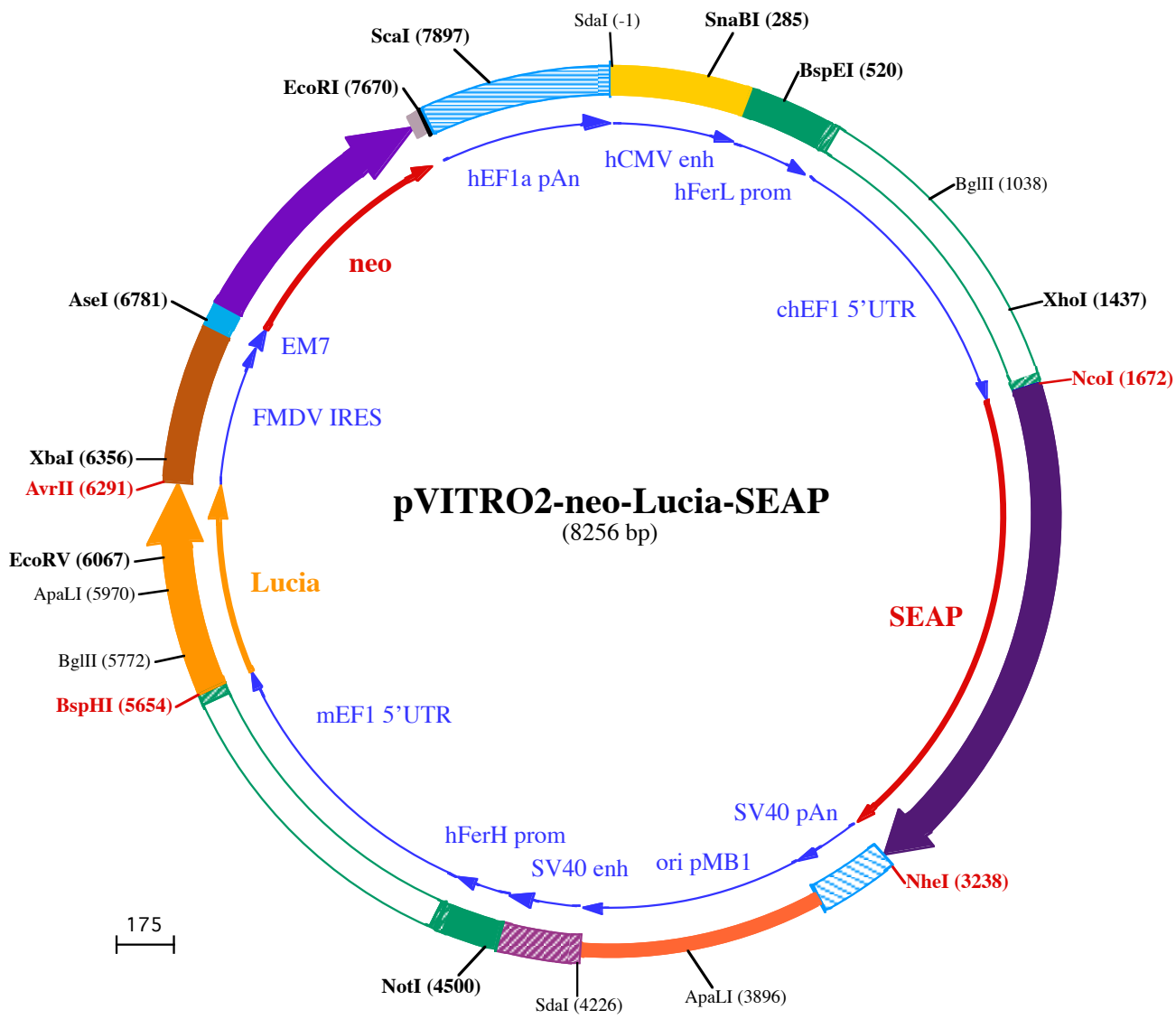
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SdaI (-1)
1 CCTGCAGGCGTTACATAA CTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
101 CGCCAATAGGGACTTTCATTGACGTCAATGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC

SnaBI (285)

201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTACTCACGGGGATTCCAAGTCTCCACCCCAATTGACGTCAATG
401 GGAGTTTGTGTTGACTAGTCAGGGCCCAACCCCAAGCCCAATTTACAACACGCTGGCGCTACAGGCGGTGACTTCCCCTTGTCTTGGGCGGG

BspEI (520)

501 GGGCTGAGACTCCTATGTGCTCCGATTGTGTCAGGCACGGCTTCGGCCCGCTCCTGCCACCGAGATTGGCCGCTAGGCCTCCCGAGCCCTGCC
601 TCCGAGGCGCGCCACATAAAGAAGCCGCTTAGCCACGTCCTCCGAGTTCGGCGGTCCCGGGTCTGTCTAAGTTGCCCGCAGAACACAGg
701 taagtgcgctgtgtggttcccggggctggcctctttacgggttatggccttgcgtgccttgaattacttccatgccctggctgcagtagctgattc
801 ttgatcccagacttcgggttgaagtgggtggagagttcaggccttgcgttaaggagcccttcgctcgtgcttgagttgaggcctggcctgggagc
901 ctggggccgcccgtgtaactctggtggcaccttcgctcgtctcgtcgtcttgcctaagtctctagccatttaaaatgttataaacagctgcagc

BglII (1038)

1001 cttttttctggcgagatagctttaaatacgggccaagatctgcacactggtatctcggtttttggggcgcgggcgagcgggcccgtgcgtccc
1101 agcgcacatggtcggcaggcgggctgcgagcggccaccgagaatcggcggggtagtctcaactggcggcctgctcgtgctgcctgcctcgc
1201 gccgctgtatcggccgcttggggcgaaggctggccggctggcaccagttcgtgagcggaaagatggcggcttccggccctgctgcaggagc
1301 tcaaaatggaggacggcggcggggagagcggggggtagtcaccacacaaaaggaaaggcccttctcctcatccgtcgttcatgtgactcca

XhoI (1437)

1401 cggagtagccggcgccgtccaggcacctcattagttctcgcagcttttggagtagctcgtctttaggttggggggggggttttatgcatggagttcc
1501 ccacactgagtggtggagactgaagagtaggagcagcttggcacttgatgtaattctccttgaatttgcctttttaggttggatcttgcctcattc

NcoI (1672)

1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAACCTACCCCTAAAAGCCACCATGGTCTGGGCCCTGCATGCTGCT
1701 GCTGCTGCTGCTGGCCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACC CGGACTTCTGGAACCGCAGGCGAGCCGAGGCC
1801 9▶ L L L L 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
CTGGTGCCGCAAGAAGCTGCAGCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGTGTCTACGGTACAGCTGCCA
1901 43▶ L G A A K 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
GGATCTAAAAGGCGAGAAGAAGGACAAACTGGGCGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGA
2001 76▶ R I L K G Q 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
CAAATGTGCCAGACAGTGGGCCACAGCCACGGCTACTGTGCGGGTCAAGGCAACTCCAGACCATGGCTTGTGAGTGCAGCCGCCCTTTAAC
2101 109▶ K H V P D 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
CAGTCAACACGACAGCAGCGGCAACGAGTCTCCTGTGATGAATCGGGCAAGAAAGCAGGGAAGTCAAGTGGGAGTGGTAACCACACAGAGTGCAGC
2201 143▶ Q C N T T R 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
ACGCCCTGCCAGCCGACCTACGCCACACGGTGAACCGCACTGTACTCGGACCGCAGCTGCCTGCCTCGGCCGCCAGGAGGGTGCAGGACAT
2301 176▶ H A S P A G T 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
CGTACGACGCTCATCTCCAACATGGACATTGATGTGATCTGGGTGGAGGCCAAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAGAT
2401 209▶ A T Q L I S 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
GACTACAGCCAAGTGGGACCAGGCTGGACGGGAAGATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCTGGTATGTGTGGAACCGCACTGAGC
2501 243▶ D Y S Q G G T 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
TCATGACGGCTTCCCTGGACCGCTGTGACCCATCTCATGGTCTCTTGGACCTGGAGACATGAAATACGAGATCCACGAGACTCCACTGACCC
2601 276▶ L M Q A S L D P 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
CTCCCTGATGGAGATGACAGAGGCTGCCCTCGCCTGCTGAGCAGGAACCCCGGGCTTCTTCTTCTGAGGGTGGTGCATCGACACGGTCAT
2701 309▶ S L M E M T E 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
CACGAAAGCAGGGCTTACCGGCACTGACTGAGACGATCATGTTCTGACGACGCCATTGAGAGGGCGGGCCAGCTACCCAGCGAGGAGGACGCTGAGCC
2801 343▶ H E S R A Y R A 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
TCGCTACTGCCACTCCACGCTTCTCCTCGGAGGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCCGGACAGGAA
2901 376▶ L V T A D H S H V 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
GGCCTACACGGTCTCTATACGAAACGGTCCAGGCTATGTCTCAAGACGGCCCGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTAT
409▶ A Y T V L L Y G 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

3001 CGGCAGCAGTCAGCAGTGCCTGGACGAAGAGACCCACGACGAGCGAGGACGTGGCGGTGTTGCGCGCGGCCCGCAGGGCGACCTGGTTCACGGCGTGC
443▶ R Q Q S A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V
3101 AGGAGCAGACCTTCATAGCGCACGTATGGCTTCGCCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCCGGCACCCGACCGCCGC
476▶ Q E Q T F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A
NheI (3238)
3201 GCACCCGGGGCGTCCCCTGCAAGCGTCTGGATTGAAGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCA
509▶ H P G R S R S K R L D •
3301 GTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACAATGCATTCAT
3401 TTTATGTTTCAGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGAAATGTTAATTAAGTACCATGACCAA
3501 AATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAGGATCTTCTGAGATCCTTTTTTCTGCGGTAATCTGCTGC
3601 TTGCAAAACAAAAAACCCCGCTACCAGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGA
3701 TACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCCCTACATACCTCGCTCTGCTAATCTGTTACCAGT
ApaLI (3896)
3801 GGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAACGGGGGTTCTGTGC
3901 ACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGAGAAAGCGGACA
4001 GGTATCCGGTAAGCGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGCTGGGTTTCGCCACCT
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SdaI (4226)
4201 CCTTTTGCTCACATGTTCTTAATTAACTGCAGGGCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGTACCTTCTGAGGCTGAAAGAACCAGCTGTG
4301 GAATGTGTGCAGTTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACAGGTGTGAAAG
4401 TCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCCGCCAGAGCGCGGAGGGCTCCA
NotI (4500)
4501 GCGGCCGCCCTCCCCACAGCAGGGGGGGTCCCGGCCACCGAAGGAGCGGGTCCGGGCGGGCGCGTATTGGCCGGGGCGGGCTGACGCC
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5501 GAGACTAGTCAGGCCAGCTGGCGTGAAGTCATTTTTGGAATTTGTCCTTGAAGTTTGGAGCGGAGCTAATTCGGGCTTCTTAGCGTTCAAAGG
BspHI (5654)
5601 TATCTTTAAACCTTTTTAGGTGTTGTGAAAACCCGCTAATCAAGCAATCATGATGAAATCAAGGTGCTGTTGCCCTCATCTGTATTGCTGT
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BglII (5772)
5701 TGCTGAGGCAAAACCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACCAGATCTTGAGACTGACCTGTTCAACC
15▶ A E A K P T E I N E D L N I A A V A S N F A T T D L E T D L F T N
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49▶ W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P P D V L
ApaLI (5970)
5901 GGGAGCTGGAGGCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCC
82▶ R E L E A N A R R A G C T R G C L I C L S H I K C T P K M K K F I P
EcoRV (6067)
6001 TGGCAGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCTGGCTCAAGGATAAG
115▶ G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G F K D K

6101 GAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGTCTGACCTCCTGA
149▶ E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D L L
AvrII (6291)

6201 AGAAGTGGCTTCCCCAGAGGTGTACCACCTTTTCCAGCAAGATTGAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATACCTAGGAGC
182▶ K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R •
XbaI (6356)

6301 AGGTTTCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTCCAGGCTAGAGGGTAACACTTTGTAAGTGGCTCCACGCTCGA
6401 TCCACTGGCGAGTGTAGTAACAGCACTGTTGCTTCTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCC
6501 ACGCCACACGGGCCGTATGTGTCAACCCAGCACGGGACTTTACTGCGAAACCACTTTAAAGTGACATTGAAACTGGTACCCACACTGGTGA
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AseI (6781)

6701 TCTATGCCTGAATAGGTGACCGGAGTCCGGCACCTTTCCTTTGCAATTACTGACCCATGAATAACA**CTGACTGTTTGACAATTAATCATCGGCATAGTA**
6801 **TATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACC**ATGATTGAACAAGTGGATTGCACGCAGGTTCTCCGGCCGTTGGGTGGAGAGGCTATT
1▶ M I E Q D G L H A G S P A A W V E R L F

6901 CGGCTATGACTGGGCACAACAGACAATCGGCTGCTGATGCCCGGTGTTCCGGTGTGACGCGAGGGGCCCGGTTCTTTTGTCAAGACCGACCTG
20▶ G Y D W A Q Q T I G C S D A A V F R L S A Q G R P V L F V K T D L
7001 TCCGGTGCCCTGAATGAAGTGAAGACGAGGAGCGCGGCTATCGTGGCTGGCCACGACGGCGTTCCTTGCAGCTGTGCTCGAGTGTGACCTGAAG
54▶ S G A L N E L Q D E A A R L S W L A T T G V P C A A V L D V V T E
7101 CGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGCGAGGATCTCTGTATCTCACCTTGCTCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT
87▶ A G R D W L L L G E V P G Q D L L S S H L A P A E K V S I M A D A M
7201 GCGGGCTGCATACGCTTATCGCGTACCTGCCATTGACCCACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTG
120▶ R R L H T L D P A T C P F D H Q A K H R I E R A R T R M E A G L V
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154▶ D Q D D L D E E H Q G L A P A E L F A R L K A S M P D G E D L V V
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187▶ T H G D A C L P N I M V E N G R F S G F I D C G R L G V A D R Y Q D
7501 CATAGGTTGGTACCCGTGATATTGCTGAAGAGCTTGGCGGCAATGGGCTGACCGCTTCTCGTGTTCACGGTATCGCCGCTCCCGATTCCGAGCGC
220▶ I A L A T R D I A E E L G G E W A D R F L V L Y G I A A P D S Q R
EcoRI (7670)

7601 ATCGCCTTCTATCGCCTTCTGACGAGTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGAATTCGCTAGG**ATTATCCCTAATACTGTC**
254▶ I A F Y R L L D E F F •
7701 CACCCACTCTTAATCAGTGGTGAAGAAGCGTCTCAGAAGTGTGTTTCAATTGGCCATTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATG
ScaI (7897)

7801 CATCGTAAACCTCAGAAGGAAAGGAGAATGTTTGTGGACCACTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGT
7901 ACTTTTTAATGAAACAACCTGACCAAAAATTTGTACAGAATTTTGGACCCATTAAGTAAATGAGAAACCTGTGTTCCTTTGGTCAACACC
8001 GAGACATTTAGGTGAAAGACATCTAATCTGGTTTTACGAATCTGGAACCTCTTGAAGTAAATCTTGAAGTTAACACTTCTGGGTGGAGAATAGGGT
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