

pVITRO2-neo-GFP/SEAP

An expression plasmid encoding the GFP and SEAP reporter genes

Catalog code: pvitro2-ngfpseap

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

For research use only

Version 19L18-MM

PRODUCT INFORMATION

Contents

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

Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year 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

pVITRO1 and pVITRO2 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-GFP/SEAP contains the reporter genes GFP and SEAP and can be used as a control vector.

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

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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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

• **GFP gene:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

• **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 G418	Competent <i>E. coli</i> cells Selection antibiotic	gt116-11 ant-gn-1

TECHNICAL SUPPORT

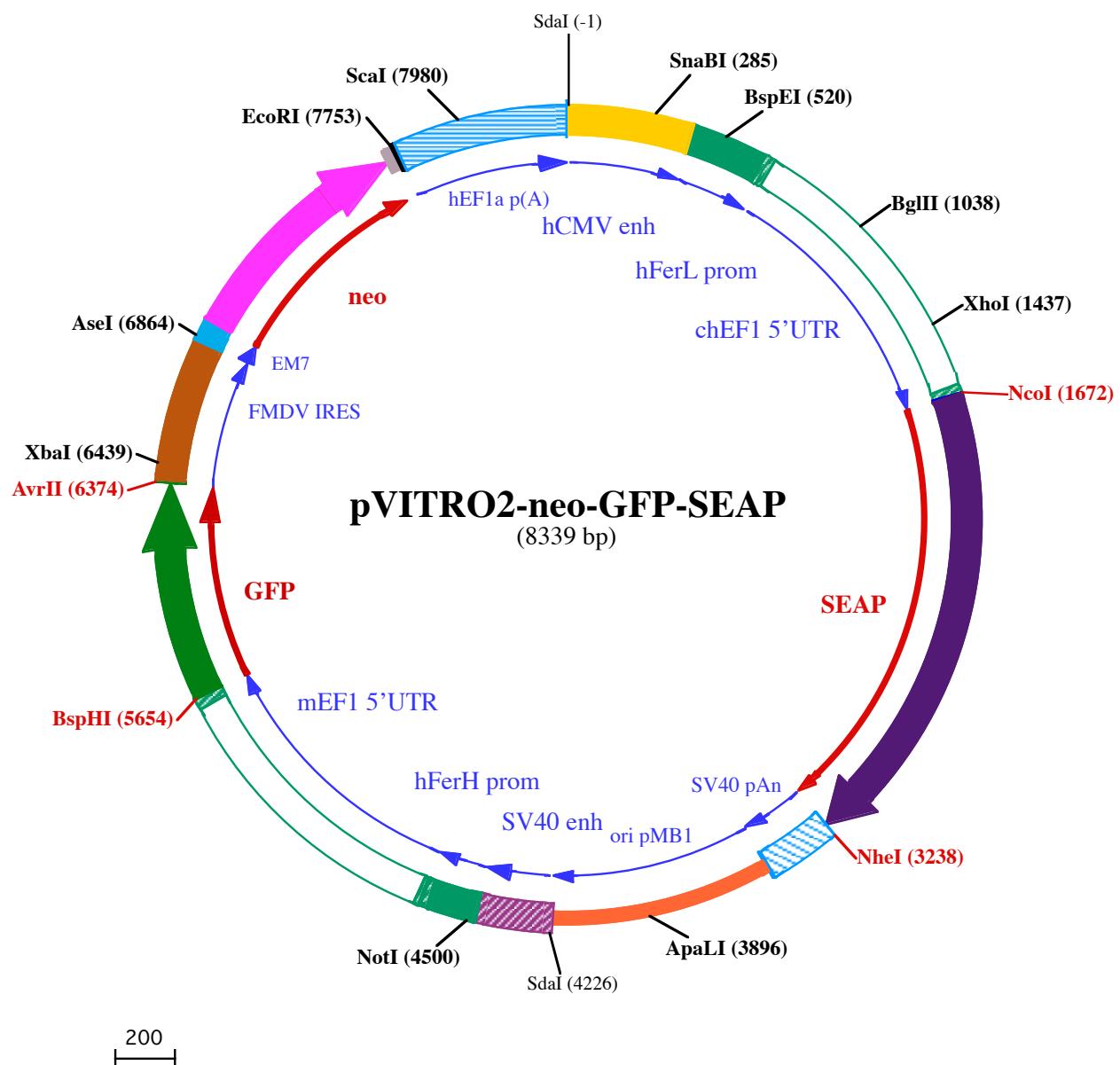
InvivoGen USA (Toll-Free): 888-457-5873

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



SdAI (-1)
1 CCTCAGGGCTTACATAACTTACCGTAAATGGCCGCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCAGTAGTA

101 CGCCAATAGGGACTTCCATTGACGTCAATGGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTAGCCCC
201 TATTGACGTCAATGACGGTAAATGCCCGCTGGATTGCCCCAGTACATGACCTTATGGACTTCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCCGGTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCAGGGATTCCAAGTCTCACCCCTTGACGTCAATG
401 GGAGTTGTTGACTAGTCAGGGCCCAAGCCCCCAAGCCCCCATTACAACACGCTGGCCTACAGGCCTGACTTCCCTGCTTGGGGCGGG
→ BspEI (520)
501 GGGCTGAGACTCTATGTGTCGGATTGGTCAGGCACGGCCTCGGCCCGCCTCTGCCACCGCAGATTGCCGCTAGGCCCTCCGAGGCCCTGCC
601 TCCAGGGCCGGCCACCATAAAAGAACGCCCTAGCCACGTCCCTCGAGTCGGCGTCCCGGGCTGTCTCAAGCTGCCAGAACACAGG
701 taatgtccgttgtgtttcccgccgtggcttacgggttatggcccttgctgtgccttaatttcatgcccgtggctgcagtagtgcatt
801 ttgtatcccagactcgggttggaaagtgggtggagagttcgaggcctgcgttaaggagccccctgcctcgtgcgttagttggccctggctgg
901 ctggggccgcgcgtctaattctgtggcacctcgcgtgtctcgctgtttcgtaagtctctagccattaaaattttgtataaccagtcgcac
→ BglIII (1038)
1001 cttttttctggcagatagtctgttaatgcggccaagatctgcacactgttatcggtttttggggccggggccgcacggggccgtgcgtcc
1101 agcgcacatgttcggcaggcgccgtcgagcgcggccaccggagaatcgacggggtagtctcaactggccggcgtctggcctggccctgc
1201 gcccgcgtgtatgcggccctggcgcaaggctggccggcaccagttgcgtgagcggaaagatggccgttccggccctgtcgcaggag
1301 tcaaatggaggacgcggcccccggagacggccgggtgactcaccacacaaggaaaaggcccttcctcatccgtcgttgcatt
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1501 ccacactgatgggtggagactgaagagttaggccagttgcactgtatgttaattctcttggaaatttgccttttagttggatctgccttcat
→ XbaI (1437)
1601 tcaagcctcagacagtggttcaaaatgttttcttccatttcagGTGCGTAAAACCTAAAGGCCACCATGTTCTGGGGCTGCATGCTGCT
→ Neor (1672)
1701 GCTGCTGCTGCTGCTGGCCTGAGGCTACAGCTCCCTGGCATCATCCAGTTGAGGAGAGAACCGGACTCTGGAACCGCAGGCCAGGG
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
1801 CTGGGTGCCAAGAGCTGCAGCTGCACAGACAGCCAAAGAACCTCATCTTCTGGCATGGATGGGTGTCTACGGTACAGCTGCCA
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
1901 GGATCCTAAAGGGCAGAAGAACGGAAACTGGGGCTGAGATACCCCTGGATGGCTCCATATGGCTCTGGCAAGACATACAATGTAGA
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
2001 CAAACATGTGCCAGACAGTGGAGCCACGCCACGGCTACCTGTGCGGGTCAAGGGCACTTCCAGACCATTGGCTGAGTGCAGGCCGCTTAA
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
2101 CAGTGAACACGACAGCGGCAACGAGGTATCTCGTGTGAATCGGCAAGAACGGAGTCAGTGGAGTGGTAACCACACGAGTCAGC
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 R V Q
2201 ACGCTCGCCAGCCGACCTACGCCACACGGTAACCGCAACTGGTACTCGGACGCCACGTGCTGCCCTGGCCCGCAGGGTGCAGGACAT
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
2301 CGCTACGAGCTCATCTCAACATGGACATTGTGATGTGATCTGGTGGAGGCCAAGTACATGTTGCTGGCATGGAAACCCAGACCTGAGTACCCAGAT
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
2401 GACTACAGCCAAGGTGGGACAGGGCTGGACGGGAAGAATCTGGTCAAGGAATGGCTGGCAAGCAGCCAGGGTGCCTGGTATGTGAGTGGAAACCGACTGAGC
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
2501 TCATGCAGGCTCCCTGGACCCGTGTGACCCATCTCATGGTCTTGGAGCAGACATGAAATACGAGATCACCGAGACTCCACACTGGACCC
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
2601 CTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGTGAGCAGGAACCCCGCGCTCTCTCGTGGAGGGTGTGAGTGCATGCCACCGTCAT
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
2701 CACGAAAGCAGGGCTTACCGGGACTGACTGAGACGATCATGTTGACGCCATTGAGAGGGCGGCCAGCTCACAGCAGGAGGACAGCTGAGC
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
2801 TCGTCACTGCCGACCACTCCACGTCTCTCTGGAGGCTACCCCTGGAGGGAGTCATCTCGGCTGGCCCTGGCAAGGCCCCGGACAGGA
376▶ I V T A D H S H V E S F G G Y P I R G S S I F G I A P G K A R D R K

2901 GGCCTACAGGTCTTCTATCGAAACGGTCCAGGCTATGCTCAAGGACGGCCGGGATTTACCGAGAGCGAGAGCGGGAGCCCCGAGTAT
 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 CGCAGCAGTCAGCAGTGCCTGGACGAAGAGACCCACGCAGGCAGGACGTGGCGTGGCGCCGAGCGCACCTGGTCACGGCGTGC
 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 AGGAGCAGACCTTCATAGCGACGTATGCCCTGCCGCTGCCTGGAGGCCACCGCCTGCACCTGGCGCCCGCAGCGCACCTGGTCACGGCGTGC
 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 GCACCCGGGCGGTCCCGTCAAGCGTCTGGATTGAAGCTAGCTGCCAGACATGATAAGATAACATTGATGAGTTGGACAACCACACTAGAATGCA
 509▶ H P G R S R S K R L D •
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ApaLI (3896)
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 4301 GAATGTGTCAGTTAGGTGTGAAAGTCCCAGGCTCCAGCAGGAGAGTATGCAAAGCATGCAATTAGTCAGCAACCACATGCCACTAGTCC
 4401 TCCCCAGGCTCCCAAGCAGGAGAGTATGCAAAGCATCTCAATTAGTCAGCAACCACATGCCACTAGTCCAGAGCGCGAGGGCTCCA
NotI (4500)
 4501 GCGCCGCCCTCCCCACAGCAGGGCGGGTCCCGGCCACCGAACGGAGGCGGGCTGGGGCGCGCTGATTGGCGGGCGGGCTGACGCC
 4601 GACCGGGCTATAAGAGACCAAGCGACCCGAGGCCAGACGTTCTCGCCGAAGCTTCCGCTCAGAACGCGAGGTGAGGGGGGGTGGCTCCCG
 4701 GCCCGCAGCTGGAGGCTCTGCTCCAGGGGGCCGGCCCTGCTCGCCGGGATTAGCTCGCAGCATTCCGCTCAGTTGGGGGGGGGGGGGG
 4801 AGGCAGAGTGCAGGGCTAGCGAACCCGTAGCTCGCTGTCCGGCTGAGGCCTAGCGTGGTCCGCCGCCGCGTGTACTCCGCC
 4901 GCACTCTGGTTTTTTTTTTGTTGTTGCTGCTGCTGCTGATTGCGCTCAGCAATAGGGCTAACAAAGGGAGGGTGGGGCTTGCTCGCC
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BspHI (5654)
 5601 TATTTAAACCTTTAGGTGTTGAAACACCGCTAACTCAAAGCAATCATGAGCAAGGGAGAAGAACTCTTACTGGTGTGCTTCAATTCT
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 15▶ V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I C
 5801 ACAACAGGAAGCTGCCAGTGCCTGCCACTCTGGTACCCCTGACTTATGGTCAATGTTCACTGAGCGTACCCGACCATGAAGCAGCATG
 49▶ T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H
 5901 ACTTCTTAAATCTGCAATGCCAGAAGGTATGTCAGGAGAGGACAATCTCTTAAAGGATGATGAAAGTATAAGACAAGGGAGAAGTGAAGTTGA
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 115▶ G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H

6101 AATGTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAAGTTAATTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAATGGCAGACCATT

149▶ N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H
6201 ACCAGCAGAACACCCCTATTGGTATGGCCAGTCTCCCTCCAGATAATCACTATCTCCGACTCAATCTGCTCTGTCCAAGACCTAATGAGAAAAG

182▶ Y Q Q N T P I G D G P V L L P D N H Y L R T Q S A L S K D P N E K R
AvrII (6374)

6301 AGACCATGGCTCTGGAGTTGTGACAGCAGCAGGAATTACTCTGGAATGGATGAGCTGTACAAGTAACCTAGGAGCAGGTTCCCCAATGACA

215▶ D H M V L L E F V T A A G I T L G M D E L Y K •

XbaI (6439)

6401 CAAACGTCAACTGAAACTCCGCCGGCTTTCCAGGTCTAGAGGGTAACACTTGTACTCGCTGGCTCACGCTCGATCCACTGGCAGTGTAA

6501 GTAAACAGCACTGTTGCTCGTAGCGGAGCATGACGGCGTGGAACTCCTCTTGGTAACAAGGACCCACGGGGCAAAGCCACGCCACACGGGCCG

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6701 TCAGGTACCCGAGGTAAACACGCGACACTGGGATCTGAGAAGGGACTGGGCTCTATAAAAGCGCTGGTTAAAAGCTTCTATGCCTGAATAGGT

AseI (6864)

6801 GACCGGAGGTGGCACCTTCCTTGCATTACTGACCTATGAATACAA**CTGACTGTTGACAATTAAATCATGGCATAGTATATCGCATAGTATAAT**

6901 ACGACTCACTATAGGAGGGCACC**ATGATTGAAACAAGATGGATTGCCAGGTTCCGGCGCTTGGTGGAGAGGCTATTGGCTATGACTGGCAC**

1▶ M I E Q D G L H A G S P A A W V E R L F G Y D W A

7001 AACAGACAATGGCTGCTGTGATGCCCGTGTCCGGCTGTAGCCAGGGCGCCGGTTCTTGTCAAGACCGACCTGTCGGTGCCTGAATGAA

26▶ 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 S G A L N E
7101 ACTGAAAGACGAGGCAGCGCGCTATCGTGGCTGGCACGACGGCGTTCTGGCAGCTGTGCTCGACGTTGTACTGAAGCGGGAGGGACTGGCTG

59▶ 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 A G R D W L
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126▶ 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 D Q D D L D
7401 CGAAGAGCATCAGGGCTCGGCCAGCCACTGTTGCCAGGCTCAAGCGAGCATGCCGACGGCAGGGATCTGCTGTGACACATGGCGATGCC

159▶ 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 T H G D A C
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193▶ 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 I A L A T
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226▶ 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 I A F Y R L

EcoRI (7753)

7701 TCTTGACGAGTTCTCTGAGCGGACTCTGGGTTGAAATGACCGACCAAGC**GAATTGCTAGGATTATCCCTAATACCTGCCACCCACTCTTAATCA**

259▶ L D E F F •

7801 GTGGTGAAGAACGGTCTCAGAACACTGTTGTTCAATTGCCATTAAAGTTAGTAGTAAAGACTGGTAATGATAACAATGACATCGTAAACCTTCAG

ScaI (7980)

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8001 ACTTGACCAAAATTGTCACAGAATTGAGACCCATTAAAAAGTAAATGAGAAACCTGTGTGTTCTGGTCAACACCGAGACATTAGTGAA

8101 GACATCTAATTCTGGTTACGAATCTGAAACTCTGAAATGTAATTCTGAGTTAACACTCTGGTGGAGAATAGGGTTGTTCCCCCACATA

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