STOP

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The purchase of the pVITRO1-neo-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.

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InvivoGen
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pVITRO1-neo-Lucia/SEAP

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

Catalog code: pvitro1-nlucsp

https://www.invivogen.com/pvitro1-luciaseap

For research use only
Version 19L17-MM

PRODUCT INFORMATION

Contents:
- 20 µg of pVITRO1-neo-Lucia/SEAP provided as lyophilized DNA

Storage and stability:
- Product is shipped at room temperature.
- Lyophilized DNA is stable for 3 months at -20˚C.
- Resuspended DNA is stable more than one year at -20˚C.

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 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.

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

pVITRO1-Lucia/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Nco*I/*Avr*II for Lucia luciferase and *Bsp*HI/*Nhe*I for SEAP) that allow for convenient cloning of ORF's

PLASMID FEATURES

• **rEF1 and mEF1 prom:** pVITRO1-neo-GFP/LacZ plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart[1], both promoters display a strong activity that yield similar levels of expression. EF-1α promoters are expressed at high levels in all cell cycles and lower levels during G0 phase. EF-1α promoters are also non-tissue specific; they are highly expressed in all cell types.

• **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].

• **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.*[4]

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

• **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[5].

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

• **Neo:** The neo gene from Tn5 encodes an aminoglycoside 3'-phospho-transferase (3' APH II) that confers resistance to the antibiotics kanamycin in bacteria 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 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.

• **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.

• **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™.

1. Kim DW. *et al.,* 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system Gene 91(2):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(2):521-30. **4. Carswell S. & Alwine JC.** ,1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 9(10): 4248-58. **5.** Ramesh N. *et al.,* 1996. High-riter bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700.

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_2O. 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 *E. coli* cells	gt116-11
G418	Selection antibiotic	ant-gn-1

InvivoGen
www.invivogen.com

pVITRO1-neo-Lucia-SEAP
(8446 bp)

SdaI (-1)
ScaI (8087)
EcoRI (7860)
neo
AseI (6971)
EM7
FMDV IRES
XbaI (6547)
AvrII (6482)
EcoRV (6258)
ApaLI (6161)
Lucia
BglII (5963)
NcoI (5848)
rEF1 prom
hEF1 pAn
SV40 enh
mEF1 prom
BspHI (1562)
NdeI (1856)
SEAP
SV40 pAn
NheI (3128)
ori pMB1
ApaLI (3786)
hCMV enh
SdaI (4116)
NdeI (4296)
SnaBI (4401)
ApaLI (3786)

200
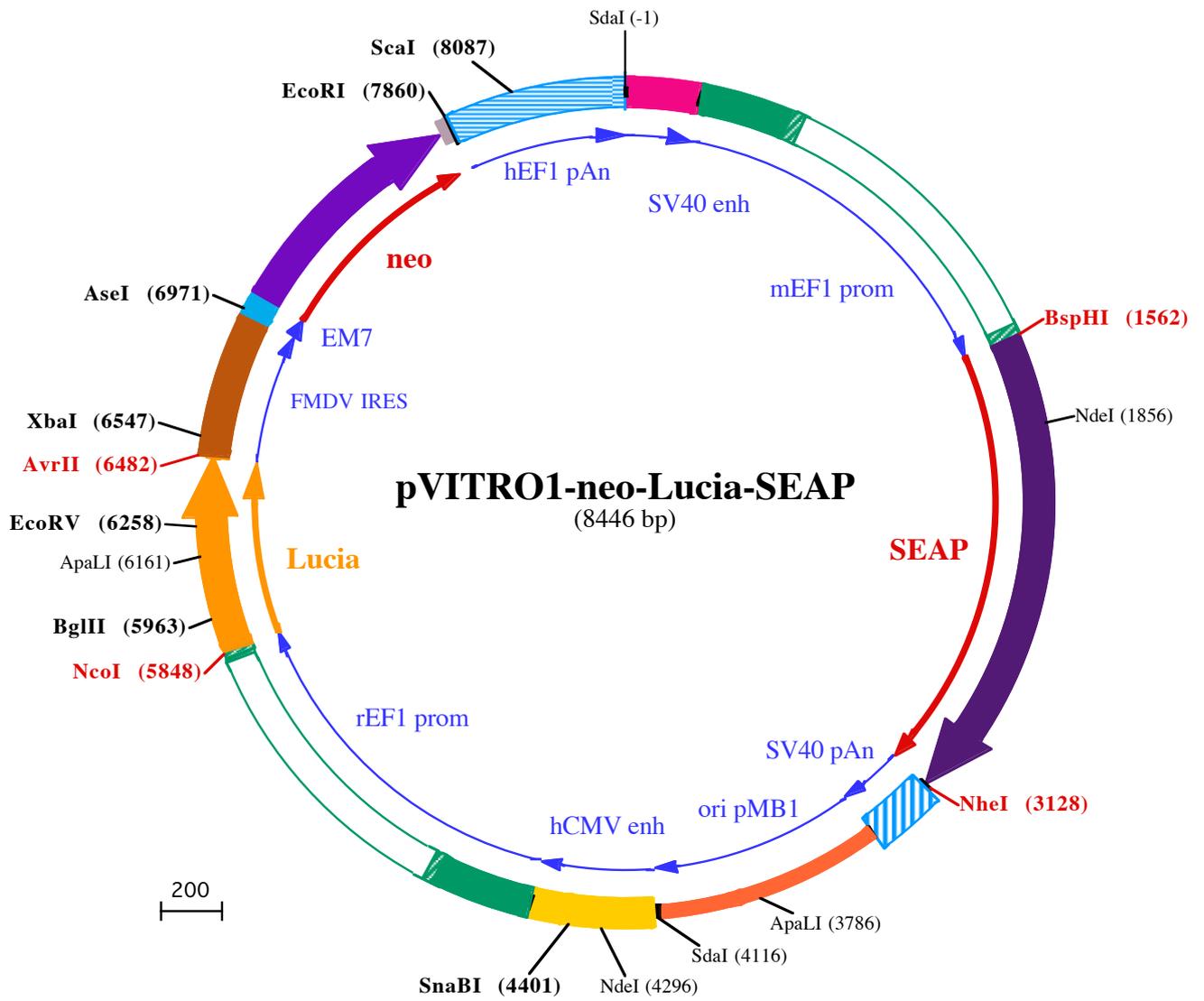
SdaI (-1)
1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG

301 CCCAGGGACCGTCCCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCCCACGACAGCGCGAGGAGCATGCGCTCAGGGCTGAGCGCGGGGAGAGCAGA

401 GCACACAAGCTCATAGACCCTGGTCGTGGGGGGGAGGACCGGGGAGCTGGCGCGGGGCAAACTGGGAAAGCGGTGTCGTGTGCTGGCTCCGCCCTCTTCC

501 CGAGGGTGGGGGAGAACGGTATATAAGTGCGGCAGTCGCCTTGGACGTTCTTTTTCGCAACGGGTTTGCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGC

601 TTCCGCGGGCCGCCGAGCTGGAGGTCCTGCTCCGAGCGGGCCGGGCCCCGCTGTCGTCGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGC

701 GGCGCGGGAGGCAGAGTGCGAGGCCTAGCGGCAACCCCGTAGCCTCGCCTCGTGTCCGGCTTGAGGCCTAGCGTGGTGTCCGCGCCGCCGCCGCGTGCTA

801 CTCCGGCCGCACTCTGGTCTTTTTTTTTTTTGTTGTTGTTGCCCTGCTGCCTTCGATTGCCGTTCAGCAATAGGGGCTAACAAAGGGAGGGTGCGGGGCT

901 TGCTCGCCCGGAGCCCGGAGAGGTCATGGTTGGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCCGCCTTCGGAGCACATGTCCGACGCCAC

1001 CTGGATGGGGCGAGGCCTGGGGGTTTTTCCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCG

1101 GCGCCGCGTTGCCCTGCCTCCCTAACTAGGGTGAGGCCATCCCGTCCGGCACCAGTTGCGTGCGTGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGA

1201 GCTCAAAATGGAGGACGCGGCAGCCCGGTGGAGCGGGCGGGTGAGTCACCCACACAAAGGAAGAGGGCCTGGTCCCTCACCGGCTGCTGCTTCCTGTGAC

1301 CCCGTGGTCCTATCGGCCGCAATAGTCACCTCGGGCTTTTGAGCACGGCTAGTCGCGGCGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTT

1401 GGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGGAAGTCATTTTTGGAATTTGTCCCCTTGAGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGG

1501 TTCAAAGGTATCTTTTAAACCCTTTTTTAGGTGTTGTGAAAACCACCGCTAATTCAAAGCAAT**CATGA**TTCTGGGGCCCTGCATGCTGCTGCTGCTGCTG
    BspHI (1562)
    1▶ M I L G P C M L L L L L

1601 CTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCG
    13▶ 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 L G A

1701 CCAAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAA
    46▶ 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 R I L K

    NdeI (1856)
1801 AGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTG
    79▶ 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 K H V

1901 CCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACA
    113▶ 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 Q C N

2001 CGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCC
    146▶ 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 H A S P

2101 AGCCGGCACCTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAG
    179▶ 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 A T Q

2201 CTCATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCC
    213▶ 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 D Y S

2301 AAGGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGC
    246▶ 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 L M Q A

2401 TTCCCTGGACCCGTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATG
    279▶ 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 S L M

2501 GAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCA
    313▶ 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 H E S

2601 CGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACG
    346▶ 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 L V T A

2701 GTCCTCCTATCGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGT
    379▶ 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 A Y T

2801 GTCCTCCTATCGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGT
    413▶ 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 R Q Q

2901 CAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGAC
    446▶ 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 Q E Q T

3001 CTTCATAGCGCACGTCATGGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGCACCACCGACGCGCGCACCCGGGG
    479▶ 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 H P G

                                        NheI (3128)
3101 CGGTCCCGGTCCAAGCGTCTGGATTGAA**GCTAGCTGG**CCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAA
    513▶ R S R S K R L D •

3201 TGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTC

3301 AGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAA

3401 CGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAA

3501 AAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCGAGCGCAGATACCAAATAC

3601 TGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCC

ApaLI (3786)

3701 AGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCA

3801 GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGT

3901 AAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAG

4001 CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC

SdaI (4116)

4101 ACATGTTCTTAATTAACCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG

4201 TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA

NdeI (4296)

4301 TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACAT

SnaBI (4401)

4401 CTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCAC

4501 CCCATTGACGTCAATGGGAGTTTGTTTTGACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAGCCCAGGGACCGTC

4601 CCTAAATTCTCACAGACCCAAATCCCTGTAGCCGCCCCACGACAGCGCGAGGAGCATGCGCCCAGGGCTGAGCGCGGGTAGATCAGAGCACACAAGCTCA

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5601 ccgcatagtcacctcgggcttctcttgagcaccgctcgtcgcggcggggggagggggatctaatggcgttggagtttgttcacatttggtgggtggagact

5701 agtcaggccagcctggcgctggaagtcattcttggaatttgcccctttgagtttggagcgaggctaattctcaagcctcttagcggttcaaaggtatttt

NcoI (5848)

5801 ctaaacccgtttccagGTGTTGTGAAAGCCACCGCTAATTCAAAGCAAccATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGC

1▶ M E I K V L F A L I C I A V A E A

BglII (5963)

5901 AAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACCAACTGGGAGACC

17▶ 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 W E T

6001 ATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCCTGAGGGAGCTGG

51▶ 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 R E L

ApaLI (6161)

6101 AGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTG

84▶ 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 G R C

EcoRV (6258)

6201 CCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTG

117▶ 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 E P L

6301 GACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGAAGAAGTGGC

151▶ 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 K K W

AvrII (6482)

6401 TTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATACCTAGGAGCAGGTTTCCC

184▶ 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 (6547)

6501 CAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGC

6601 GAGTGTTAGTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACA

6701 CGGGCCCGTCATGTGTGTGCAACCCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAG

6801 GATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCT

AseI (6971)

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

7000 AGTATAATACGACTCACTATAGGAGGGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGA

7100 CTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCC

23▶ 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 S G A
7200 CTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGG

57▶ 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 A G R
7300 ACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCT

90▶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 R R L
7400 GCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGAT

123▶ 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 D Q D
7500 GATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACACATGGCG

157▶ 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 T H G
7600 ATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTT

190▶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 I A L
7700 GGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTC

223▶ 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 I A F

EcoRI (7860)
7800 TATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACT

257▶ Y R L L D E F F •
7900 CTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAA

8000 ACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAA

ScaI (8087)
8100 TGGAAACAACTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTT

8200 AGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCC

8300 CCCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGT

8400 CACTAAAACAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG