

pVIVO2-GFP/SEAP

A multigenic plasmid for high levels of expression of GFP and SEAP reporter genes

Catalog code: pvivo2-gfppsp

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO2-GFP/SEAP is 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

pVIVO2 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector.

- **pVIVO2-GFP/SEAP** contains the reporter genes GFP and SEAP and can be used as a control vector.

- **pVIVO2-GFP/SEAP** also can be used for cloning of open reading frames (ORFs). Both reporter genes are flanked by unique sites (*Bsp*HI/*Avr*II for GFP and *Nco*I/*Nhe*I for SEAP) that allow for convenient cloning of ORFs which can be selected from InvivoGen's extensive list of genes.

For more information: <https://www.invivogen.com/genes>.

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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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

- **LGFP** is a new allele of the green fluorescent protein. The gene has been chemically synthesized to remove the CpGs to ensure long-lasting expression.

- **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.

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

- **Hygro-ΔCpG** is a new allele of the *hph* gene conferring resistance to hygromycin B. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Hygro-ΔCpG* gene allows the selection of *E. coli* clones transformed with a pVIVO plasmid.

Note: Stable transfection of mammalian clones cannot be performed due to the absence of a eukaryotic promoter upstream of the Hygro-ΔCpG gene.

- **Term:** The *E. coli* *rps* O terminator allows efficient transcription termination of the *Hygro-ΔCpG* gene.

- **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 (cat. code: hb-det2) or QUANTI-Blue™ (cat. code: rep-qb1).

- **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.

1. Eisenstein RS. & 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* 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.* 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 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.

TECHNICAL SUPPORT

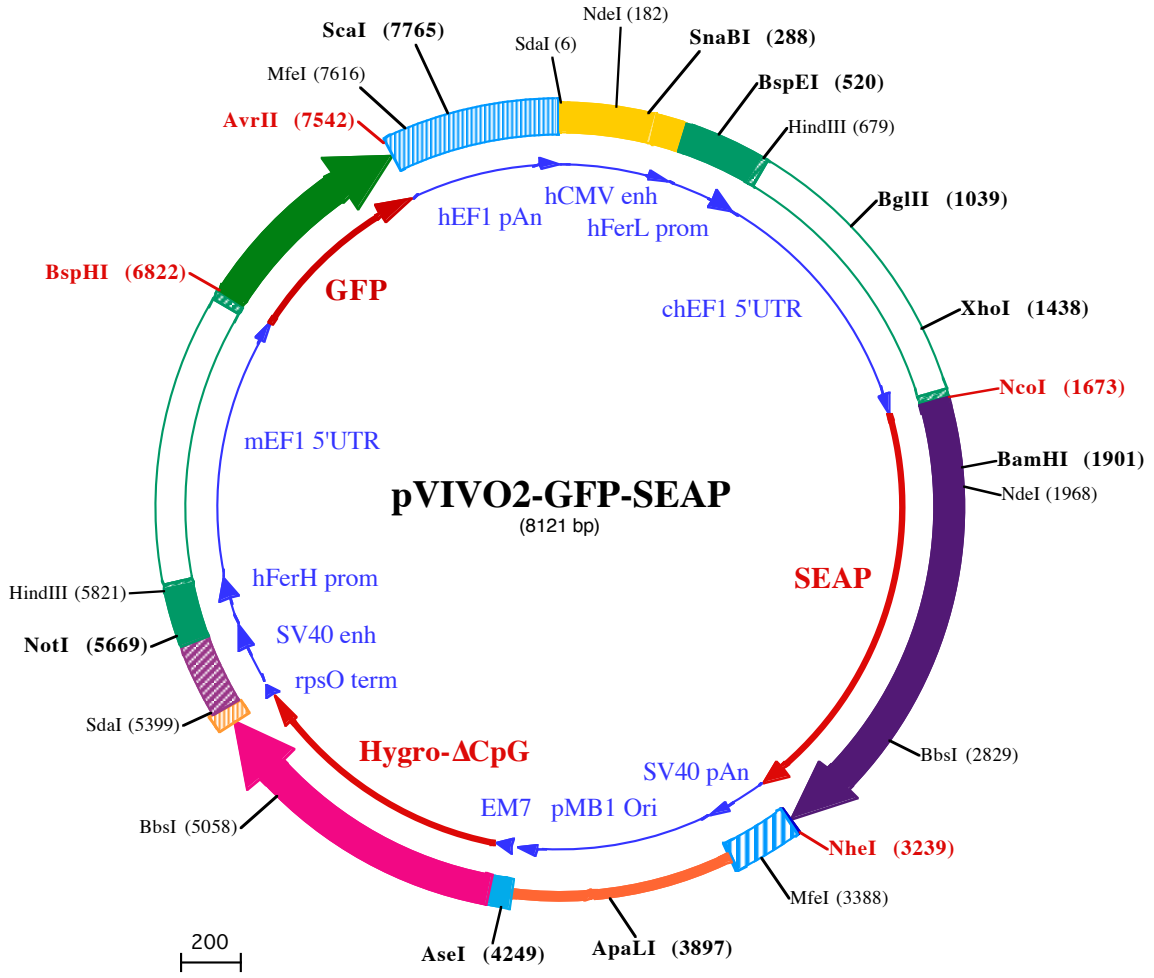
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SdaI (6)
1 **CCTGCAGG**CGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (182)
101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCCCT

SnaBI (288)
202 **ATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGC**

303 **TATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGGTGGATAGCGGTTTACTCACGGGATTTC**CAAGTCTCCACCCATTGACGTCAATGGGA

404 **GTTTGTTTTGA**CTAGTCAGGGCCCCAACCCCCCAAGCCCCATTTCAACACGCTGGCCTACAGGCGCTGACTTCCCTTGCTTTGGGCGGGGGG

BspEI (520)
504 **CTGAGACTCCTATGTGCTCGGATTGGTCAGGCACGGCCTTCGGCCCGCTCCTGCCACCGCAGATTGGCCGCTAGG**CCTCCCCGAGCGCCCTGCCTCG

HindIII (679)
605 **AGGGCGGCGCACCATAAAAGAAGCCGCCCTAGCCAGTCCCCTCGCAGTTGGCGGTCCCGGGTCTGTCTCA**AGCTTGGCGCAGAACAAGGtaagt

706 **gccgtgtgtgtgtcccgcgggctggcctctttacgggtatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattcctgatc**

807 **ccgagcttccgggttggagatgggtgggagatgagggccttgccttaaggagcccccttgcctcgtgcttgagttgaggcctggcttggcgctggggc**

908 **cgccgcgtgctaactcgtggcaccttgcgcctgtctcgtgcttctcgttaagtctctagccatttaaaatTTTTgataaccagctgcgacgctTTTTTT**

BglII (1039)
1009 **ctggcgagatagctcttgaatgctgggccaagatctgcacactggatatttcggtttttggggccgcgggcgagcgggcccgtgcgtcccagcgcacat**

1110 **gttcggcgaggggggctgcgagcgcggccaccgagaatcgagcggggtagtctcaaacctggcggcctgctctggtgcctggcctgcgccctgtgt**

1211 **atcgcccccttggcggaagctggccggctggcaccagttgcgtgagcggaaagatggccgcttcccggcctgctgcagggagctcaaatggag**

1312 **gacgcggcgccccggagagcggcggtgagtcacccacacaaagaaaaggcccttctctcatccgtgccttcatgtgactccacggagtaccggg**

XhoI (1438)
1413 **cgccgtccaggcacctcgtattagtctcgagcttttggagtacgtcgtctttaggttgggggaggggttttatgcgatggagttccccacactgagtg**

1514 **gtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaaatttgcctttttgagtttggatcttgcctcattctcaagcctcagaca**

NeoI (1673)
1615 **gtggttcaaagtTTTTTTTcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCAC**ATGGTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCT

1716 **GGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGGAGGCAGCCGAGGCCCTGGGTGCCCAAGA**
14▶ G L R L Q L S L G I I P V E E N P D F W N R E A A E A L G A A K

BamHI (1901)
1817 **AGCTGCAGCCTGCACAGACAGCCCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAG**
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

NdeI (1968)
1918 **AAGAAGGACAAACTGGGGCTGAGATACCCCTGGCTATGGACCGCTTCCATATGTGGCTCTGTCCAAGACATAAATGTAGACAAACATGTCCAGACAG**
82▶ 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 S

2019 **TGGAGCCACAGCCACGGCCTACTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTTGTAGTGACAGCCCGCTTAAACAGTGCAACACGACACGG**
115▶ 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

2120 **GCAACGAGGTCATCTCCGTGATGAATCGGGCAAGAAGCAGGGAAGTCAGTGGGAGTGGTAACCACACACAGTGACGACGCCCTGCCAGCCGGCACC**
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

2221 **TACGCCACACCGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCAGGAGGGTGCCAGGACATCGCTACGCACTCATCTCCAA**
183▶ 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 N

2322 **CATGGACATTGATGTGATCCTGGTGGAGGCCAAAGTACATGTTTCGATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCAAGGTGGACCA**
216▶ 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

2423 **GGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCAAGCGCCAGGTGCCGGTATGTGTGGAACCGCACTGAGCTCATGCAAGCTTCCCTGGACCCG**
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

2524 **TCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGC**
284▶ 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 A

2625 **TGCCCTGCGCCTGCTGAGCAGGAACCCCGCGGCTTCTCTCTTCTGAGGGTGGTGCATCGACCACGGTATCACGAAAGCAGGGCTTACCGGGCAC**
317▶ 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

BbsI (2829)
2726 **TGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACAGCTGAGCCTCGTCACTGCCACCACTCCCACGTC**
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

2827 **TTCTCCTCGGAGGCTACCCCTCGAGGGAGCTCCATCTTCCGGCTGGCCCTGGCAAGGCCGGGACAGGAAGGCCTACACGGTCTCTATACGGAAA**
385▶ 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 N

2928 **CGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTACGAGTGGCCCTGGACG**
418▶ 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

3029 **AAGAGCCACGCAAGCGAGGACGTGGCGGTGTCGCGCGCGGCCAGGCGCACTGGTTCAGGCGTGCCAGGAGCAGACCTTCATAGCCAGGTCATG**
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

3130 GCCTTCGCCGCTGCCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCCGGCACCACCGACCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCT
486▶ 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 L
NheI (3239)
3231 GGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG
519▶ D •
MfeI (3388)
3332 GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGA
3433 GGTTTTTTAAAGCAAGTAAAACTCTACAAATGTGGTATGGAATGTTAATTAACCTAGCCATGACCAAAATCCCTAACCTGAGTTTTCTGCCACTGAGC
3534 GTCAGACCCCGTAGAAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCACAAAAAACACCCGCTACCAGCGGTGG
3635 TTTGTTTCCCGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCCAGCAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTA
3736 GGCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACC GG
ApaI (3897)
3837 GTTGACTCAAGACGATGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGAGCGAACGACCTACACCGAAC
3938 TGAGATACCTACAGCGTGAAGCTATGAGAAAGCGCCACGCTCCCGAAGGGAGAAAGCGGCACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGC
4039 ACGAGGGAGCTCCAGGGGGAACCGCTGGTATCTTTATAGTCTGTGCGGTTTCCGCCACCTCTGACTTGAGCGTGCATTTTTGTGATGCTGCTCAGGGGG
4140 GCGGAGCCTATGAAAAACGCGCAACGCGGCCTTTTTACGGTTCTGGCCTTTTGTCTCATGTCTTAAATTAATTTTTCAAAGTA
AseI (4249)
4241 GTTGACAATTAATCATCGCATAGTATATCGCATAGTATAATACGACTCACTATAAGGAGGCCACCATGAAGAACTGAACTGACAGCAACTTCTGTTG
4342 AGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGACGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTGATGTTGGAGGAAGAGGT
1▶ M K K P E L T A T S V
4443 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 V G G R G
4443 TATGTTCTGAGGGTCAATTCTTGCTGATGGTTTTTACAAGACAGATATGTTTACAGACACTTTGCCTCTGCTGCTGCAATTCAGAAGTTCTGGGA
46▶ 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 I P E V L D
4544 CATTGGAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGC
79▶ 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 L P A V L
4645 AACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCAAACCTCTGGATTTGGTCTTTGGTCCCAAGGCATTGGTCACTACCACT
113▶ 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 G Q Y T T
4746 TGGAGGGATTTTCAATTTGTCATTGCTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGA
147▶ 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 A Q A L D E
4847 ACTCATGCTGTTGGCAGAAGATTTGCTGAAGTCAAGCAGCTGGTCCATGCTGATTTTGAAGCAACATGTTCTGACAGACAATGGCAGAATCACTGCAG
180▶ 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 G R I T A
4948 TCATTGACTGGTCTGAAGCCATGTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTGCTGGAACAACAACAAGA
214▶ 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 E Q Q T R
BbsI (5058)
5049 TATTTTTGAAAGAAGACACCCAGAAGTGGCTGGTCCCCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGG
248▶ 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 Q S L V D G
5150 AAATTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGCTGGTCTGGAAGTGTGGAAGAAGTCAAATTTGCAAGAAGGTTCTGCTG
281▶ N F D D A A W A Q G R C D A I V R S E G A G T V G R T Q I A R R S A
5251 CTGTTTGGACTGATGGATGTGTTGAAGTTCTGGCTGACTCTGGAACAGGAGACCTCCACAAGACCCAGAGCCAAGGAATGAATATTAGCTAGGAGTTTC
315▶ 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 •
SdaI (5399)
5352 AGAAAAGGGGGCTGAGTGGCCCTTTTTCAACTTAATTAACCTGCAAGGCTGAATAACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCT
5452 GAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGTTGGAAAGTCCCAAGCTCCCAAGCAGGAGCAAGATGCAAAGCATGCATCTCAATTAGTCAGCA
5553 ACCAGGTGTGAAAGTCCCAAGCTCCCAAGCAGGAGCAAGATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCCCGCAGAGC
NotI (5669)
5653 GCGCAGGGCTCCAGCGGCCCTCCCAAGCAGGAGGGGCTCCCGCGCCACCGGAAGGAGCGGGCTCGGGCGGGCGCGCTGATTGGCCGGG
HindIII (5821)
5754 GCGGCTGACGCCGACGGCTATAAGAGACCACAAGCGACCCGACGGCCAGACGTTCTTCCGCGAAGCTTGCCGTCAGAACGAGGTGAGGGGGGGG
5855 GTGGCTTCCGCGGCCGCCAGCTGGAGGCTCTGCTCCGAGCGGGCCGGCCCGCTGTCGTCGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGC
5956 GGGCGCGCGGGAGGCGAGGTGCGAGGCTAGCGGCAACCCGTAAGCTCGCTCGCTGTCGGCTTGAAGCCTAGCGTGGTCCCGCGCCCGCCGCGTG
6057 CTACTCCGGCCGACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCTTCGATTGCCGTTAGCAATAGGGGCTAACAAAGGAGGGTGGGGG
6158 CTTGCTCGCCGAGCCCGAGAGGTCATGTTGGGGAGGAATGGAGGACAGGAGTGGCGGTTGGGGCCCGCCGCTTCGGAGCACATGTCGACGCCA
6259 CCTGGATGGGCGAGGCTGGGGTTTTTCCGAAGCAACAGGCTGGGGTACGCTGCGGAGGCAATGTTGGCCAGCACCTGCTGCTTGGCTGGCG
6360 GCGCCGCTTGCCTGCCTCCTAACTAGGTTGAGGCCATCCCGTCCGGCACCAGTTGCTGCTGGAAGATGGCCGCTCCCGGGCTGTTGCAAGGAG
6461 CTCAAAATGGAGGACGCGGACCCGGTGGAGCGGGGGTGGATCACCCACACAAGGAAGAGGGCTGGTCCCTCACCGGCTGCTGCTTCTGTGACCC
6562 CGTGGTCTATCGGCCGAATAGTCACCTCGGGCTTTTGGACACGGCTAGTCCGCGGGGGGAGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGT
6663 GGGTGGAGACTAGTCAGCCAGCTGGCGTGAAGTCATTTTTGAATTTGTCCTTGGTGGTGGAGGCTAATTCTCGGGCTTCTTAGCGTTCA
BspHI (6822)
6764 AAGGTATCTTTAAACCTTTTTTAGGTGTTGTGAAAACCCGCTAATTCAAAGCAATCATGAGCAAGGAGAAGAACTTTTACTGGTGTGTTCCCAAT
1▶ M S K G E E L F T G V V P I
6865 TCTGTTGAGCTGGATGGTATGTAATGGCCACAAATTTCTGTGCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTT
14▶ L 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
6966 GTACAACAGGAAAGCTGCCAGTGCCTTGGCAACTCTGGTGAACACCCTGACTTATGTTGTTCAATGTTTACGAGGTACCCTGACCACATGAAGCAGCAT
48▶ C 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

7067 GACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGA
82▶ D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E
7168 AGGTGATACACTGGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGGAACATTCTGGGTCAACAAGCTGGAGTACAACATAATTCTCACA
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
7269 ATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACCTGGCAGACCATTAC
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 Y
7370 CAGCAGAACACCCCTATTGGTGATGGCCAGTTCTCCTCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGCCAAAGACCCTAATGAGAAAAGAGA
183▶ 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 D
7471 CCACATGGTCCTCTGGAGTTTGTGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGATTATCCCTAATACCTGCCACCCC
216▶ H M V L L E F V T A A G I T L G M D E L Y K •
MfeI (7616)
7572 ACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTA
ScaI (7765)
7673 AAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTGCCTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTTA
7774 ATGGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGAGACCATTAAAAAGTTAAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTT
7875 AGGTGAAAGACATCTAATTCTGGTTTTACGAATCTGGAACTTCTTGAAAATGTAATCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCC
7976 CCACATAATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTGTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCA
8077 CTA AACAGGCCAAAACTGAGTCCTTGGGTTGCATAGAAAAGCTG