

pVIVO1-GFP/SEAP

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

Catalog code: pvivo1-gfppsp

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO1-GFP/SEAP provided as lyophilized DNA
- 1 ml of Hygromycin B Gold (ultrapure Hygromycin B; 100 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20°C.
- Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles.
- Store Hygromycin B Gold at 4°C or at -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

pVIVO1 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector.

- pVIVO1-GFP/SEAP contains the reporter genes GFP and SEAP and can be used as a control vector.
- pVIVO1-GFP/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (BspHI/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, visit: <https://www.invivogen.com/genes>.

PLASMID FEATURES

- **haGRP78 and hGRP94 prom:** The hamster GRP78 and human GRP94 promoters drive weak levels of expression in normal conditions and are induced in stress conditions prevailing inside tumors, such as glucose deprivation and hypoxia¹. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly².
- **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 plasmids³.
- **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.

- **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 enhancer⁴.

- **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.*⁵

- **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 or QUANTI-Blue™ Solution.

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58. 2. Gazit G. *et al.* 1999. Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. *Cancer Res* 59: 3100-6 3. Dean DA. *et al.*, 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22. 4. 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. 5. 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.

TECHNICAL SUPPORT

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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

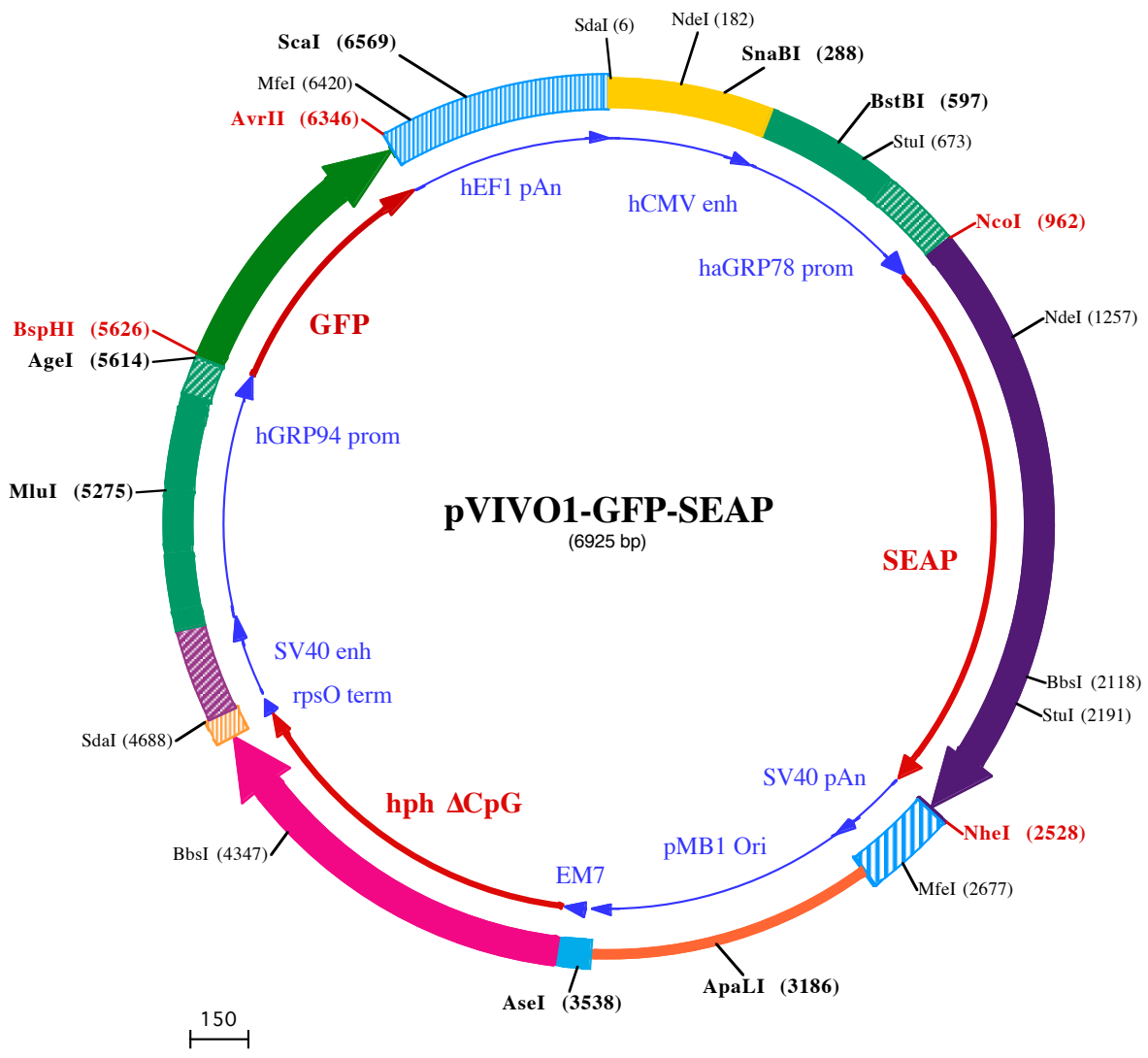
This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
Hygromycin B Gold	Selection antibiotic	ant-hg-1
pVIVO1-GFP/LacZ	Dual reporter plasmid	pvivo1-gfplacz
pVIVO1-Lucia/SEAP	Dual reporter plasmid	pvivo1-lucsp
pVIVO1-mcs	Multiple cloning site plasmid	pvivo1-mcs
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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SdaI (6)
1 **CCTGCAAGG**CGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

NdeI (182)
101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATATGGCAAGTACGCCCCCTA

SnaBI (288)
203 TTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA

305 TTACCATGATGATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGTTTTGACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTT

407 TGTTTTGA**CT**AGTTACCGCGGAAACGGTCTCGGTTGAGAGGTACCCGAGGGACAGGCACTGCTGAACCAATAGGACCGCGCACAGGGCGGATGCTG

BstBI (597)
508 CCCCTATTGGCGGCCGTTGAGAGTGACCAAGACCAATGAGTACGCCGGGGGCGTAGCAGTGACGTAAGTTGCGGAGGAGCCGCTTGAATCGGCAGC

StuI (673)
610 GGCCAGCTTGGTGGCATGGACCAATCAGCGTCTCCAACGAGGAGCGCTTCGCCAATCGGAGGCCCTCACGACGGGGCTGGGGGAGGGTATATAAGCCGA

712 GTCGGCGCGCGCGCTCCACACGGGCGAGACCACAGCGACGGGAGCGTCTGCCTCTGCGGGGCCGAGAGGTAAGCGCCGCGCTGCCCTTCCAGGCCA

814 ACTCGGAGCCCGTCTCGTGGCTCCGCCTGATCGGGGCTCTGTGCGCCTCAGATCGGTGGAACGCCGTCGCGCTCCGGGACTACAAGCTGTTGCTGGC

NeoS (962)
916 CCGGAGACTGCCGAAGGACCGCTGAGCACTGCTCAGCGCCGCA**acc**ATGGTTCTGGGCTGCATGCTGCTGCTGCTGCTGCTGGCCCTGAGGCTA

1018 CAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACC CGACTTCTGGAACCGGAGGAGCCGAGGCCCTGGTGCCCAAGAAGCTGCAGCCCTGCA

19 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 K L Q P A

1120 CAGACAGCCGCCAAGAACCTCATCATCTTCTGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGCGAAGAAGGACAACTG

53 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 K K D K L

NdeI (1257)
1222 GGGCTGAGATACCCTGGCTATGGACCGCTTCCCATATGTGGCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAG

87 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 G A T A T

1324 GCCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTTGTGAGTGCAGCCGCCGCTTAAACAGTGCAACACGACACGCGGCAACGAGGTGATCTCC

121 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 G N E V I S

1426 GTGATGAATCGGGCAAGAAAGCAGGAAGTCACTGGGAGTGGTAACACCACACGAGTGCAGCACGCTCGCCAGCCGGCACCTACGCCACACGGTGAAC

155 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 Y A H T V N

1528 CGCAACTGGTACTCGGACCGCAGTGCCTGCCTCGGCCCGCCAGGAGGGTGCAGGACATGCTACGAGCTCATCTCCAACATGGACATTGATGTGATC

189 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 M D I D V I

1630 CTGGGTGGAGGCCAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCCAAGTGGGACCAGGCTGGACGGGAAGAATCTG

223 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 R L D G K N L

1732 GTGCAGGAATGGCTGGCGAAGCGCAGGGTGGCCGGTATGTGTGAACCGCACTGAGTGCATGCAGGCTTCCCTGGACCCGCTGTGTGCCATCTCATGGGT

257 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 S V T H L M G

1834 CTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCAGACTCCACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGGCCCTGCTGAGCAGG

291 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 A L R L L S R

1936 AACCCCGCGGCTTCTTCTTCTGTTGGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCCGAC

325 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 L T E T I M F D

BbsI (2118)
2038 GACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTGCTACTGCCACCCTCCACGCTTCTCTTCCGAGGCTACCCCTG

359 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 F S F G G Y P L

StuI (2191)
2140 CGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCGGGACAGGAAGCCCTACACGGTCTCCTATACGAAACGGTCCAGGCTATGTCTCAAGGAC

393 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 G P G Y V L K D

2242 GGC GCCCGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGACGAGTCAGCAGTGCCTTGGACGAAGAGACCCACGACGGCGAGGACGTG

427 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 E E T H A G E D V

2344 GCGGTGTTGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTATAGCGCACGTATGGCTTCCGCGCTGCTGGAGCCCTAC

461 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 A F A A C L E P Y

NheI (2528)
2446 ACCGCTGCGACCTGGCGCCCGCCGGCACCCGACCGCGCACCCGGGGCGGTCCCGTCCAAGCGTCTGATTGAAGCTAGCTGG**CCAGACATGAT**

495 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 D •

2548 AAGATACATTGATGAGTTTGGCAAACCAACTAGAAATGCAAGTGAATAATGCTTTATTTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTA

MfeI (2677)
2650 AAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACAA

2752 ATGTGGTATGAAAT**G**TAAATTAAGTACCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCGTAGAAAAGATCAAAGGATCT

2854 TCTTGAGATCCTTTTTCTGCGCTAATCTGCTGCTTGAACAACAAAAACCCGCTACACGCGGTGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTT

2956 TTTCCGAAGGTAAGTGGCTTCCAGCAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCT

3058 ACATACCTCGCTCTGCTAATCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGGC

ApaLI (3186)

3160 CAGCGGTGCGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCC
3262 ACGCTTCCGAAGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTGGAAACAGGAGCGCACGAGGGAGCTCCAGGGGAAACGCTGGTATCTT
3364 TATAGTCTGTGGGTTTCGCCACCTCTGACTTGGAGCTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGGCGCTTT

AseI (3538)

3466 TTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCCATCATGTTCTTAAATTAATTTTTCAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGT
3568 ATAATACGACTCACTATAGGAGGGCCACCTGAAGAACTGAACTGACAGCACTTCTGTTGAGAAGTTTCTATTGAAAAATTTGATTCTGTTTCTGATC
3670 TCATGCAGCTGTCTGAAGGTGAAGAAAGCAGAGCCTTTCTTTGATGTTGGAGGAAGGTTATGTTCTGAGGGTCAATCTTGTGCTGATGGTTTTTACA
25▶ L M Q L S E G E E S R A F S F D V G G R G Y V L R V N S C A D G F Y
3772 AAGACAGATATGTTTACAGACACTTTCCTCTGCTGCTGCTGCAATTCAGAAAGTTCTGGACATGGAGAATTTTCTGAATCTCTCACCTACTGCATCAGCA
59▶ K D R Y V Y R H F A S A A L P I P E V L D I G E F S E S L T Y C I S
3874 GAAGAGCACAAGGAGTCACTCTCCAGGATCTCCCTGAACTGAGCTGCCAGCTGTTCTGCAACTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATC
93▶ R R A Q G V T L Q D L P E T E L P A V L Q P V A E A M D A I A A A D
3976 TGAGCCAACTCTGGATTGGTCTTTTGGTCCCAAGGCATTGGTCTGACACACTTGGAGGGATTTTCTGATGCTGATCCTCATGTCTATC
127▶ L S Q T S G F G P F G P Q G I G Y T T W R D F I C A I A D P H V Y
4078 ACTGGCAGACTGTGATGGATGACACAGATTTCTGCTTCTGTTGCTCAGGCACTGGATGAACTCATGCTGTGGGCAGAAAGATTGCTCTGAAGTGCAGACCTGG
161▶ H W Q T V M D D T V S A S V A Q A L D E L M L W A E D C P E V R H L
4180 TCCATGCTGATTTTGGAAAGCAACAATGTTGACAGACAATGGCAGAATCACTGCAGTCACTGGTCTGAAGCCATGTTTGGAGATTTCAATATGAGG
195▶ V H A D F G S N N V L T D N G R I T A V I D W S E A M F G D S Q Y E

BbsI (4347)

4282 TTGCCAACATTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAACAACAAGAATTTTTGAAAGAAGACACCCAGAACTGGCTGGTCCCCAGACTGA
229▶ V A N I F F W R P W L A C M E Q Q T R Y F E R R H P E L A G S P R L
4384 GAGCCTACATGCTCAGAAATGGCCTGGACCAACTGTATCAATCTCTGGTGTGATGAAACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTG
263▶ R A Y M L R I G L D Q L Y Q S L V D G N F D D A A W A Q G R C D A I
4486 TGAGGCTGCTGGAAGTGTGGAAGAAGTCAATTTGCAAGAAGTCTGCTGCTGTTTGGACTGATGGATGTGTTGAAATTTCTGGCTGACTCTGGAACA
297▶ V R S G A G T V G R T Q I A R R S A A V W T D G C V E V L A D S G N

SdaI (4688)

4588 GGAGACCTCCACAAGACCCAGAGCCAAGGAATGAATATTAGCTAGGAGTTTCAGAAAAGGGGCTGAGTGGCCCTTTTTTCAACTTAATTAACCTCAG
331▶ R R P S T R P R A K E •
4690 GGCCTGAAATAACCTCTGAAAGAGGAAGTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCCAG
4792 GCTCCCCAGCAGGAGAGATGCAAAGCATGCATCTCAATTAGTCAGAACAGGCTGTGAAAGTCCCCAGGCTCCCCAGCAGGAGATGCAAAGCA
4894 TGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTTCATCACCACCGCCACCCCGCCCGCCCTCTGAAAGGTTCTAGGGGATTGCAACC
4995 TCTCTCGTGTGTTCTTTTCCGAGAAGCGCCACACGAGAAGCTGGCCGCGAAAGTCTGCTGGAATCACTTCCAACGAAACCCAGGCATAGATGG
5097 GAAAGGGTGAAGAACACGTTGTCATGGCTACGTTTCCCGGTCACGGAATAAACGCTCTCTAGGATCCGGAAGTAGTCCGCGGACCTCTCTAAAAGGA

MluI (5275)

5199 TGGATGTGTTCTCTGTTACATTCATTGGACGTTTTCCCTTAGAGGCCAAGGCCCCAGGCAAAAGGGCGGTCCCACGCTGAGGGGCGCCGAGCCATT
5301 TGATTGGAGAAAAGCTGCAAACTGACCAATCGGAAGGAGCCACGCTTCGGGCATCGGTACCCGACCTGGACAGCTCCGATTGGTGGACTTCCGCCCC
5403 CTCACGAATCCTATTGGGTGCCGTGGGTGCGTGGTGGCGGCGATTGGTGGTTCATGTTTCCGTCGCCCGCCGCGAGAAGTGGGGGTGAAAAGCGGCC
5505 CGACCTGCTTGGGTGTAGTGGCGGACCGCGCGGTGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGTGGAGGCGGCTCCTGCATCGAAGGGGACTT

AgeI (5614) BspHI (5626)

5607 GAGACTCACCAGTGCACGTGAGCAAGGAGGAGAAGAACTTTACTGGTGTGTTGCCAATTTCTGTTGAGCTGGATGGTGTGATGTAATGGCCACAATTC
▶ M S K G E E L F T G V V P I L V E L D G D V N G H K F
5709 TCTGTGCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAAGCTGACTCTGAAGTTCAATTTGTACAACAGGAAAGCTGCCAGTGCCTTGGCCAACTCTGGT
28▶ S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V
5811 ACCACCTGACTTATGGTGTCAATGTTTACAGAGTACCCTGACCACATGAAGCAGCATGACTTCTTAAATCTGCAATGCCAGAAGTTATGTTCCAGGAG
62▶ T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E
5913 AGGACAATCTTCTTAAAGGATGATGAAATATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGTTAACAGAATGAGCTGAAAGGCATTGAT
96▶ R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D
6015 TTTAAGGAAGATGAAACATTTGGGTGACAAAGCTGGAGTACAATAATTCTCACAATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGGTT
130▶ F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V
6117 AATTTCAAGATTAGACACAACATTTAGGATGGATCTGTTCAACTGGCAGACCATTAACAGCAGAACCCCTATTGGTGTGATGGCCAGTTCTCTCCAGAT
164▶ N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D
6219 AATCACTATCTCCGCACTCAATCTGCTGTCAAAGACCTAATGAGAAAAGAGACCACATGTTCTCTGAGTGGTGTGACAGCAGCAGGAATTAATCTG
198▶ N H Y L R T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L

AvrII (6346)

MfeI (6420)

6321 GGAATGGATGAGCTGTACAAGTAACTAGGATATCCCTAATACCTGCCACCCCACTTAAATCAGTGGTGAAGAAGCGTCTCAGAAGTGTGTTGTTCAA
232▶ G M D E L Y K •
6423 TTGGCATTAAAGTTTAGTAGTAAAAGACTGGTTAATGATAAATGATCGTAAACCTTCAGAAAGGAAAGGAGAATGTTTTGTGACCACTTTGTTTTTC

ScaI (6569)

6525 TTTTTGCGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGAAACAACCTTGACCAAAAATTTGTACAGAATTTGAGACCATTA
6627 AAAAAGTTAAATGAGAAACCTGTGTGTTCCCTTGGTCAACACCGAGACATTTAGGTGAAAGACATCTAATCTGTTTTACGAATCTGAAACTTCTGAA
6729 ATGTAATTTCTGAGTTAACTTCTGGGTGGAGAATAGGGTGTGTTTTCCCCACATAAATGAAAGGGGAAGGAATATCAATTAAGCTATGGGAGGGTTC
6831 TTTGATTACAACAGGTGGAGAGAAATGCAGCATGTTGCTGATTGCTGTCACTAAAACAGGCCAAAACCTGAGTCTTGGGTTGCATAGAAAGCTG