

pVITRO1-blasti-GFP/LacZ

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

Catalog code: pvitro1-bgfpplacz

<https://www.invivogen.com/pvitro1-gfpplacz>

For research use only

Version 20F22-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO1-GFP/LacZ provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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

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

pVITRO1-GFP/LacZ also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Nco* I/*Avr* II for GFP and *Bsp*H I/*Nhe* I for LacZ) that allow for convenient cloning of ORFs.

PLASMID FEATURES

- **rEF1 and mEF1 prom:** pVITRO1-mcs plasmid carries two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins. Similarly to their human counterpart¹, 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 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 several-fold more active than the SV40 enhancer³.

- **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 expression⁵.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr gene** confers resistance to Blasticidin both in *E. coli* and mammalian cells. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* 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.
- **LacZ gene:** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope.
- **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).

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-titer 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 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.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

TECHNICAL SUPPORT

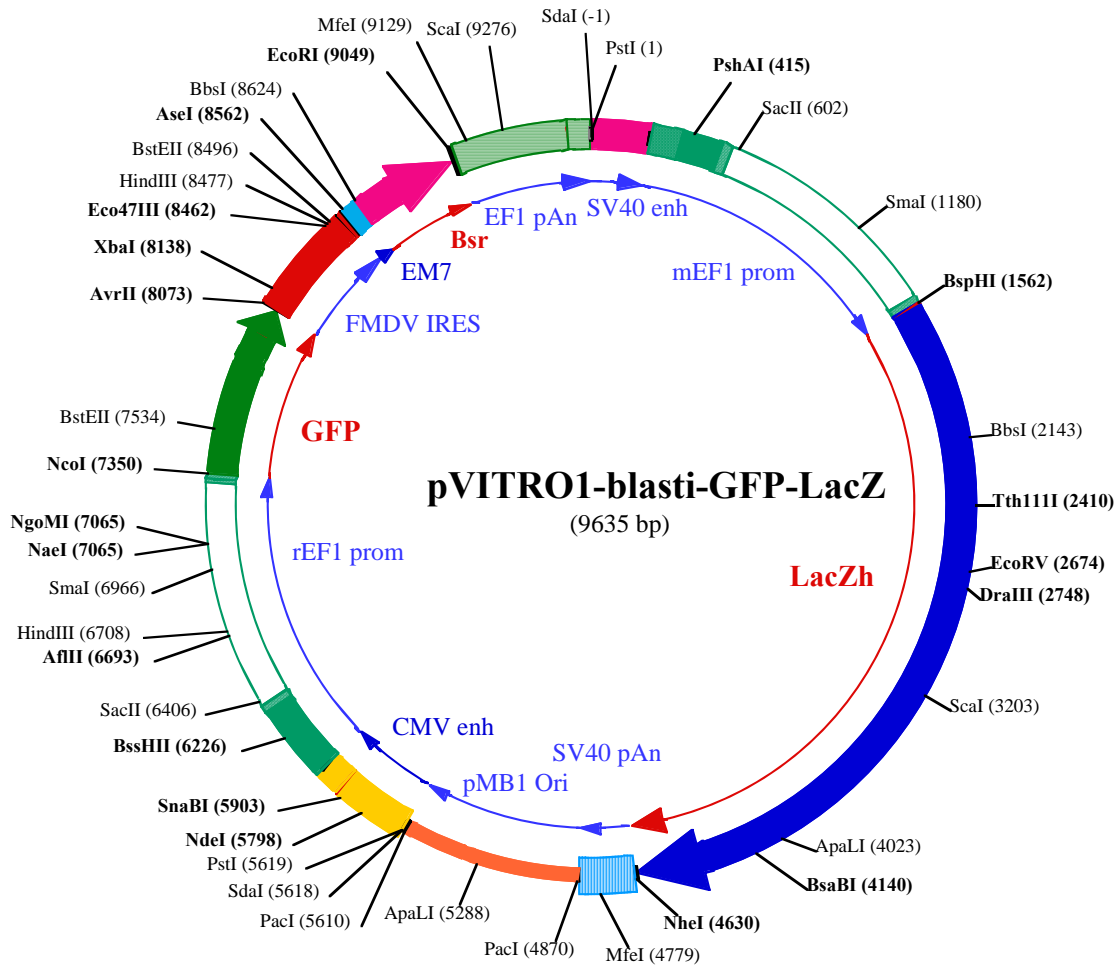
InvivoGen USA (Toll-Free): 888-457-5873

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



250

PstI (1)
SdaI (-1)

1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCGAAAGAACACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACACAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAG

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAG

301 CCCAGGGACCGTCCCTAAATTTCTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGCGAGGAGCATGCGCTCAGGGCTGAGCGGGGAGAGCAGA

PshAI (415)

401 GCACACAAGCTCATAGACCCTGGTCTGGGGGGAGGACCGGGGAGCTGGCGCGGGCAAACCTGGGAAAGCGGTGTCGTGTGCTGGCTCCGCCCTCTTCC

501 CGAGGGTGGGGGAGAACGGTATATAAGTGCAGCAGTGCCTTGGACGTTCTTTTTCGCAACGGGTTTTCGCTCAGAACCGAGGTGAGGGGCGGGTGTGGC

SacII (602)

601 TTCGCGGGCCCGGAGCTGGAGGTCCTGCTCGAGCGGGCCGGGCCCGCTGTGTCGTCGGCGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGC

701 GCGCGGGGAGGCAGAGTGCAGGCTAGCGGCAACCCCGTAGCCTCGCTCGTGTCCGGCTTGGGCTAGCGTGGTGTCCGCGCCGCGCGCGCTGCTA

801 CTCGCGCCGACTCTGGTCTTTTTTTTTTTTGTGTGTGTGCGCTGCTGCCCTCGATTGCGGTTTCAGCAATAGGGGCTAACAAAGGAGGGTGCGGGGCT

901 TGCTCGCCCGAGCCCGAGAGGTCATGGTTGGGAGGAATGGAGGACAGGAGTGGCGGCTGGGGCCCGCCCTTCGAGCAGCATGTCCGACGCCAC

1001 CTGGATGGGGGAGGCTGGGGTTTTTCCCGAAGCAACAGGCTGGGGTTAGCGTCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCG

SmaI (1180)

1101 GCGCCGCTTGCCTCCCTAAGTGGGTGAGGCCATCCCGTCCGGCACAGTTGCGTGCCTGGAAGATGGCCGCTCCCGGCCCTGTTGCAAGGA

1201 GCTCAAAATGGAGGACGCGGCAGCCCGGTGGAGCGGGCGGGTGGAGTACCCACACAAAGGAAGAGGGCTGGTCCCTCACCGGCTGCTGCTTCTGTGAC

1301 CCCGTTGCTCTATCGGCCGCAATAGTACCTCGGGCTTTTGAGCACGGCTAGTCCGCGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTT

1401 GGTGGTGGAGACTAGTCAGGCCAGCCTGGCGTGAAGTCATTTTTGGAATTTGTCCTTGGATTGAGTTTTGAGCGGAGCTAATTTCTCGGGCTTCTTAGCGG

BspHI (1562)

1501 TTCAAAGGTATCTTTTAAACCTTTTTTTAGGTGTTGTGAAAACCCCGCTAATTCAAAGCAATCATGAGCCTGGCTGTTGTGCTGCAAGGAGAGACTGG

1601 GAGAACCTGGAGTGACCAGCTCAACAGACTGGCTGCCACCCCTCCCTTTGCTCTTTGGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCACGACCCAG

13▶ E N P G V T Q L N R L A A H P P F A S W R N S E E A R T D R P S Q

1701 AGCTCAGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCTCGCCCTGAAGCTGTGCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACAC

46▶ Q L R S L N G E W R F A W F P A P E A V P E S W L E C D L P E A D T

1801 TGTGTGGTGCACCAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTACCTACCCCATCACTGTGAACCCCTTTTGTGCCCACT

79▶ V V V P S N W Q M H G Y D A P I Y T N V T Y P I T V N P P F V P T

1901 GAGAACCCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGACCAGGATCATCTTTGATGGAGTCAACTCTGCCT

113▶ E N P T G C Y S L T F N V D E S W L Q E G Q T R I I F D G V N S A

2001 TCCACCTCTGGTGAATGGCAGGTGGGTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGACCTCTGCTTCCCTCAGAGCTGGAGAGAACAG

146▶ F H L W C N G R W V G Y G Q D S R L P S E F D L S A F L R A G E N R

BbsI (2143)

2101 GCTGGCTGTCATGGTCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCCTGCTGCAC

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

2201 AAGCCACCAACCAGATTTCTGACTTCCATGTTGCCACCAAGTTCATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAAC

213▶ K P T T Q I S D F H V A T R F N D D F S R A V L E A E V Q M C G E

2301 TCAGAGACTACCTGAGAGTCAAGTGGCCTCTGGCAAGGTGAGACCCAGGTGGCTCTGGCACAGCCCTTGGAGGAGAGATCATTTGATGAGAGAGG

246▶ L R D Y L R V T V S L W Q G E T Q V A S G T A P F G G E I I D E R G

Tth111I (2410)

2401 AGGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGGCTGTTGTGGAGCTGCAC

279▶ G Y A D R V T L R L N V E N P K L W S A E I P N L Y R A V V E L H

2501 ACTGCTGATGGCACCCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTTCAGAGAAAGTCAAGGATTGAGAATGGCCTGCTGCTCAATGGCAAGCCTCTGC

313▶ T A D G T L I E A E A C D V G F R E V R I E N G L L L L N G K P L

EcoRV (2674)

2601 TCATCAGGGGAGTCAACAGGCATGAGCACCCCTCTGCAATGACCAAGTATGATGATGAACAGACAATGGTGAAGATATCTGCTAATGAAGCAGAACAA

346▶ L I R G V N R H E H H P L H G Q V M D E Q T M V Q D I L L M K Q N N

DraIII (2748)

2701 CTTCAATGCTGTGAGGTGCTCTCACTACCCCAACCCCTCTCTGGTACACCCCTGTGTGACAGGTATGGCCTGTATGTTGTTGATGAAGCCAACATTGAG

379▶ F N A V R C S H Y P N H P L W Y T L C D R Y G L Y V V D E A N I E

2801 ACACATGGCATGGTGCCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCTGCCATGTCTGAGAGAGTGACCAGGATGGTGAGAGACAGGAACC

413▶ T H G M V P M N R L T D D P R W L P A M S E R V T R M V Q R D R N
2901 ACCCTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAACCATGATGCTCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACC

446▶ H P S V I I W S L G N E S G H G A N H D A L Y R W I K S V D P S R P
3001 TGTGCAGTATGAAGGAGGTGGAGCAGACCAACAGCCACAGACATCATCTGCCCATGTATGCCAGGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCC

479▶ V Q Y E G G G A D T T A T D I I C P M Y A R V D E D Q P F P A V P
3101 AAGTGGAGCATCAAGAAGTGGCTCTCTGCTGGAGAGACCAGACCTCTGATCCTGTGTGAATATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTG

513▶ K W S I K K W L S L P G E T R P L I L C E Y A H A M G N S L G G F
ScaI (3203)
3201 CCAAGTACTGGCAAGCCTTCAGACAGTACCCAGGCTGCAAGGAGGATTGTGTGGGACTGGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAA

546▶ A K Y W Q A F R Q Y P R L Q G G F V W D W V D Q S L I K Y D E N G N
3301 CCCCTGGTCTGCCTATGGAGGAGACTTTGGTGACACCCCAATGACAGGCAGTTCATGATGAATGGCTGGTCTTTGAGAGCAGGACCCCTCACCCCTGCC

579▶ P W S A Y G G D F G D T P N D R Q F C M N G L V F A D R T P H P A
3401 CTCACAGAGGCCAAGCACCAGCAACAGTTCCTCCAGTTCAGGCTGTCTGGACAGACCATTGAGGTGACATCTGAGTACCTCTTCAGGCACCTCTGACAAAT

613▶ L T E A K H Q Q Q F F Q F R L S G Q T I E V T S E Y L F R H S D N
3501 AGCTCTGCACTGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGGGTCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAAGTGGC

646▶ E L L H W M V A L D G K P L A S G E V P L D V A P Q G K Q L I E L P
3601 TGAGTGCCTCAGCCAGAGTCTGTGGACAACCTGTGGTAAACAGTGGGTTGAGGTTGAGCCCAATGCAACAGCTGGTCTGAGGCAGGCCACATCTCTGCA

679▶ E L P Q P E S A G Q L W L T V R V V Q P N A T A W S E A G H I S A
3701 TGGCAGCAGTGGAGGCTGGCTGAGAACTCTCTGTGACCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTG

713▶ W Q Q W R L A E N L S V T L P A A S H A I P H L T T S E M D F C I
3801 AGCTGGCAACAAGAGATGGCAGTTCAACAGGCAGTCTGGCTTCCTGTCTCAGATGTGGATTGGAGACAAGAAGCAGCTCCTCACCCCTCTCAGGGACCA

746▶ E L G N K R W Q F N R Q S G F L S Q M W I G D K K Q L L T P L R D Q
3901 ATTCACAGGGTCTCTCTGGACAATGACATTTGGAGTGTCTGAGGCCACAGGATGACCCAAATGCTGGGTGGAGAGGTGGAAGGCTGCTGGACACTAC

779▶ F T R A P L D N D I G V S E A T R I D P N A W V E R W K A A G H Y
ApaLI (4023)
4001 CAGGCTGAGGCTGCCTGCTCCAGTGCACAGCAGACCCCTGGCTGATGCTGTTCTGATCACCACAGCCCATGCTTGGCAGCACAAGGCAAGACCCCTGT

813▶ Q A E A A L L Q C T A D T L A D A V L I T T A H A W Q H Q G K T L
BsaBI (4140)
4101 TCATCAGCAGAAAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCAAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGG

846▶ F I S R K T Y R I D G S G Q M A I T V D V E V A S D T P H P A R I G
4201 CCTGAAGTGTCAACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGGCTTAGGCCCTCAGGAGAACTACCTGACAGGCTGACAGCTGCCTGCTTTGAC

879▶ L N C Q L A Q V A E R V N W L G L G P Q E N Y P D R L T A A C F D
4301 AGGTGGACCTGCCTCTGTCTGACATGTACACCCCTTATGTGTTCCCTTCTGAGAAATGGCTGAGGTGGCACCAGGAGCTGAACTATGGTCTCACC

913▶ R W D L P L S D M Y T P Y V F P S E N G L R C G T R E L N Y G P H
4401 AGTGGAGGGGAGACTTCCAGTTC AACATCTCCAGTACTCTCAGCAACAGCTCATGGAAACCTCTCACAGGCACCTGCTCCATGAGAGGAGGGAACCTG

946▶ Q W R G D F Q F N I S R Y S Q Q Q L M E T S H R H L L H A E E G T W
4501 GCTGAACATTTGATGGCTTCCACATGGGCATTGGAGGAGTACTCTTGGTCTCCTTCTGTGTCTGCTGAGTTCCAGTTATCTGCTGGCAGGTACCACATAT

979▶ L N I D G F H M G I G G D D S W S P S V S A E F Q L S A G R Y H Y
NheI (4630)
4601 CAGCTGGTGTGGTGCAGAAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAGTGAATGCAAGTGAAGAAA

1013▶ Q L V W C Q K •

MfeI (4779)
4701 AATGCTTTTATTGTGAAATTTGTGATGCTATTGCTTTTATTGTAAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTT

PacI (4870)
4801 TCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGCAAAATGTTAATTAACTAGCCATGACCAAAATCCCTT

4901 AACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAAC

5001 AAAAAAACCCCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCCAGAGAGCGCAGATACCAAAAT

5101 ACTGTTCTTCTAGTGTAGCCGTAGTTTAGGCCACCACTTCAAGAACTCTGTAGCACCCCTACATACCTCGCTCTGCTAATCTGTTTACCAGTGGCTGCTG

5201 CCAAGTGGCATAAGTCTGTCTTACCAGGTTGGACTCAAGACGATAGTTACCAGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTACACAGCC

5301 CAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGGCTATGAGAAAGCGCCACGCTTCCCGAAGGGGAGAAAGGCGGACAGGTATCCG

5401 GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTCCGGTTTCGCCACCTCTGACTTG

5501 AGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACCGCGGCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTTCG

SdaI (5618)
PacI (5610) PstI (5619)

5601 TCACATGTTCTTAATTAACCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCATAATGA

NdeI (5798)

5701 CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCA

5801 TATGCCAAGTACGCCCTTATGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTAC

SnaBI (5903)

5901 ATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTGACTCAGCGGGATTTCGAAGTCTCC

6001 ACCCCATTGACGTCAATGGGAGTTTGTGTTGACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAGCCAGGGACCG

6101 TCCTTAAATTCACAGACCCAAATCCCTGTAGCCGCCACGACAGCGCAGGAGCATGCGCCAGGGCTGAGCGCGGTAGATCAGAGCACACAAGCT

BssHII (6226)

6201 CACAGTCCCGCGGTGGGGGGAGGGGCGCTGAGCGGGGCCAGGGAGCTGGCGCGGGGCAAACTGGGAAAAGTGGTGTCTGTGCTGGCTCCGCCCTC

6301 TTCCCAGGGTGGGGGAGAACGGTATATAAGTGCCTGAGTGCCTTGGACGTTCTTTTCGCAACGGGTTTGGCGTCAGAACGCAGgtgagtggcgggtg

SacII (6406)

6401 tggcttcgcgggccccggagctggagccctgctctgagcgggccccgctgatatgacgagtgctcgcgagggttagctgtgagcattcccacttoga

6501 gtggcgggctgccccgggtgagagtgagagcctagcggcaaccccgtagcctcgctcgtgtccggttgaggcctagcgtggtgtccgcccccgcgt

AflII (6693)

6601 gccactccggccgactatgctgtttttgtccttgcctcgatgcttccagcagcatgggctaacaaaggagggtgtggggctcactcttaagg

HindIII (6708)

6701 agcccatgaagcttacgttggataggaatggaagggcaggagggcgactgggccccccgcttcggagcacatgtccgacgccacctggatggggcg

6801 aggcctgtggcttccgaagcaatcgggcgtgagtttagcctacctggccatgtggccctagcactgggacggctggcctggcggtgcccgttccc

SmaI (6966)

6901 ttgctcccaacaagggtgagggcgtccccccggcaccagttgcttgcgcggaagatggccgctccccgggcccctgttgaaggagctcaaaatggag

NgoMI (7065)

NaeI (7065)

7001 gacgcggcagcccgggtggagcgggccccggtgagtcacccacacaaaggaagagggccttgcccctcgccggccgctgcttccctgtgaccccggtgctctatc

7101 ggccgcatagtcacctcgggcttctcttgagcaccgctcgtcgcggcgggggaggggatctaatggcgttggagttagttcacatttgggtgggtggaga

7201 ctagtccagccagcctggcgtggaagtcattcttggaaattgcccctttagtttggagcagggctaatctcaagcctcttagcgttcaaaggtatt

NcoI (7350)

7301 ttctaaaccggttccagGTGTTGTGAAAGCCACCGCTAATTCAAAGCAACcATGGTTTCTAAGGGAGAAGAACTCTTTACTGGTGTGTCCCAATTCG

1 M V S K G E E L F T G V V P I L

7401 GTTGAAGTGGATGGTGAATGGCCACAAATTCCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTGTGA

17 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

BstEII (7534)

7501 CAACAGGAAAGTGCAGTGCCTTGGCCAACCTCTGGTGACCAACCTGACTTATGFTGTTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGA

50 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 D

7601 CTTCTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAAATATAAGACAAAGGCAGAAGTGAAGTTTGAA

83 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

7701 GGTGATACACTGGTTAACAGAAATGAGCTGAAAGGCATTGATTTTAAAGGAAGATGGAAAACATCTGGGTCAACAGCTGGAGTACAACTATAATTCTCAC

117 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

7801 ATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGGTTAATTCAGATTAGACACAAACATGAGGATGGATCTGTCCAACCTGGCAGACCATTA

150 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

7901 CCAGCAGAACACCCCTATGGTGTAGGCCAGTTCTCCCTCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCCTAATGAGAAAAAGA

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

AvrII (8073)

8001 GACCACATGGTCTCTGGAGTTTGTGACAGCAGCAGGAATTAATCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCAATGACAC

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

XbaI (8138)

8101 AAAACGTGCAACTTGAAACTCCGCTGGTCTTCCAGGCTAGAGGGGTAAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTATG

8201 TAACAGCACTGTGCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTTAACAAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCCGT

8301 CATGTGTGCAACCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTT

8401 CAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTG

Eco47III (8462) HindIII (8477) BstEII (8496)

8501 ACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCTATGAATACACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATAC

AseI (8562)

8601 GACTCACTATAGGAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTGCCACTGAGAAGATCACCATGCTCTATGAG

BbsI (8624)

1 M K T F N I S Q Q D L E L V E V A T E K I T M L Y E

8701 GACAACAAGCACCATGTGGGGCGGCCATCAGGACCAAGACTGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGTCACCTGTCTGTG

27 D N K H H V G A A I R T K T G E I I S A V H I E A Y I G R V T V C

8801 CTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGACAGATC

60 A E A I A I G S A V S N G Q K D F D T I V A V R H P Y S D E V D R S

8901 CATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGACTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAA

93 I R V V S P C G M C R E L I S D Y A P D C F V L I E M N G K L V K

EcoRI (9049)

9001 ACCACCATTGAGGAACCTATCCCCCTCAAGTACACCAGGAACATAACCTGAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCAGTGG

127 T T I E E L I P L K Y T R N •

MfeI (9129)

9101 TGGAGAACGGTCTCAGAACTGTTTGTTCATTTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGG

ScaI (9276)

9201 AAAGGAGAATGTTTTGTGGACCACCTTTGGTTTTCTTTTTTTCGCTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAAACAACCTT

9301 GACCAAAAATTTGTCACAGAATTTGAGACCCATTAATAAGTTAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAAGACA

9401 TCTAATCTGGTTTTACGAATCTGGAACTTCTTGAAAATGTAATTTCTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCAATAATTG

9501 GAAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGG

9601 CCAAAAACCTGAGTCCTTGGTTGCATAGAAAGCTG