

pVIVO2-mcs

A multigenic cloning plasmid for strong and sustained expression in normal tissues

Catalog # pvivo2-mcs

For research use only

Version # 04F30-SV

PRODUCT INFORMATION

Content:

pVIVO2-mcs is provided as 20 µg of lyophilized DNA.
- 4 pouches of *E. coli* Fast-Media® Hygro

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA is stable 1 year when stored at -20°C. Resuspended DNA is stable 12 months when stored at -20°. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Hygro pouches at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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 new generation multigenic vector with TWO transcription units allowing the combined expression of TWO genes of interest from a SINGLE vector.

pVIVO2-mcs may be used for:

- **cloning two genes of interest.** pVIVO2-mcs contains two multiple cloning sites (MCS) for convenient insertion of two genes.
- **co-expression of two genes in vivo.** Both multiple cloning sites are located downstream of the strong, ubiquitous Ferritin composite promoters, allowing high levels of expression of the cloned transgenes. Expression may be achieved *in vivo*, and *in vitro* in transient transfection experiments.

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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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.
- **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.
- **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.
- **MCS:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

Bsp HI, *Xba* I, *Bsr* G I, *Bst* 1107I, and *Avr* II.
Bsp HI is compatible with *Nco* I and *Bsp* LU11I.
Xba I is compatible with *Nhe* I, *Spe* I and *Avr* II.
Bsr G I is incompatible with *Acc*65 I, *Ban* I and *Bsi*W I.
Bst 1107I is compatible with any other blunt-end restriction enzymes.
Avr II is compatible with *Xba* I, *Spe* I and *Nhe* I.

MCS2 contains the following restriction sites:

Nco I, *Bam*H I, *Eco*R I, *Eco*R V, and *Nhe* I
Nco I is compatible with *Bsp*H I and *Bsp*LU11 I.
*Bam*H I is compatible with *Bgl* II, *Bst*Y I, *Dpn* II, and *Bcl* I.
*Eco*R I is compatible with *Apo* I, *Mfe* I and *Tsp*509 I.
*Eco*R V is compatible with any other blunt-end restriction enzymes.
Nhe I is compatible with *Xba* I, *Spe* I and *Avr* II.

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.

Selection of bacteria with *E. coli* Fast-Media:

E. coli Fast-Media® Hygro is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Hygro is a TB (liquid) or LB (solid) based medium with hygromycin B, and contains stabilizers.
E. coli Fast-Media® Hygro can be ordered separately (catalog code # fas-hg-1, fas-hg-s).

Method:

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid over-boiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

References:

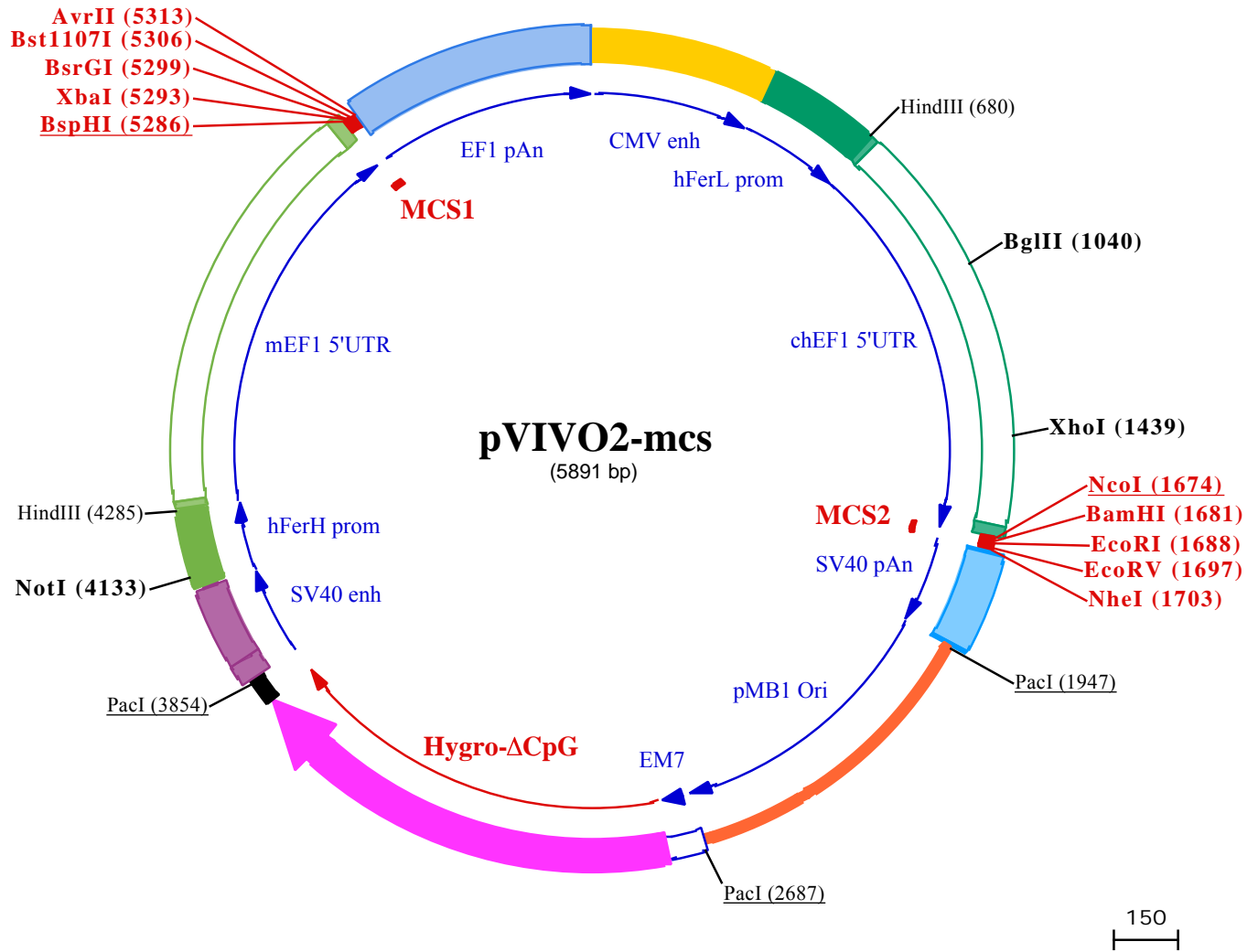
1. Eisenstein RS. and Munro HN. 1990. *Enzyme* 44(1-4):42-58
2. Dean DA. *et al.* 1999. *Exp. Cell. Res.* 253:713-22
3. Boshart M. *et al.* 1985. *Cell* 141(2):521-30
4. Carswell S., and Alwine JC. 1989. *Mol. Cell Biol.* 10: 4248-4258

TECHNICAL SUPPORT

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA



1 CCTGCAGGCGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
 101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCC
 201 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
 301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCAGCGGGATTCCAAGTCTCCACCCATTGACGTCAATG
 401 GGAGTTTGTFTTACTAGTCAGGGCCCAACCCCAAGCCCACTTACAACACGCTGGCGGTACAGGCGGTGACTTCCCTTGCTTTGGGGCGGG
 501 GGGCTGAGACTCTATGTGCTCCGATTGGTCAGGCACGGCTTCGGCCCGCCTCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCTGCC
 601 TCCGAGGCGCGGCACCATAAAAAGAAGCCGCTAGCCACGTCCCTCGCAGTTCGGCGGGTCCCGCGGTCTGTCTCAAGCTTCCCGCCAGAACACAGg
 HindIII (680)
 701 taagt gccgtgtgt ggttcccggggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc
 801 ttgatcccagacttccgggttggaaagtgggtgggagagttcgaggccttgcgttaaggagccccttcgctcgtgcttgagttgaggcctggcttggggc
 901 ctggggcgcgccgtgctaactcgggtggcaccttcgctcgtctcgtgctttcgttaagtctctagccatttaaaatTTTTgataaccagctgcgacg
 BglII (1040)
 1001 cttttttctggcgagatagcttctgtaaatgcccgaagatctgcacactggtatctcggttttggggcccgggcgccgacggggcccgtgcgtccc
 1101 agcgcacatgctcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggggtagtctcaactggccggcctgctctggtgcctggcctcgc
 1201 gccgcctgtatcggccgcctggcgaggcaagctggcccggctggcaccagttgcgtgagcggaaagatggccgcttcccggcctgctgcagggagc
 1301 tcaaaatggaggacgcccggcgaggagcggcggggtgagtcaccacacaaaaggaaaaggccttctctcctcatccgtcgttcatgtgactcca
 XhoI (1439)
 1401 cggagtaccggcgccgtccaggcacctcgattagttctcgagcttttggagtacgtcgtcttttaggttggggggagggttttatgcatggagtttcc
 1501 ccacactgagtgggtggagactgaagagttaggccagcttggcacttgatgtaattctccttgggaatttgcctttttgagtttgatcttgcctcattc
 BamHI (1681) EcoRV (1697)
 NcoI (1674) EcoRI (1688)
 1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAACCTACCCCTAAAAGCCA **CCATGGAGGATCCAGAATTCAGATATCA**
 NheI (1703)
 1701 **GGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCT**
 1801 **ATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCTTTTATGTTTCAGGTTCCAGGGGAGGTGTGGAGGTTT**
 PacI (1947)
 1901 **TTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGAAAATGTTAATTAACCTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA**
 2001 **GACCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGCGTAACTGCTGCTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTT**
 2101 **GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGG**
 2201 **CCACCCTTCAAGAACTCTGTAGCACCCTACATACCTCGCTGCTGTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGG**
 2301 **TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAAC**
 2401 **TGAGATACCTACAGCGTGAGCTATGAGAAAGCCACGCTCCCGAAGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGTCGGAACAGGAGAGCG**
 2501 **CACGAGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG**
 PacI (2687)
 2601 **GGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGCTCACATGTTCTTAATTAATTTTTCAAAA**
 2701 **GTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAAGTACAGCAACTTCT**
 2801 **PTTGAGAAGTTTCTCATTGAAAAATTTGATCTGTTTCTGATCTCATGCAGCTGCTGAAAGTGAAGAAAGCAGAGCCTTTCTTTTGTGATGTTGGAGGAA**
 11 ▶ ValGluLysPheLeuI leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArgAlaPheSerPheAspValGlyGlyA
 2901 **GAGGTTATGTTCTGAGGGTCAATTCTTGTGCTGATGGTTTTTACAAGACAGATATGTTACAGACACTTTGCCTCTGCTGCTGCCAATTCAGAAGT**
 44 ▶ rgGlyTyrValLeuArgValAsnSerCysAlaAspGlyPheTyrLysAspArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProl leProGluVa
 3001 **TCTGGACATTGGAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGAGCAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCT**
 77 ▶ lLeuAspl leGlyGluPheSerGluSerLeuThrTyrCysl leSerArgArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGluLeuProAla
 3101 **GTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACCTCTGGATTGGTCCTTTTGGTCCCCAAGGCATTGGTCACT**
 111 ▶ ValLeuGlnProValAlaGluAlaMetAspAlal leAlaAlaAlaAspLeuSerGlnThrSerGlyPheGlyProPheGlyProGlnGlyI leGlyGlnT
 3201 **ACACCCTTGGAGGATTTCAATTTGTGCCATTGCTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTTGCTGCTCAGG**
 144 ▶ yrThrThrTrpArgAspPheI leCysAlal leAlaAspProHisValTyrHisTrpGlnThrValMetAspAspThrValSerAlaSerValAlaGlnAl

3301 ACTGGATGAAGCTCATGCTGTGGGCAGAAAGATTGCTCTGAAGTCAGACACCTGGTCCATGCTGATTTTGGAAAGCAACAATGTTCTGACAGACAATGGCAGA
177▶ aLeuAspGluLeuMetLeuTrpAlaGluAspCysProGluValArgHisLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAspAsnGlyArg
3401 ATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAAC
211▶ l l e ThrAlaVal l l e AspTrpSerGluAlaMetPheGlyAspSerGlnTyrGluValAlaAsn l e PhePheTrpArgProTrpLeuAlaCysMetGluG
3501 AACAAACAAGATATTTTGAAGAAGACACCCAGAAGCTGGCTGTTCCCCAGACTGAGAGCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATC
244▶ InGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArg l e GlyLeuAspGlnLeuTyrGlnSe
3601 TCTGGTTGATGAAACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGTCTGGTGGTGGAACTGTTGGAAGAAGCTCAAATTGCA
277▶ rLeuValAspGlyAsnPheAspAlaAlaTrpAlaGlnGlyArgCysAspAla l e ValArgSerGlyAlaGlyThrValGlyArgThrGln l e Ala
3701 AGAAGTCTGCTGCTGTTTGGACTGATGGATGTGTTGAAGTCTGGCTGACTCTGAAAACAGGAGACCCCTCCAAGACCCAGAGCCAAGGAATGAATAT
311▶ ArgArgSerAlaAlaValTrpThrAspGlyCysValGluValLeuAlaAspSerGlyAsnArgArgProSerThrArgProArgAlaLysGlu•••

PaeI (3854)

3801 TAGCTAGGAGTTTCAGAAAAGGGGGCCTGAGTGGCCCTTTTTTCAACTTAATTAACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAAGCTTGGTTAGG
3901 TACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCAT
4001 CTCAATTAGTCAGCAACCAGGTGTGAAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCAC

NotI (4133)

4101 TAGTTCGCCAGAGCGCGGAGGGCCTCCAGCGCGGCCCTCCCCACAGCAGGGGGGGTCCCGGCCACCAGGAAGGAGCGGCTCGGGGGCGGGC

HindIII (4285)

4201 GCGCTGATTGGCCGGGGCGGCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGAGGGCCAGACGTTCTTCGCCGAAGCTTCCCGTCAGAAC
4301 GCAGGTGAGGGCGGGTGTGGCTTCCGCGGGCCCGAGCTGGAGGTCTGCTCCGAGCGGGCGGGCCCGCTGTGCTCGCGGGGATTAGCTGGCAGC
4401 ATTCCCGCTTCGAGTTGCGGGCGCGGGAGGCAGAGTGCAGGGCTAGCGGCAACCCGTAGCCTCGCCTCGTGTCCGGCTTGAGGCATAGCGTGGTG
4501 TCCGCGCCCGCCCGCTGCTACTCCGGCCACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCCGTTACAGAAATAGGGGCT
4601 AACAAAGGGAGGGTGGCGGGCTTGCTCGCCCGAGCCCGAGAGGTCATGGTTGGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCGCTT
4701 CGGAGCACATGTCCGACGCCACCTGGATGGGGCGAGGCTGGGGTTTTTCCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCAAGCA
4801 CCCGGCACGATCTGGCTTGGCGCGCCGCTTGCCTGCCTCCCTAACTAGGGTGAAGCCATCCCGTCCGGCACAGTTCGCTGCGTGAAAGATGGCCG
4901 CTCCCGGGCCCTGTGCAAGGAGCTCAAAATGGAGGACGCGGAGCCCGTGGAGCGGGCGGGTGAAGTACCCACACAAGGAAGAGGGCCTGGTCCCTC
5001 ACCGGCTGCTGCTTCTGTGACCCGCTGGTCTATCGGCCGAATAGTCACCTCGGGCTTTTGAAGCAGGCTAGTCGCGGGCGGGGAGGGGATGTAATG
5101 GCGTTGGAGTTTGTTCACATTTGGTGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGAAAGTCATTTTTGGAATTTGTCCCCTTGAGTTTTGAGCGGAGC

XbaI (5293)

BspHI (5286)

BsrGI (5299)

5201 TAATTCCTGGGCTTCTTAGCGTTCAAAGGTATCTTTTAAACCCTTTTTAGGTGTTGTGAAAACCACCGCTAATTCAAAGCAATCATGAATCTAGACTG

AvrII (5313)

Bst1107I (5306)

5301 TACAAGTATACCCTAGGATTATCCCTAATACCTGCCACCCACTCTAATCAGTGGTGAAGAACGGTCTCAGAAGCTTTGTTTCAATTGGCCATTTAA
5401 GTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTGTGGACCACTTGGTTTTCTTTTTGCGT
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5601 AAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGTGAAAAGACATCTAATCTGGTTTACGAATCTGAAAACCTCTTGAAAATGTAA
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5801 ATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACCTGAGTCCTTGGGTTGCATAGAAGCTG