

pVIVO2-mcs

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

Catalog code: pvivo2-mcs
<https://www.invivogen.com/pvivo-mcs>

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO2-mcs 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-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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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 *Bs*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.

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

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

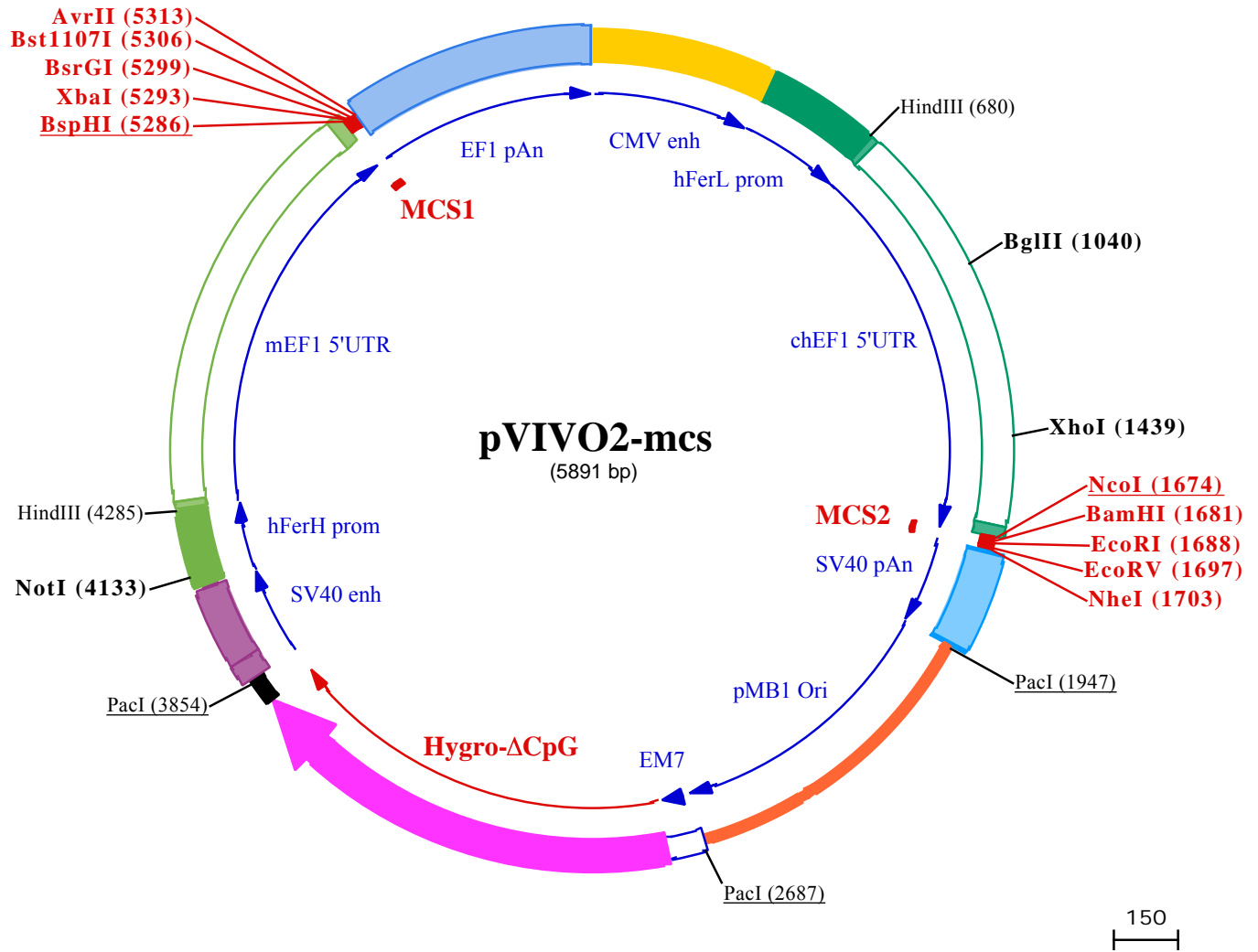
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1 CCTGCAGGCGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
 101 CGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCC
 201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
 301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCAGCGGGATTCCAAGTCTCCACCCATTGACGTCAATG
 401 GGAGTTTGTFTTGACTAGTCAGGGCCCAACCCCAAGCCCCATTTCACAACACGCTGGCGGTACAGGCGGTGACTTCCCTTGCTTTGGGGCGGG
 501 GGGCTGAGACTCTATGTGCTCCGATTGGTCAGGCACGGCTTCGGCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCTGCC
 601 TCCGAGGCGCGGCACCATAAAAAGAAGCCGCTAGCCACGTCCCTCGCAGTTCGGCGGGTCCCGCGGTCTGTCTCAAGCTTGGCCGACAGACAGg
 HindIII (680)
 701 taagt gccgtgtgt ggttcccggggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc
 801 ttgatcccagacttccgggttggaaagtgggtgggagagttcgaggccttgcgttaaggagccccttcgctcgtgcttgagttaggacctggcttggggc
 901 ctggggcgcgccgtgctaactcgggtggcaccttcgcgctgtctcgtgctttcgttaagtctctagccatttaaaattttgataaccagctgcgacg
 BglII (1040)
 1001 cttttttctggcgagatagcttctgtaaatgcccgaagatctgcacactggtatctcggttttggggccgggcgccgacggggcccgtgctccc
 1101 agcgcacatgctcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggggtagtctcaactggccggcctgctcgggtgcctggcctcgc
 1201 gccgcctgtatcggccgcccctggcgaggcaagctggcccggctggcaccagttgcgtgagcggaaagatggccgcttcccggcctgctgcagggagc
 1301 tcaaaatggaggacgcccggcgggagagcggcggggtgagtcaccacacaaaaggaaaaggccttctctcctcatccgtcgttcatgtgactcca
 XhoI (1439)
 1401 cggagtaccggcgccgtccaggcacctcgattagttctcgagcttttgagtagctcgtcttttaggttggggggagggttttatgcatggagtctcc
 1501 ccacactgagtggtgggagactgaagagttaggccagcttggcacttgatgtaattctccttgggaatttgcctttttgagtttgatcttgcctcattc
 BamHI (1681) EcoRV (1697)
 NcoI (1674) EcoRI (1688)
 1601 tcaagcctcagacagtggttcaaagttttttcttccatttcagGTGTCGTGAAAACCTACCCCTAAAAGCCA CATTGGAGGATCCAGAATTCAGATATCA
 NheI (1703)
 1701 GGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCT
 1801 ATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCTTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTT
 PacI (1947)
 1901 TTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGAAAATGTTAATTAACCTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA
 2001 GACCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGCGTAACTGCTGCTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTT
 2101 GTTTGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGTAGTTAGG
 2201 CCACCCTTCAAGAACTCTGTAGCACCCTACATACCTCGCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGG
 2301 TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAAC
 2401 TGAGATACCTACAGCGTAGCTATGAGAAAGCCACGCTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGTCGGAACAGGAGAGCG
 2501 CACGAGGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG
 PacI (2687)
 2601 GGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGCTCACATGTTCTTAATTAATTTTTCAAAA
 2701 GTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCACCATGAAGAAACCTGAAGTACAGCAACTTCT
 2801 GTTGAGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGCTGAAGGTGAAGAAAGCAGAGCCTTTTCTTTTGTGATTTGGAGGAA
 11 ValGluLysPheLeuI leGluLysPheAspSerValSerAspLeuMetGlnLeuSerGluGlyGluGluSerArgAlaPheSerPheAspValGlyGlyA
 2901 GAGGTTATGTTCTGAGGGTCAATTCTTGTGCTGATGGTTTTTACAAGACAGATATGTTACAGACACTTTGCCTCTGCTGCTGCCAATTCAGAAGT
 44 rgGlyTyrValLeuArgValAsnSerCysAlaAspGlyPheTyrLysAspArgTyrValTyrArgHisPheAlaSerAlaAlaLeuProl leProGluVa
 3001 TCTGGACATTGGAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGACACAAGGAGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCT
 77 lLeuAspl leGlyGluPheSerGluSerLeuThrTyrCysl leSerArgArgAlaGlnGlyValThrLeuGlnAspLeuProGluThrGluLeuProAla
 3101 GTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCGAGCAGCTGATCTGAGCCAAACCTCTGGATTGGTCCCTTTTGGTCCCCAAGGCATTGGTCACT
 111 ValLeuGlnProValAlaGluAlaMetAspAlal leAlaAlaAlaAspLeuSerGlnThrSerGlyPheGlyProPheGlyProGlnGlyI leGlyGlnT
 3201 ACACCCTTGGAGGATTTTCATTTGTGCCATTGCTGATCCTCATGCTATCACTGGCAGACTGTGATGGATGACACAGTTTCTGCTTCTGTGCTCAGGG
 144 yrThrThrTrpArgAspPheI leCysAlal leAlaAspProHisValTyrHisTrpGlnThrValMetAspAspThrValSerAlaSerValAlaGlnAl

3301 ACTGGATGAAGCTCATGCTGTGGGCAGAAAGATTGCTCTGAAGTCAGACACCTGGTCCATGCTGATTTTGGAAAGCAACAATGTTCTGACAGACAATGGCAGA
177▶ aLeuAspGluLeuMetLeuTrpAlaGluAspCysProGluValArgHisLeuValHisAlaAspPheGlySerAsnAsnValLeuThrAspAsnGlyArg
3401 ATCACTGCAGTCATTGACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCAACATTTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAAC
211▶ l l e Thr Ala Val l l e Asp Trp Ser Glu Ala Met Phe Gly Asp Ser Gln Tyr Glu Val Ala Asn l e Phe Phe Trp Arg Pro Trp Leu Ala Cys Met Glu G
3501 AACAAACAAGATATTTTGAAGAAGACACCCAGAAGCTGGCTGTTCCCCAGACTGAGAGCCTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATC
244▶ InGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAlaTyrMetLeuArg l e GlyLeuAspGlnLeuTyrGlnSe
3601 TCTGGTTGATGAAACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGTGAGGTCTGGTGTGGAACTGTTGGAAGAAGCTCAAATTGCA
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 GCAGGTGAGGGCGGGTGTGGCTTCCGCGGGCCCGAGCTGGAGGTCTGCTCCGAGCGGGCCGGGCCCGCTGTGCTCGCGGGGATTAGCTGCGAGC
4401 ATTCCCGCTTCGAGTTGCGGGCGCGGGAGGCAGAGTGCAGGGCTAGCGGCAACCCGTAGCCTCGCCTCGTGTCCGGCTTGAGGCCTAGCGTGGTG
4501 TCCGCGCCCGCCCGCTGCTACTCCGGCCACTCTGGTCTTTTTTTTTTTTGTGTTGTTGCCCTGCTGCCTTCGATTGCCGTTACAGAAATAGGGCT
4601 AACAAAGGGAGGGTGGCGGGCTTGCTCGCCCGAGCCCGAGAGGTCATGGTTGGGGAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCGCTT
4701 CGGAGCACATGTCCGACGCCACCTGGATGGGGCGAGGCTGGGGTTTTTCCCGAAGCAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCAAGCA
4801 CCCGGCACGATCTGGCTTGGCGCGCCGCTTGCCTGCCTCCCTAACTAGGGTGAAGCCATCCCGTCCGGCACCAAGTTCGCTGCGTGAAAGATGGCCG
4901 CTCCCGGGCCCTGTGCAAGGAGCTCAAAATGGAGGACGCGGAGCCCGTGGAGCGGGCGGGTGAAGTACCCACACAAGGAAGAGGGCTGTTCCCTC
5001 ACCGGCTGCTGCTTCTGTGACCCGCTGGTCTATCGGCCGAATAGTCACTCGGGCTTTTGAACAGGCTAGTCCGCGGGGGGAGGGGATGTAATG
5101 GCGTTGGAGTTTGTTCACATTTGGTGGTGGAGACTAGTCAGGCCAGCCTGGCGCTGAAAGTCATTTTTGGAATTTGTCCCCTTGAGTTTTGAGCGGAGC

XbaI (5293)

BspHI (5286)

BsrGI (5299)

5201 TAATTCCTCGGGCTTCTTAGCGTTCAAAGGTATCTTTTAAACCCTTTTTTAGGTGTTGTGAAAACCACCGCTAATTCAAAGCAAATCATGAATCTAGACTG

AvrII (5313)

Bst1107I (5306)

5301 TACAAGTATACCCTAGGATTATCCCTAATACCTGCCACCCACTCTAATCAGTGGTGAAGAACGGTCTCAGAAGCTTTGTTTCAATTGGCCATTTAA
5401 GTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTGTGGACCACTTGGTTTTCTTTTTGCGT
5501 GTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAAACAACCTGACCAAAAATTTGTACAGAATTTTGTGACCCATTAATAAAGTT
5601 AAATGAGAAACCTGTGTGTTCTTTGGTCAACACCGAGACATTTAGTGAAAAGACATCTAATCTGGTTTACGAATCTGAAAACCTTGTGAAAATGTAA
5701 TTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGGAAGGGGAAGGAATATCATTAAAGCTATGGAGGGTTTCTTTG
5801 ATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACCTGAGTCCTTGGGTTGCATAGAAGCTG