pVITRO2-hygro-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro2-mcs

https://www.invivogen.com/pvitro2-mcs

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-hygro-mcs provided as lyophilized DNA
- 1 ml 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

pVITRO is a family of plasmids developed mainly for in vitro studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

pVITRO2-hygro-mcs plasmid is selectable with hygromycin B in both *E. coli* and mammalian cells. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

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.

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 protein[1]. 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[2]. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.

• **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 severalfold more active than the SV40 enhancer[3].

• **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:** 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 expression[5].

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

• ***hph* gene** confers resistance to Hygromycin B both in *E. coli* and mammalin cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* is transcribed from the CAG 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.

• **MCS1 and MCS2:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

Age I, Eco RV, Bam HI, Sal I and Avr II

- Age I is compatible with Bsp EI and Sgr AI.
- Eco RV (blunt-end restriction enzyme).
- Bam HI is compatible with Bgl II, Bst YI and Bcl I.
- Sal I is compatible with Ava I and Xho I.
- Avr II is compatible with Xba I, Spe I and Nhe I.

MCS2 contains the following restriction sites:

Sgr AI, Bgl II, Xho I and Nhe I

- *Sgr AI is compatible with Bsp EI and Age I.*
- *Bgl II is compatible with Bam HI, Bst YI and Bcl I.*
- *Xho I is compatible with Ava I and Sal I.*
- *Nhe I is compatible with Xba I, Spe I and Avr II.*

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91:217-23. **2. Moreau P. *et al.*, 1981.** The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res. 9:6047-68. **3. Boshart M. *et al.* 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 141:521-30. **4. Carswell S., and Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 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.

TECHNICAL SUPPORT
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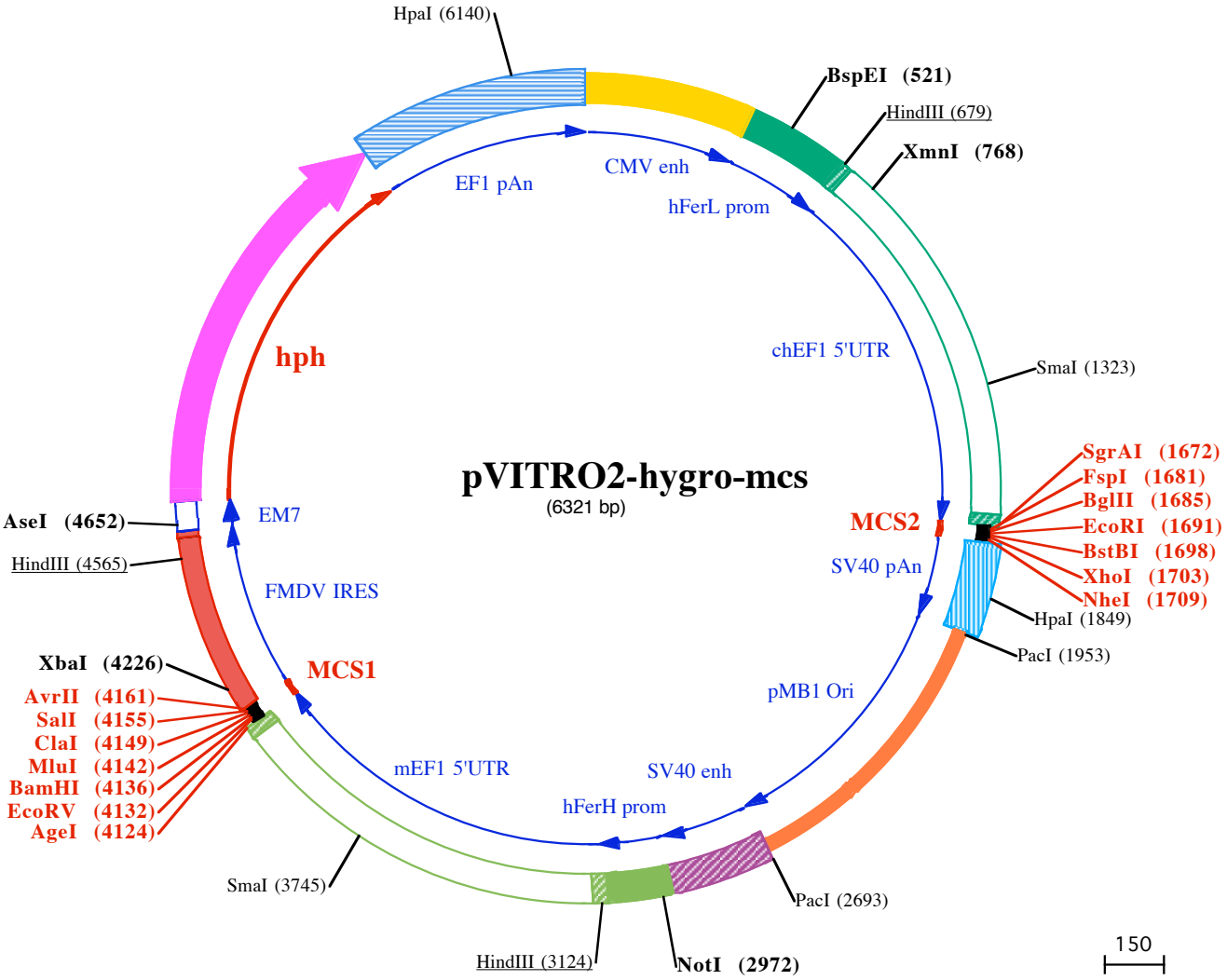
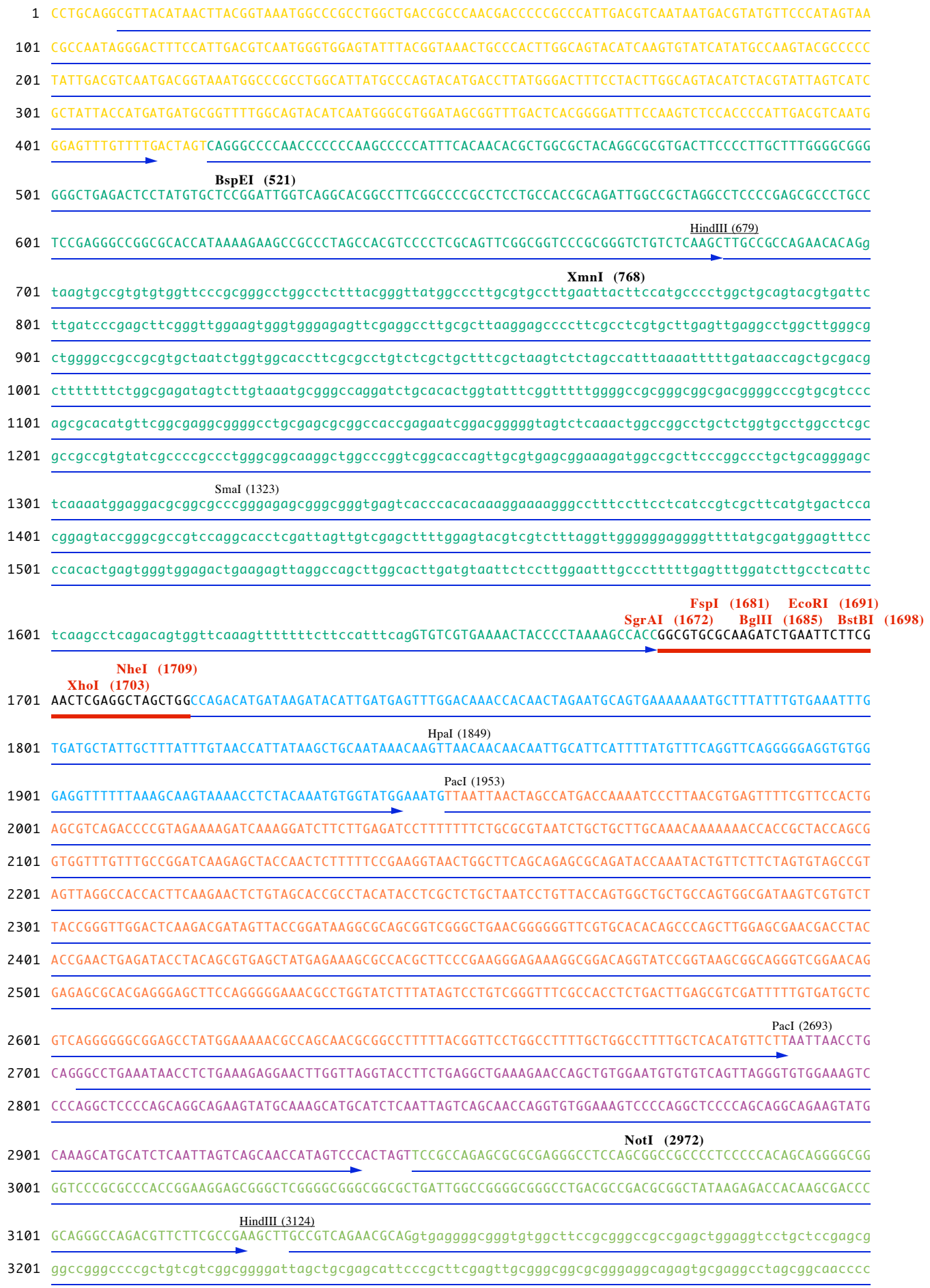

pVITRO2-hygro-mcs
(6321 bp)
HpaI (6140)
BspEI (521)
HindIII (679)
XmnI (768)
CMV enh
hFerL prom
chEF1 5'UTR
SmaI (1323)
SgrAI (1672)
FspI (1681)
BglII (1685)
EcoRI (1691)
BstBI (1698)
XhoI (1703)
NheI (1709)
HpaI (1849)
PacI (1953)
MCS2
SV40 pAn
pMB1 Ori
SV40 enh
hFerH prom
PacI (2693)
NotI (2972)
HindIII (3124)
SmaI (3745)
mEF1 5'UTR
MCS1
XbaI (4226)
AvrII (4161)
SalI (4155)
ClaI (4149)
MluI (4142)
BamHI (4136)
EcoRV (4132)
AgeI (4124)
FMDV IRES
EM7
AseI (4652)
HindIII (4565)
hph
EF1 pAn
150

InvivoGen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101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
401 GGAGTTTGTTTTGACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGG
BspEI (521)
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCC
HindIII (679)
601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGg
XmnI (768)
701 taagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc
801 ttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcg
901 ctggggccgccgcgtgctaatctggtggcaccttcgcgcctgtctcgctgctttcgctaagtctctagccatttaaaatttttgataaccagctgcgacg
1001 ctttttttctggcgagatagtcttgtaaatgcgggccaggatctgcacactggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtccc
1101 agcgcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaaactggccggcctgctctggtgcctggcctcgc
1201 gccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagc
SmaI (1323)
1301 tcaaaatggaggacgcggcgccccgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccttcctcatccgtcgcttcatgtgactcca
1401 cggagtaccgggcgccgtccaggcacctcgattagttgtcgagcttttggagtacgtcgtctttaggttgggggggaggggttttatgcgatggagtttcc
1501 ccacactgagtgggtggagactgaagagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttgcctcattc
FspI (1681) EcoRI (1691)
SgrAI (1672) BglII (1685) BstBI (1698)
1601 tcaagcctcagacagtggttcaaagttttttttcttccatttcagGTGTCGTGAAAACTACCCCTAAAAGCCACCGGCGTGCGCAAGATCTGAATTCTTCG
NheI (1709)
XhoI (1703)
1701 AACTCGAGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTG
HpaI (1849)
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGG
PacI (1953)
1901 GAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTG
2001 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCG
2101 GTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGT
2201 AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT
2301 TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC
2401 ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAG
2501 GAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTC
PacI (2693)
2601 GTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAACCTG
2701 CAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTC
2801 CCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATG
NotI (2972)
2901 CAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGGGCGG
3001 GGTCCCGCGCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGCTGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCC
HindIII (3124)
3101 GCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAGgtgaggggcgggtgtggcttccgcgggccgccgagctggaggtcctgctccgagcg
3201 ggccgggccccgctgtcgtcggcggggattagctgcgagcattcccgcttcgagttgcgggcggcgcgggaggcagagtgcgaggcctagcggcaacccc


SmaI (3745)
EcoRV (4132) MluI (4142) SalI (4155)
AgeI (4124) BamHI (4136) ClaI (4149) AvrII (4161)
XbaI (4226)
HindIII (4565)
AseI (4652)
HpaI (6140)