pUNO2-mcs

A plasmid containing a multiple cloning site and the Zeocin™ resistance gene

Catalog code: puno2-mcs

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

For research use only

Version 21EL19-MM

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 1 ml of Zeocin™ at 100 mg/ml

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable at least for 1 year.
- Store Zeocin™ 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 full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

pUNO2-mcs is a ready-made expression vector containing the Zeocin™ resistance gene, the hybrid EF1α/HTLV promoter and a multiple cloning site.

pUNO2-mcs may be used for:

Cloning in a gene of interest. Six unique restriction sites comprise the MCS facilitating cloning of genes. Cloned genes will be under the control of the EF1α/HTLV promoter.

As an "empty" control vector. pUNO2-mcs plamids were designed to serve as experimental control vectors for the pUNO2 plasmid family that feature a wide choice of native and fusion genes.

METHODS

Plasmid resuspension

Briefly 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.

Zeocin™ usage

Zeocin™ should be used at 25 µg/ml in bacteria and 50-400 µg/ml in mammalian cells. Zeocin™ is supplied as 100 mg/ml solution in HEPES buffer.

PLASMID FEATURES

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[1] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[2] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• MCS: The multiple cloning site contains the following unique restriction sites:

5' - SalI, BamHI, Eco47III, NcoI, XcmI, NheI - 3'

Each restriction site is compatible with many other enzymes, increasing the cloning options.

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[3].

• **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **CMV promoter & enhancer** drives the expression of the Zeocin™ resistance in mammalian cells.

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

• Sh ble (Zeocin™ resistance gene): Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, Zeocin™ can be used to select stable mammalian cells transfectants and *E. coli* transformants.

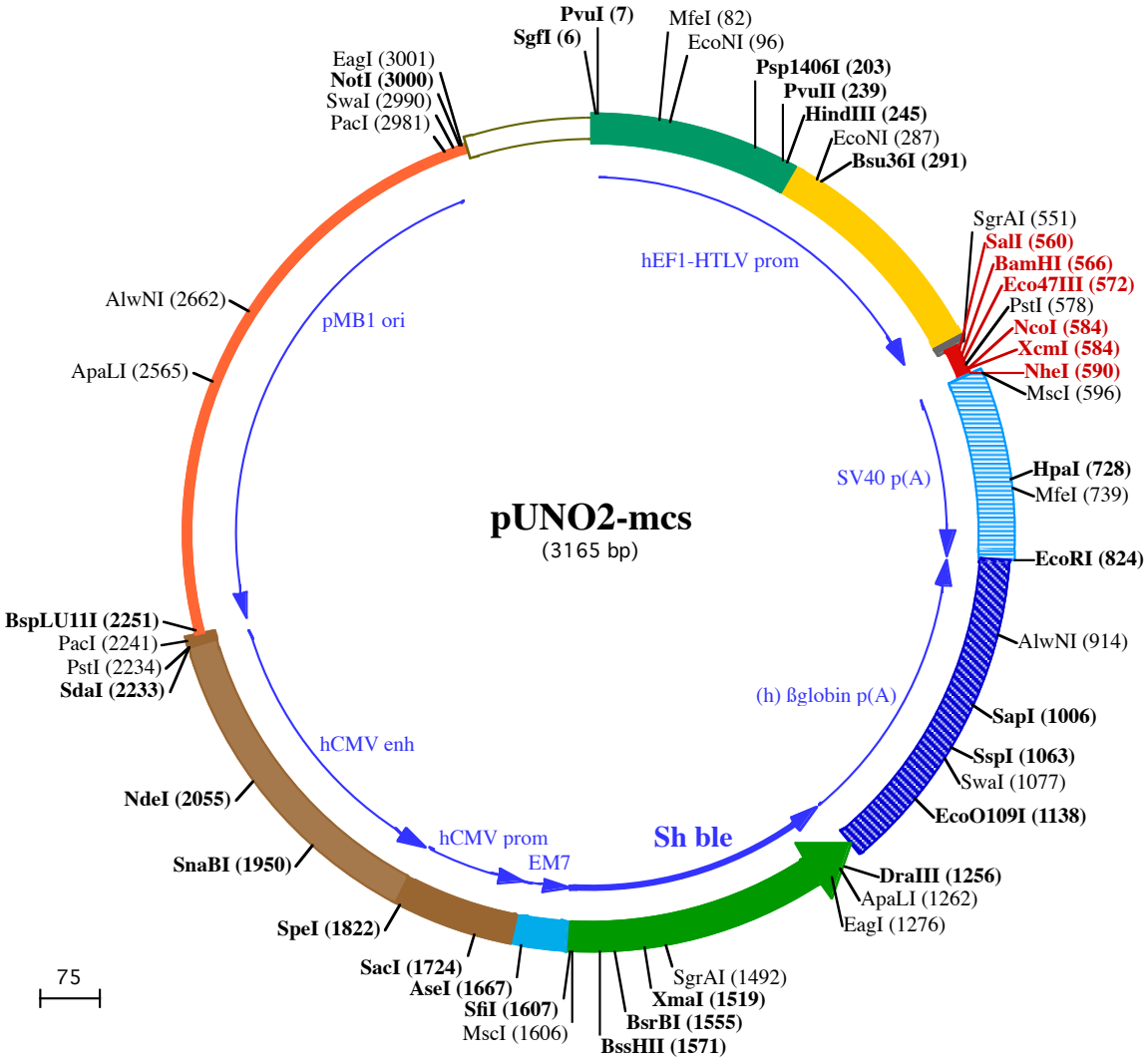
• **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of *Sh ble*. The use of beta-globin pAn minimizes interference[4] and possible recombination events with the SV40 polyadenylation signal.

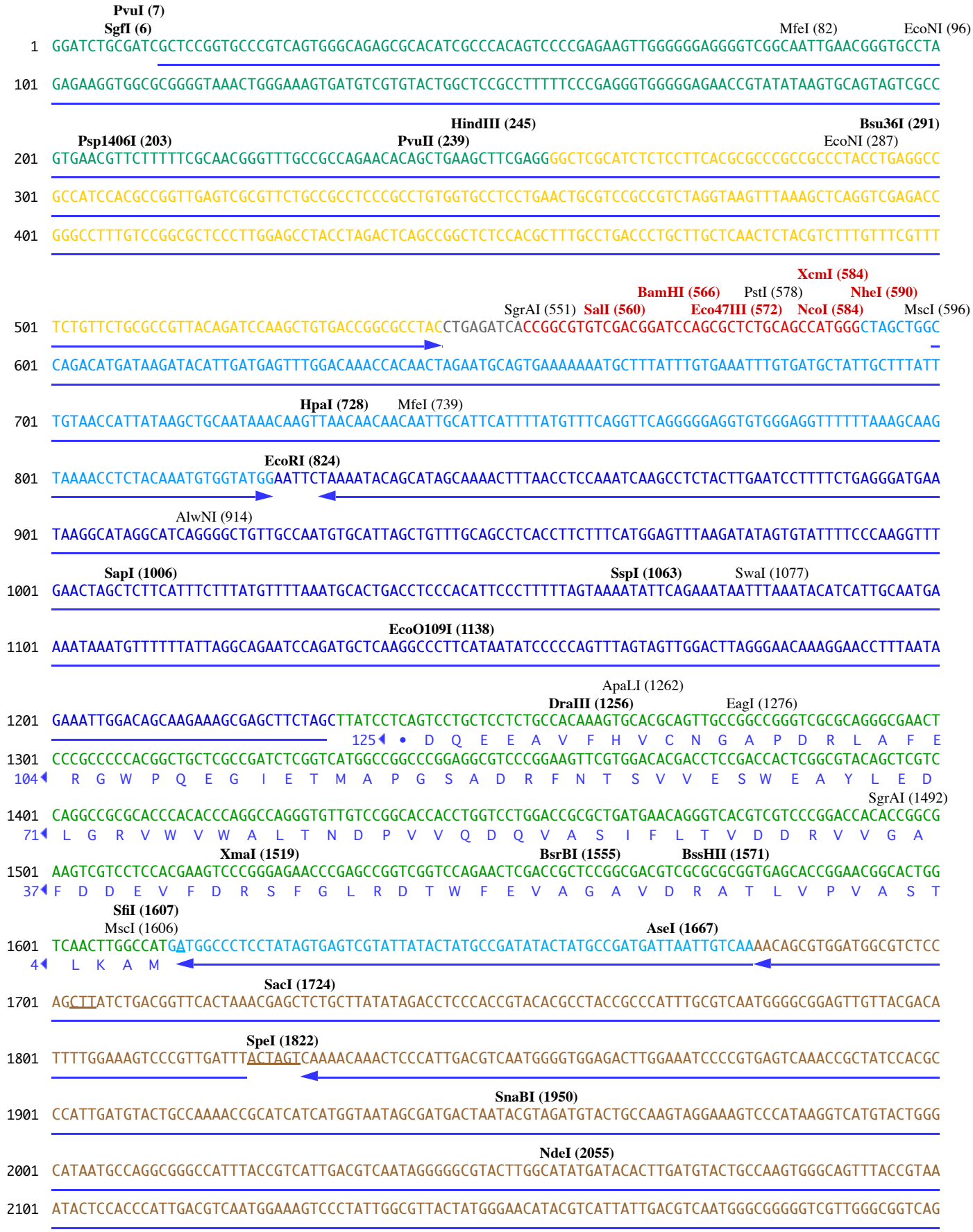
1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. **2. Takebe Y. *et al.*, 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **3. 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. **4. Yu J. & Russell JE., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin™	Selection antibiotic	ant-zn-1
ChemiComp GT116	Competent *E. coli*	gt116-11

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 Asia: +852 3622-3480
E-mail: info@invivogen.com


pUNO2-mcs
(3165 bp)
PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
Psp1406I (203)
PvuII (239)
HindIII (245)
EcoNI (287)
Bsu36I (291)
EagI (3001)
NotI (3000)
SwaI (2990)
PacI (2981)
AlwNI (2662)
ApaLI (2565)
BspLU11I (2251)
PacI (2241)
PstI (2234)
SdaI (2233)
NdeI (2055)
SnaBI (1950)
SpeI (1822)
SacI (1724)
AseI (1667)
SfiI (1607)
MscI (1606)
BssHII (1571)
BsrBI (1555)
XmaI (1519)
SgrAI (1492)
EagI (1276)
ApaLI (1262)
DraIII (1256)
EcoO109I (1138)
SwaI (1077)
SspI (1063)
SapI (1006)
AlwNI (914)
EcoRI (824)
MfeI (739)
HpaI (728)
MscI (596)
NheI (590)
XcmI (584)
NcoI (584)
BamHI (566)
SalI (560)
Eco47III (572)
PstI (578)
SgrAI (551)
hEF1-HTLV prom
SV40 p(A)
(h) ßglobin p(A)
Sh ble
EM7
hCMV prom
hCMV enh
pMB1 ori
75


PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Bsu36I (291)
Psp1406I (203)
PvuII (239)
EcoNI (287)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
XcmI (584)
BamHI (566)
PstI (578)
NheI (590)
SgrAI (551)
SalI (560)
Eco47III (572)
NcoI (584)
MscI (596)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGTGTCGACGGATCCAGCGCTCTGCAGCCATGGGCTAGCTGGC
601 CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT
HpaI (728)
MfeI (739)
701 TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAG
EcoRI (824)
801 TAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA
AlwNI (914)
901 TAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTT
SapI (1006)
SspI (1063)
SwaI (1077)
1001 GAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGA
EcoO109I (1138)
1101 AAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATA
ApaLI (1262)
DraIII (1256)
EagI (1276)
1201 GAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACT
125 • D Q E E A V F H V C N G A P D R L A F E
1301 CCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTC
104 R G W P Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D
SgrAI (1492)
1401 CAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCG
71 L G R V W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A
XmaI (1519)
BsrBI (1555)
BssHII (1571)
1501 AAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGG
37 F D D E V F D R S F G L R D T W F E V A G A V D R A T L V P V A S T
SfiI (1607)
MscI (1606)
AseI (1667)
1601 TCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCC
4 L K A M
SacI (1724)
1701 AGCTCTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACA
SpeI (1822)
1801 TTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGC
SnaBI (1950)
1901 CCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGG
NdeI (2055)
2001 CATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAA
2101 ATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAG

PacI (2241)
PstI (2234)
SdaI (2233) **BspLU11I (2251)**

2201 CCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGC

2301 GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC

2401 CAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC

ApaLI (2565)

2501 TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGC

AlwNI (2662)

2601 CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA

2701 GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA

2801 AAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCA

PacI (2981) SwaI (2990)

2901 AGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA

EagI (3001)
NotI (3000)

3001 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA

3101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA