pUNO3-mcs

A plasmid containing a multiple cloning site and the hygromycin resistance gene

Catalog # puno3-mcs

For research use only

Version # 15J27v40-JC

PRODUCT INFORMATION

Contents:

- 20 µg of pUNO3-mcs provided as lyophilized plasmid DNA
- 4 pouches of *E. coli* Fast-Media® Hygro (2 TB and 2 Agar)

Storage and Stability:

- Product is shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Resuspended DNA is stable for up to 1 year when stored at -20 °C .
- Store *E. coli* Fast-Media® at room temperature in a dry and cool place. Fast-Media® pouches are stable for 2 years when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

pUNO3-mcs is a ready-made expression vector containing the hygromycin-resistance gene (*hph*) resistance gene, the hybrid EF1α/HTLV promoter and a multiple cloning site.

pUNO3-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. pUNO3-mcs plamids were designed to serve as experimental control vectors for the pUNO3 plasmid family.

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.

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

E. coli Fast-Media® Hygro is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave.

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 400 Watts), 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 overboiling 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.*

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 restriction sites:
5' - SgrAI, SalI, BamHI, Eco47III, NcoI, 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 hygromycin resistance in mammalian cells.

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

• Hygro (hygromycin B resistance gene): Resistance to hygromycin B is conferred by the *hph* gene from *E. coli* which encodes a phosphotransferase. The *hph* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, hygromycin B 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 *hph*. 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	Catalog Code
Hygromycin B Gold (solution)	ant-hg-1
ChemiComp GT116	gt116-11
Fast-Media™ Hygro Agar	fas-hg-s
Fast-Media™ Hygro TB	fas-hg-l

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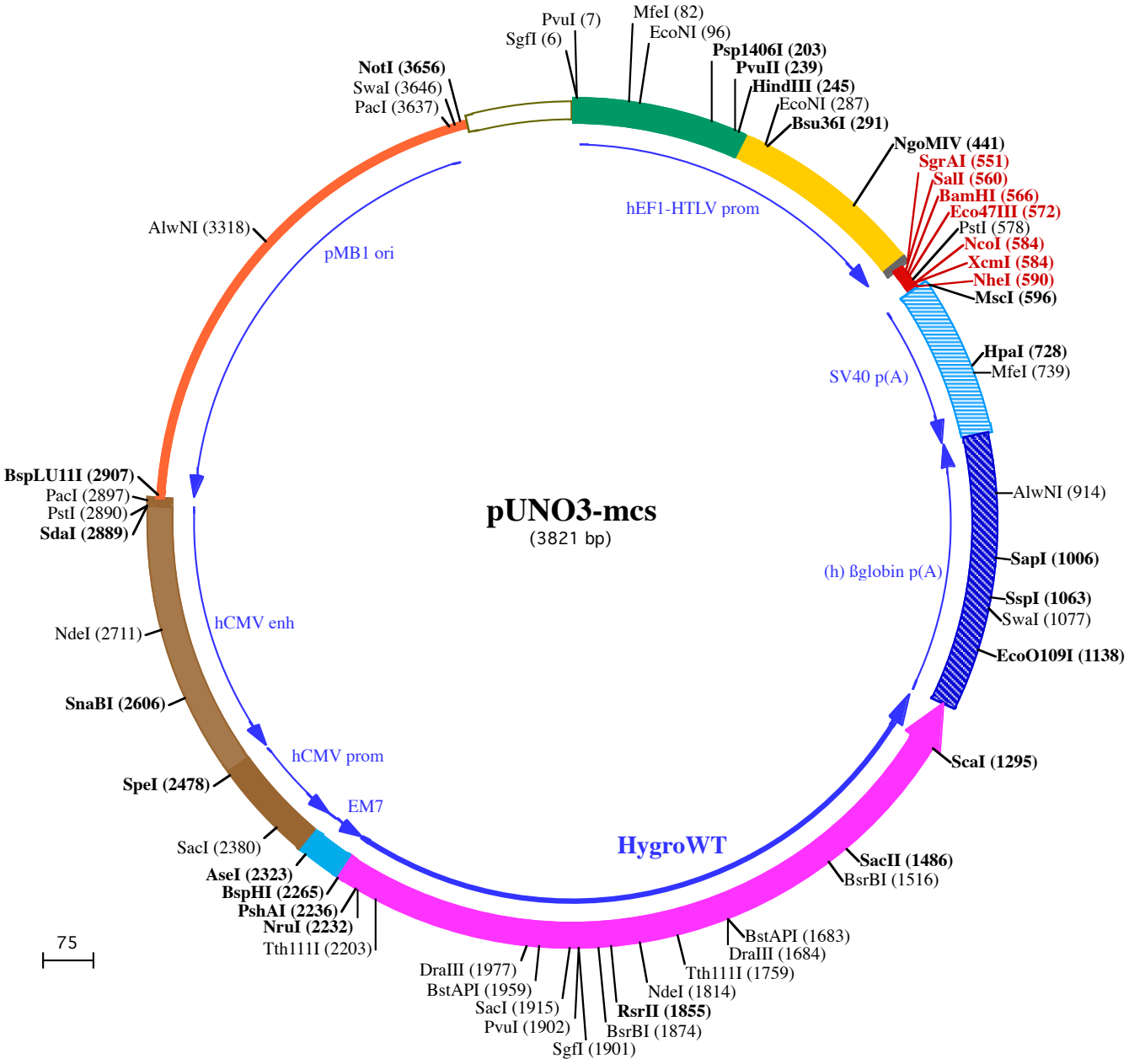
pUNO3-mcs
(3821 bp)
PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
Psp1406I (203)
PvuII (239)
HindIII (245)
EcoNI (287)
Bsu36I (291)
NotI (3656)
SwaI (3646)
PacI (3637)
NgoMIV (441)
SgrAI (551)
SalI (560)
BamHI (566)
Eco47III (572)
PstI (578)
NcoI (584)
XcmI (584)
NheI (590)
MscI (596)
AlwNI (3318)
pMB1 ori
hEF1-HTLV prom
HpaI (728)
MfeI (739)
SV40 p(A)
AlwNI (914)
SapI (1006)
SspI (1063)
SwaI (1077)
EcoO109I (1138)
(h) ßglobin p(A)
ScaI (1295)
SacII (1486)
BsrBI (1516)
BstAPI (1683)
DraIII (1684)
Tth111I (1759)
NdeI (1814)
RsrII (1855)
BsrBI (1874)
SgfI (1901)
PvuI (1902)
SacI (1915)
BstAPI (1959)
DraIII (1977)
Tth111I (2203)
NruI (2232)
PshAI (2236)
BspHI (2265)
AseI (2323)
SacI (2380)
EM7
hCMV prom
SpeI (2478)
SnaBI (2606)
NdeI (2711)
hCMV enh
BspLU11I (2907)
PacI (2897)
PstI (2890)
SdaI (2889)
HygroWT
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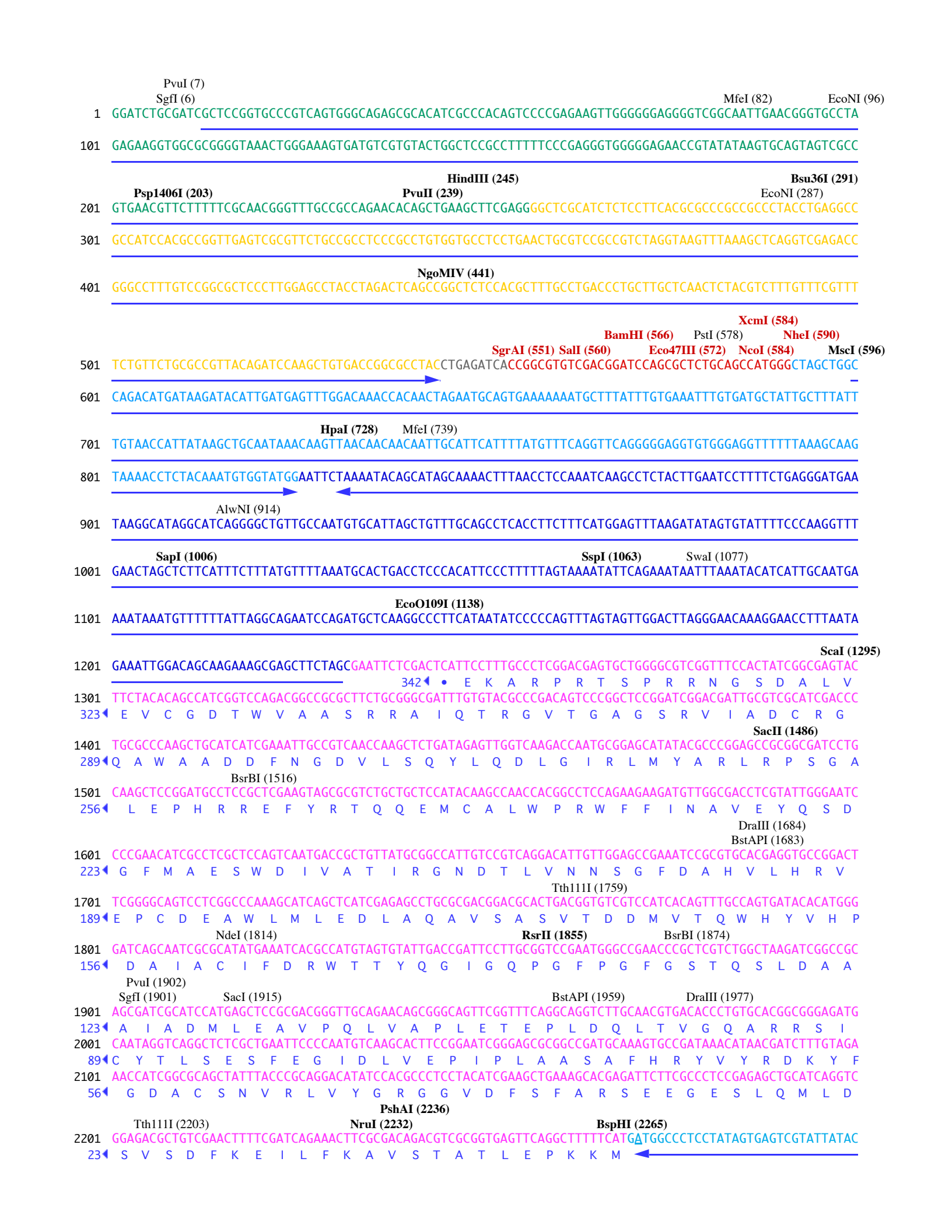
PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
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101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Bsu36I (291)
Psp1406I (203)
PvuII (239)
EcoNI (287)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
NgoMIV (441)
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
801 TAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA
AlwNI (914)
901 TAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTT
SapI (1006)
SspI (1063)
SwaI (1077)
1001 GAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGA
EcoO109I (1138)
1101 AAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATA
ScaI (1295)
1201 GAAATTGGACAGCAAGAAAGCGAGCTTCTAGCGAATTCTCGACTCATTCCTTTGCCCTCGGACGAGTGCTGGGGCGTCGGTTTCCACTATCGGCGAGTAC
342 • E K A R P R T S P R R N G S D A L V
1301 TTCTACACAGCCATCGGTCCAGACGGCCGCGCTTCTGCGGGCGATTTGTGTACGCCCGACAGTCCCGGCTCCGGATCGGACGATTGCGTCGCATCGACCC
323 E V C G D T W V A A S R R A I Q T R G V T G A G S R V I A D C R G
SacII (1486)
1401 TGCGCCCAAGCTGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATACGCCCGGAGCCGCGGCGATCCTG
289 Q A W A A D D F N G D V L S Q Y L Q D L G I R L M Y A R L R P S G A
BsrBI (1516)
1501 CAAGCTCCGGATGCCTCCGCTCGAAGTAGCGCGTCTGCTGCTCCATACAAGCCAACCACGGCCTCCAGAAGAAGATGTTGGCGACCTCGTATTGGGAATC
256 L E P H R R E F Y R T Q Q E M C A L W P R W F F I N A V E Y Q S D
DraIII (1684)
BstAPI (1683)
1601 CCCGAACATCGCCTCGCTCCAGTCAATGACCGCTGTTATGCGGCCATTGTCCGTCAGGACATTGTTGGAGCCGAAATCCGCGTGCACGAGGTGCCGGACT
223 G F M A E S W D I V A T I R G N D T L V N N S G F D A H V L H R V
Tth111I (1759)
1701 TCGGGGCAGTCCTCGGCCCAAAGCATCAGCTCATCGAGAGCCTGCGCGACGGACGCACTGACGGTGTCGTCCATCACAGTTTGCCAGTGATACACATGGG
189 E P C D E A W L M L E D L A Q A V S A S V T D D M V T Q W H Y V H P
NdeI (1814)
RsrII (1855)
BsrBI (1874)
1801 GATCAGCAATCGCGCATATGAAATCACGCCATGTAGTGTATTGACCGATTCCTTGCGGTCCGAATGGGCCGAACCCGCTCGTCTGGCTAAGATCGGCCGC
156 D A I A C I F D R W T T Y Q G I G Q P G F P G F G S T Q S L D A A
PvuI (1902)
SgfI (1901)
SacI (1915)
BstAPI (1959)
DraIII (1977)
1901 AGCGATCGCATCCATGAGCTCCGCGACGGGTTGCAGAACAGCGGGCAGTTCGGTTTCAGGCAGGTCTTGCAACGTGACACCCTGTGCACGGCGGGAGATG
123 A I A D M L E A V P Q L V A P L E T E P L D Q L T V G Q A R R S I
2001 CAATAGGTCAGGCTCTCGCTGAATTCCCCAATGTCAAGCACTTCCGGAATCGGGAGCGCGGCCGATGCAAAGTGCCGATAAACATAACGATCTTTGTAGA
89 C Y T L S E S F E G I D L V E P I P L A A S A F H R Y V Y R D K Y F
2101 AACCATCGGCGCAGCTATTTACCCGCAGGACATATCCACGCCCTCCTACATCGAAGCTGAAAGCACGAGATTCTTCGCCCTCCGAGAGCTGCATCAGGTC
56 G D A C S N V R L V Y G R G G V D F S F A R S E E G E S L Q M L D
PshAI (2236)
Tth111I (2203)
NruI (2232)
BspHI (2265)
2201 GGAGACGCTGTCGAACTTTTCGATCAGAAACTTCGCGACAGACGTCGCGGTGAGTTCAGGCTTTTTCATGATGGCCCTCCTATAGTGAGTCGTATTATAC
23 S V S D F K E I L F K A V S T A T L E P K K M


AseI (2323)
SacI (2380)
SpeI (2478)
SnaBI (2606)
NdeI (2711)
PacI (2897)
PstI (2890)
SdaI (2889)
BspLU11I (2907)
AlwNI (3318)
PacI (3637)
SwaI (3646)
NotI (3656)
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