pUNO1ss-mcs

A plasmid containing a multiple cloning site and the hIL2 signal sequence

Catalog code: puno1ss-mcs

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

For research use only

Version 19K09-MM

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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

pUNO1ss-mcs is a ready-made expression vector containing the blasticidin resistance gene, the human IL2 signal sequence, the hybrid EF1α/HTLV promoter and a multiple cloning site.

pUNO1ss-mcs may be used for **cloning a gene of interest that lacks the secretion signal**.

pUNO1ss-mcs contains the human IL2 signal sequence and a multiple cloning site (MCS) comprising six unique restriction sites facilitating cloning of genes. Cloned genes will be under the control of the EF1α/HTLV promoter.

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.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied as a 10 mg/ml colorless 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.
- **IL2 ss:** The human IL2 signal sequence shares common characteristics with signal peptides of other secretory proteins.
- **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 blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin 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 *bsr*. 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
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11

TECHNICAL SUPPORT
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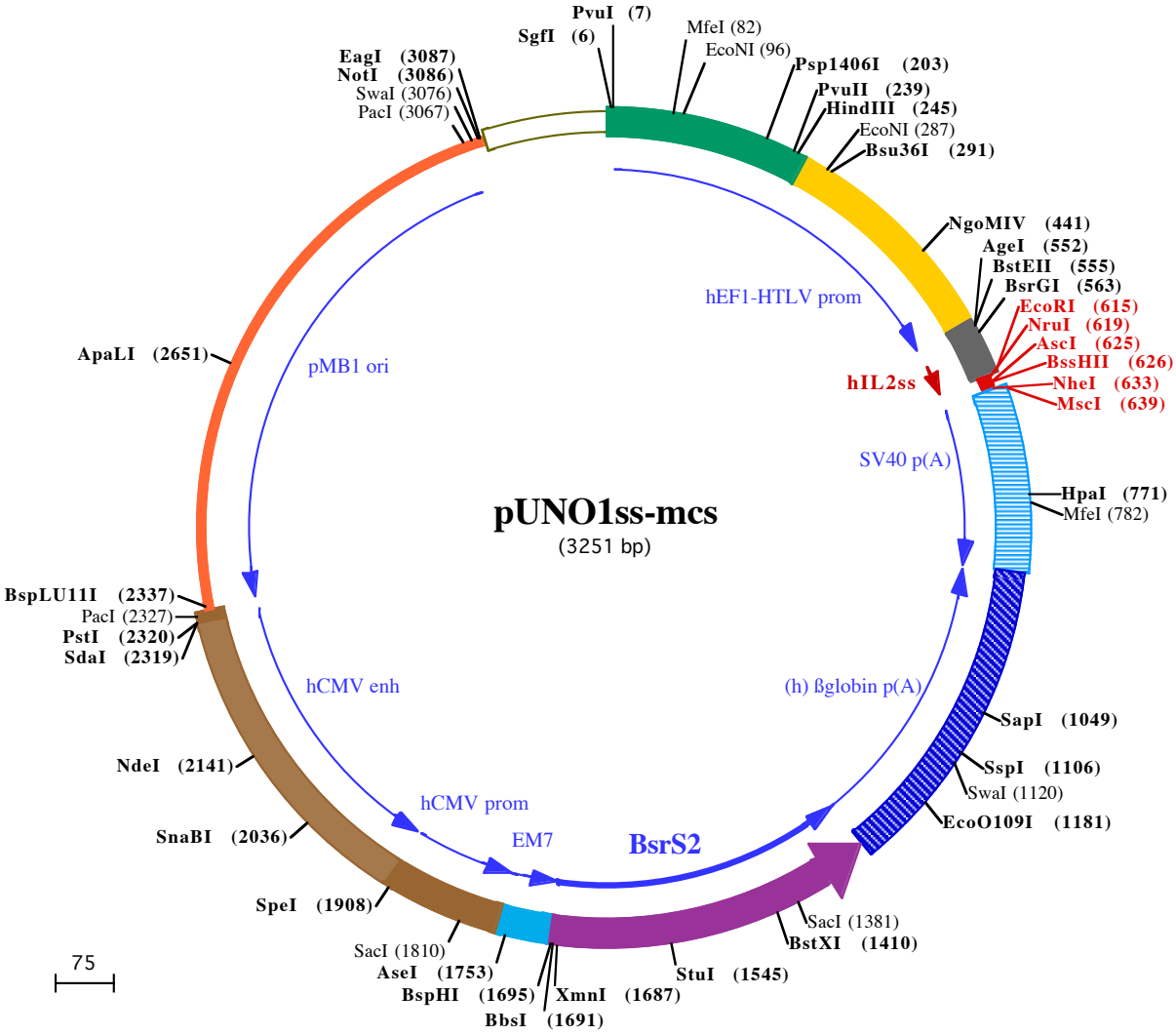
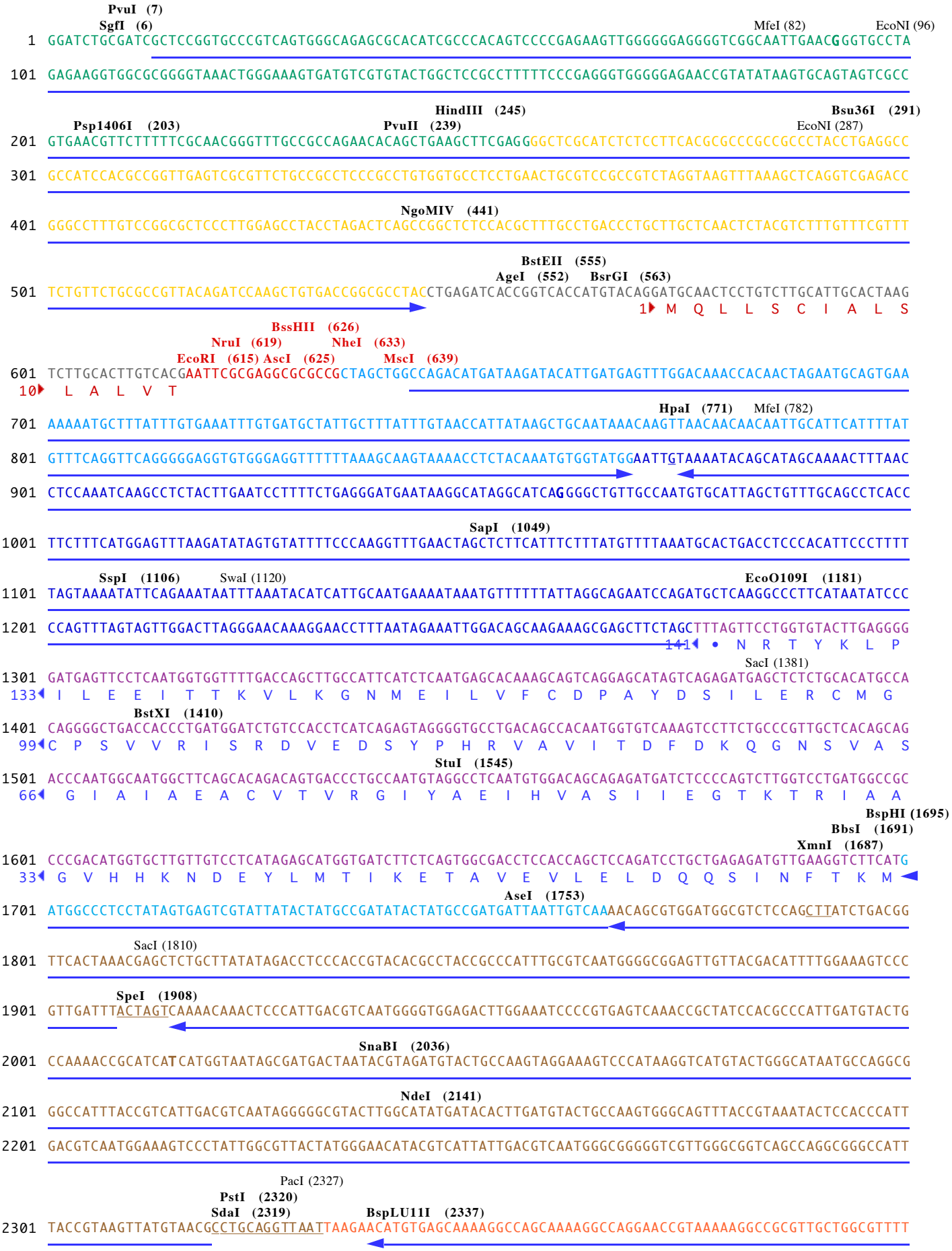

pUNO1ss-mcs
(3251 bp)
PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
Psp1406I (203)
PvuII (239)
HindIII (245)
EcoNI (287)
Bsu36I (291)
EagI (3087)
NotI (3086)
SwaI (3076)
PacI (3067)
NgoMIV (441)
AgeI (552)
BstEII (555)
BsrGI (563)
EcoRI (615)
NruI (619)
AscI (625)
BssHII (626)
NheI (633)
MscI (639)
hEF1-HTLV prom
hIL2ss
SV40 p(A)
HpaI (771)
MfeI (782)
SapI (1049)
SspI (1106)
SwaI (1120)
EcoO109I (1181)
(h) ßglobin p(A)
BsrS2
SacI (1381)
BstXI (1410)
StuI (1545)
XmnI (1687)
BspHI (1695)
BbsI (1691)
AseI (1753)
SacI (1810)
SpeI (1908)
SnaBI (2036)
NdeI (2141)
hCMV prom
EM7
hCMV enh
BspLU11I (2337)
PacI (2327)
PstI (2320)
SdaI (2319)
ApaLI (2651)
pMB1 ori
75


PvuI (7)
SgfI (6)
MfeI (82)
EcoNI (96)
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101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Bsu36I (291)
Psp1406I (203)
PvuII (239)
EcoNI (287)
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301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
NgoMIV (441)
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
BstEII (555)
AgeI (552)
BsrGI (563)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAG
1 M Q L L S C I A L S
BssHII (626)
NruI (619)
NheI (633)
EcoRI (615)
AscI (625)
MscI (639)
601 TCTTGCACTTGTCACGAATTCGCGAGGCGCGCCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAA
10 L A L V T
HpaI (771)
MfeI (782)
701 AAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTAT
801 GTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTGTAAAATACAGCATAGCAAAACTTTAAC
901 CTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACC
SapI (1049)
1001 TTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTT
SspI (1106)
SwaI (1120)
EcoO109I (1181)
1101 TAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCC
1201 CCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGG
141 • N R T Y K L P
SacI (1381)
1301 GATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCA
133 I L E E I T T K V L K G N M E I L V F C D P A Y D S I L E R C M G
BstXI (1410)
1401 CAGGGGCTGACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAG
99 C P S V V R I S R D V E D S Y P H R V A V I T D F D K Q G N S V A S
StuI (1545)
1501 ACCCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCCTGATGGCCGC
66 G I A I A E A C V T V R G I Y A E I H V A S I I E G T K T R I A A
BspHI (1695)
BbsI (1691)
XmnI (1687)
1601 CCCGACATGGTGCTTGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATG
33 G V H H K N D E Y L M T I K E T A V E V L E L D Q Q S I N F T K M
AseI (1753)
1701 ATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGG
SacI (1810)
1801 TTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCC
SpeI (1908)
1901 GTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTG
SnaBI (2036)
2001 CCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCG
NdeI (2141)
2101 GGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATT
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PacI (2327)
PstI (2320)
SdaI (2319)
BspLU11I (2337)
2301 TACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTT

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2401 TCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCC

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                                                    ApaLI   (2651)
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2801 GTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC

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                                                                                    EagI   (3087)
                                                                PacI (3067)  SwaI (3076)  NotI   (3086)
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