pORF9-HSV1tk

An expression vector containing the HSV1tk open reading frame

Catalog # porf-hsv1tk

For research use only

Version # 10L16-JC

PRODUCT INFORMATION

Contents:

- 1 disk of lyophilized *E. coli* bacteria, strain GT116 transformed by pORF9-HSV1tk
- Strain genotype is: *F-, mcrA , Δ(mrr-hsdRMS-mcrBC) , Ø80lacZΔM15 , ΔlacX74, recA1 , endA1, Δdcm, ΔsbcC-sbcD .*
- 4 pouches of *E. coli* Fast-Media® Amp.

Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Amp at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and ORF sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pORF is a ready-made expression vector containing a gene of interest.

pORF may be used for:

Obtaining a gene to subclone into another vector. Two unique restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Gene expression in mammalian cells. Cells may be transiently transfected with pORF. The secreted protein may be harvested in the cell culture supernatant as all secreted proteins in pORF possess a signal sequence.

HSV1tk gene may be cut out by using NcoI and NheI enzymes.
Age I is compatible with Xma I, BspE I, NgoM IV and SgrA I.
SgrA I is compatible with Xma I, BspE I, NgoM IV, and Age I.
Nco I is compatible with BspH I and BspLU11 I.
BspH I is compatible with Nco I and BspLU11 I.
Nhe I is compatible with Xba I, Spe I, and Avr II.

PLASMID FEATURES

• **EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1a (EF-1α) promoter[1] and 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. The promoter is stronger than CMV and 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.

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **Amp (ampicillin resistance gene):** The ampicillin resistance gene allows the selection of bacteria carrying the pORF plasmid.

• **HSV1tk gene:**
Intronless ORF from the ATG to the stop codon.
ORF Size (bp): 1131
Cloning fragment size (bp): 1139

• **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.[3]

References

1- Kim et al (1990). Gene 2: 217-223.
2- Takebe et al (1988). Mol. Cell Biol. 1: 466-472.
3- Carswell et al (1989). Mol. Cell Biol. 10: 4248-4258.

METHODS

Growth of pORF-transformed bacteria:

Use sterile conditions to do the following:

1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
2- Streak bacteria taken from this suspension on an ampicillin LB agar plate prepared with the *E. coli* Fast-Media® Amp agar provided (see below).
3- Place the plate in an incubator at 37°C overnight.
4- Isolate a single colony and grow the bacteria in TB supplemented with ampicillin using the Fast-Media® Amp liquid provided (see below).
5- Extract the pORF plasmid DNA using the method of your choice.

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

E. coli Fast-Media® Amp is a new, fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Amp is a TB (liquid) or LB (solid) based medium with ampicillin, and contains stabilizers.
E. coli Fast-Media® Amp can be ordered separately (catalog code # fas-am-l, fas-am-s, fas-am-x).

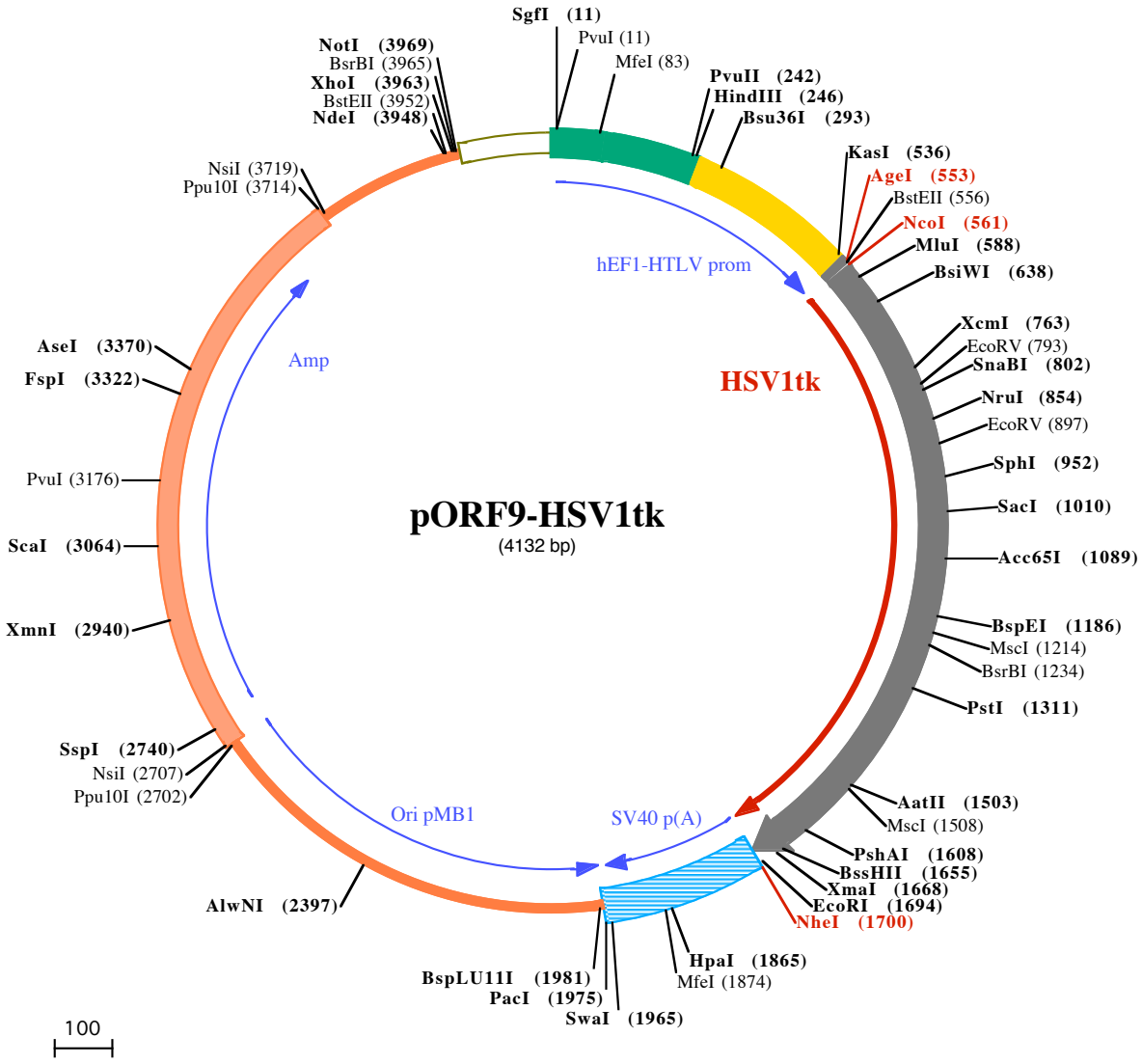
Method:

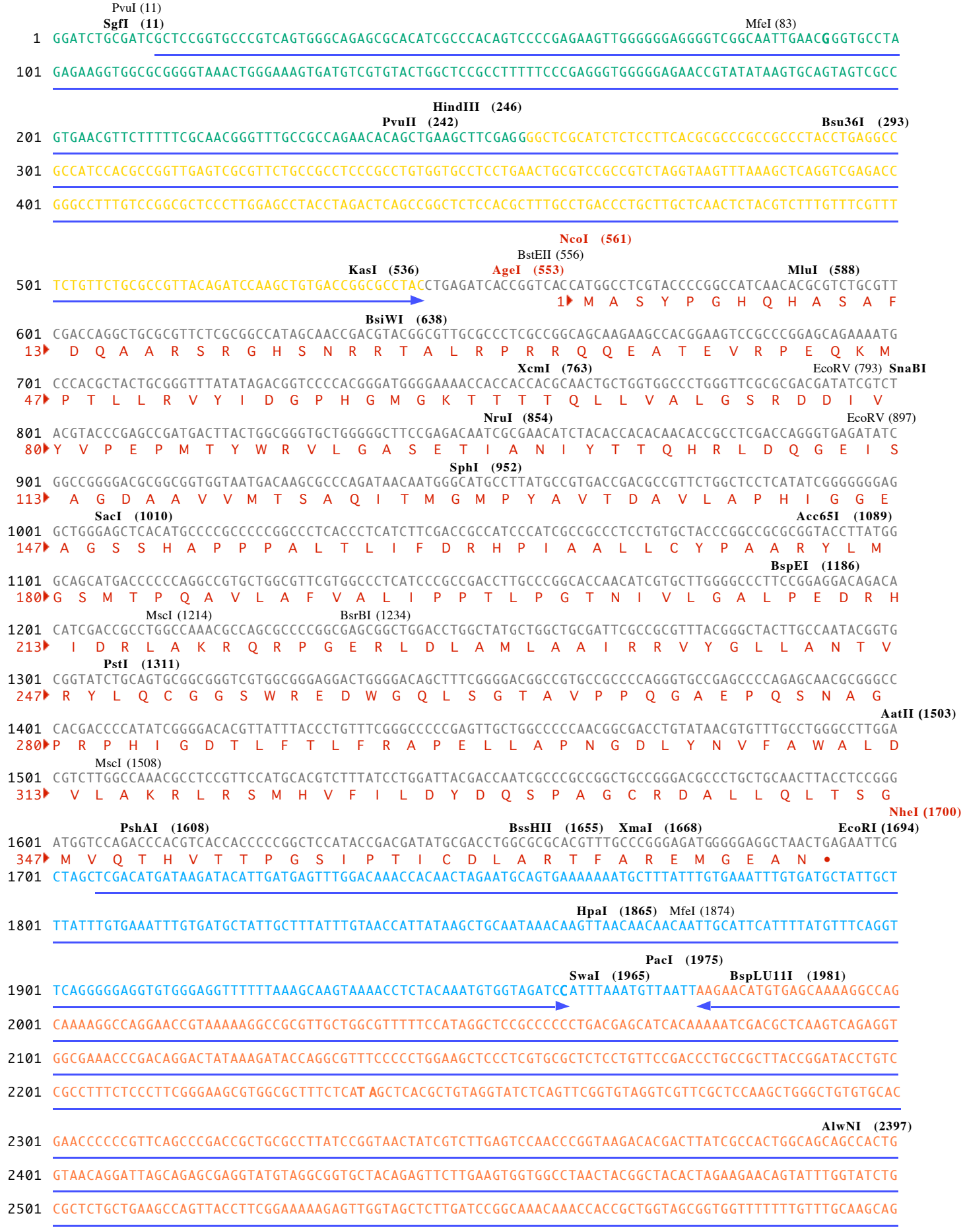
1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
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.*

TECHNICAL SUPPORT
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com

InvivoGen


pORF9-HSV1tk
(4132 bp)
HSV1tk
hEF1-HTLV prom
Amp
Ori pMB1
SV40 p(A)
SgfI (11)
PvuI (11)
MfeI (83)
PvuII (242)
HindIII (246)
Bsu36I (293)
KasI (536)
AgeI (553)
BstEII (556)
NcoI (561)
MluI (588)
BsiWI (638)
XcmI (763)
EcoRV (793)
SnaBI (802)
NruI (854)
EcoRV (897)
SphI (952)
SacI (1010)
Acc65I (1089)
BspEI (1186)
MscI (1214)
BsrBI (1234)
PstI (1311)
AatII (1503)
MscI (1508)
PshAI (1608)
BssHII (1655)
XmaI (1668)
EcoRI (1694)
NheI (1700)
HpaI (1865)
MfeI (1874)
SwaI (1965)
PacI (1975)
BspLU11I (1981)
AlwNI (2397)
SspI (2740)
NsiI (2707)
Ppu10I (2702)
XmnI (2940)
ScaI (3064)
PvuI (3176)
FspI (3322)
AseI (3370)
NsiI (3719)
Ppu10I (3714)
NotI (3969)
BsrBI (3965)
XhoI (3963)
BstEII (3952)
NdeI (3948)
100


PvuI (11)
SgfI (11)
MfeI (83)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (246)
PvuII (242)
Bsu36I (293)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
NcoI (561)
BstEII (556)
KasI (536)
AgeI (553)
MluI (588)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGGCCTCGTACCCCGGCCATCAACACGCGTCTGCGTT
1 M A S Y P G H Q H A S A F
BsiWI (638)
601 CGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGACGTACGGCGTTGCGCCCTCGCCGGCAGCAAGAAGCCACGGAAGTCCGCCCGGAGCAGAAAATG
13 D Q A A R S R G H S N R R T A L R P R R Q Q E A T E V R P E Q K M
XcmI (763)
EcoRV (793)
SnaBI
701 CCCACGCTACTGCGGGTTTATATAGACGGTCCCCACGGGATGGGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGCGACGATATCGTCT
47 P T L L R V Y I D G P H G M G K T T T T Q L L V A L G S R D D I V
NruI (854)
EcoRV (897)
801 ACGTACCCGAGCCGATGACTTACTGGCGGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATC
80 Y V P E P M T Y W R V L G A S E T I A N I Y T T Q H R L D Q G E I S
SphI (952)
901 GGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAG
113 A G D A A V V M T S A Q I T M G M P Y A V T D A V L A P H I G G E
SacI (1010)
Acc65I (1089)
1001 GCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCCTCATCTTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGCGGTACCTTATGG
147 A G S S H A P P P A L T L I F D R H P I A A L L C Y P A A R Y L M
BspEI (1186)
1101 GCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCATCCCGCCGACCTTGCCCGGCACCAACATCGTGCTTGGGGCCCTTCCGGAGGACAGACA
180 G S M T P Q A V L A F V A L I P P T L P G T N I V L G A L P E D R H
MscI (1214)
BsrBI (1234)
1201 CATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAGCGGCTGGACCTGGCTATGCTGGCTGCGATTCGCCGCGTTTACGGGCTACTTGCCAATACGGTG
213 I D R L A K R Q R P G E R L D L A M L A A I R R V Y G L L A N T V
PstI (1311)
1301 CGGTATCTGCAGTGCGGCGGGTCGTGGCGGGAGGACTGGGGACAGCTTTCGGGGACGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCC
247 R Y L Q C G G S W R E D W G Q L S G T A V P P Q G A E P Q S N A G
AatII (1503)
1401 CACGACCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCCCCGAGTTGCTGGCCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCCTTGGA
280 P R P H I G D T L F T L F R A P E L L A P N G D L Y N V F A W A L D
MscI (1508)
1501 CGTCTTGGCCAAACGCCTCCGTTCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCCGGCTGCCGGGACGCCCTGCTGCAACTTACCTCCGGG
313 V L A K R L R S M H V F I L D Y D Q S P A G C R D A L L Q L T S G
NheI (1700)
PshAI (1608)
BssHII (1655)
XmaI (1668)
EcoRI (1694)
1601 ATGGTCCAGACCCACGTCACCACCCCCGGCTCCATACCGACGATATGCGACCTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGCTAACTGAGAATTCG
347 M V Q T H V T T P G S I P T I C D L A R T F A R E M G E A N •
1701 CTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCT
HpaI (1865)
MfeI (1874)
1801 TTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGT
PacI (1975)
SwaI (1965)
BspLU11I (1981)
1901 TCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAAGAACATGTGAGCAAAAGGCCAG
2001 CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGT
2101 GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC
2201 CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCAC
AlwNI (2397)
2301 GAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
2401 GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTG
2501 CGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG


Ppu10I (2702)
NsiI (2707)
SspI (2740)
XmnI (2940)
ScaI (3064)
PvuI (3176)
FspI (3322)
AseI (3370)
Ppu10I (3714)
NsiI (3719)
NotI (3969)
BstEII (3952)
BsrBI (3965)
NdeI (3948)
XhoI (3963)