pDRIVE5SEAP-rNSE-RU5'

A plasmid with a rat neuron-specific enolase (NSE) / RU5' promoter

Catalog # pdrive5s-rnseru5

For research use only

Version # 13A08-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* transformed by a pDRIVE5-SEAP
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1 Δdcm ΔsbcC-sbcD.*
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

Shipping and storage:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20˚C. Bacteria are stable up to one year when properly stored.
- Store *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

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

GENERAL PRODUCT USE

pDRIVE5-SEAP is an expression plasmid containing a native or composite promoter of interest. **pDRIVE5-SEAP** may be used to:

- Subclone a promoter of interest into another vector. Unique restriction sites are present at each end of the promoter allowing convenient excision. The 5' site is *Spe* I. *Spe* I is compatible with *Avr* II, *Nhe* I and *Xba* I. The 3' restriction site is *Nco* I which includes the ATG start codon, and is compatible with *BspH* I and *BspL*U11 I.

- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5-SEAP promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (*Nco* I and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Rat neuron-specific enolase / RU5' promoter

Complete Promoter size: 1743bp

Specificity: Mature neurons

Neuron-specific enolase, an isoenzyme form of enolase, occurs in mature neurons and paraneurons. Transgenic mouse studies have shown that a 1.8 kb fragment of the rat NSE promoter is able to express a heterologous gene exclusively in postmitotic neurons and neuro-endocrine cells in parallel with endogenous NSE[1]. The NSE promoter contains a TATA-like sequence, no CAAT box and sequences for the AP-1 binding motif, AP-2 binding element, SP-1 binding sequence and cAMP response element[2]. The NSE promoter was used to create transgenic mice exhibiting the neuropathological phenotypes of Alzheimer's disease[3].

1. Forss-Petter S. *et al.*, 1990. Transgenic mice expressing beta-galactosidase in mature neurons under neuron-specific enolase promoter control. Neuron 5(2):187-97. **2. Sakimura K. *et al.*, 1995.** Upstream and intron regulatory regions for expression of the rat neuron-specific enolase gene. Brain Res Mol B 28(1):19-28. **3. Hwang DY. *et al.*, 2004.** Aberrant expressions of pathogenic phenotype in Alzheimer's diseased transgenic mice carrying NSE-controlled APPsw. Exp Neurol. 186(1):20-32.

PLASMID FEATURES

• **SEAP gene** encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods

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

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

• **EM2K** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

• ***Zeo*** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE plasmid.

Note: *Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.*

METHODS

Growth of pDRIVE5-SEAP-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 a zeocin LB agar plate prepared with the *E. coli* Fast-Media® Zeo 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 zeocin using the Fast-Media® Zeo liquid provided (see below).

5- Extract the pDRIVE5-SEAP plasmid DNA using the method of your choice.

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

E. coli Fast-Media® Zeo 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® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

E. coli Fast-Media® Zeo can be ordered separately (catalog code # fas-zn-l, fas-zn-s).

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
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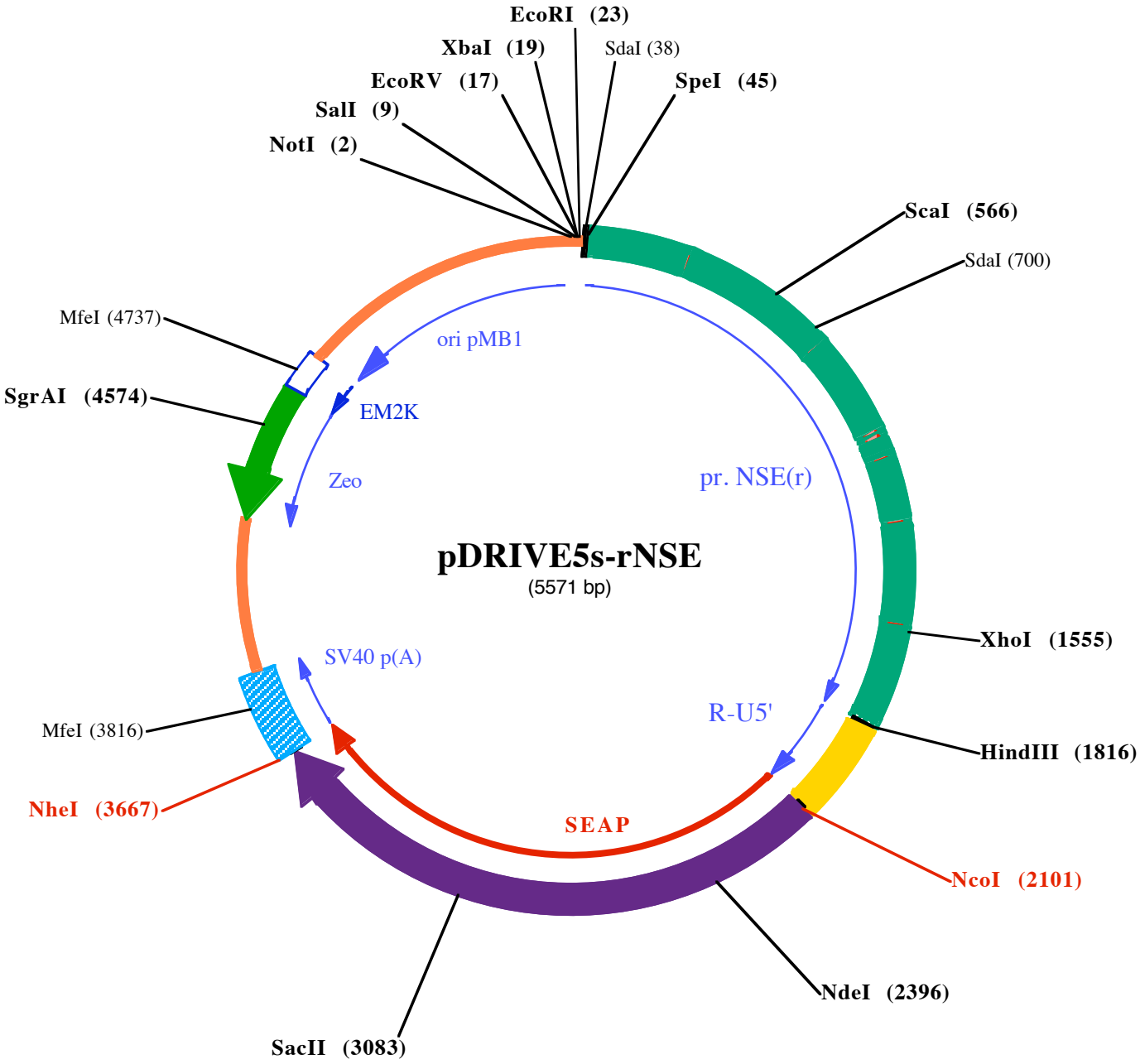
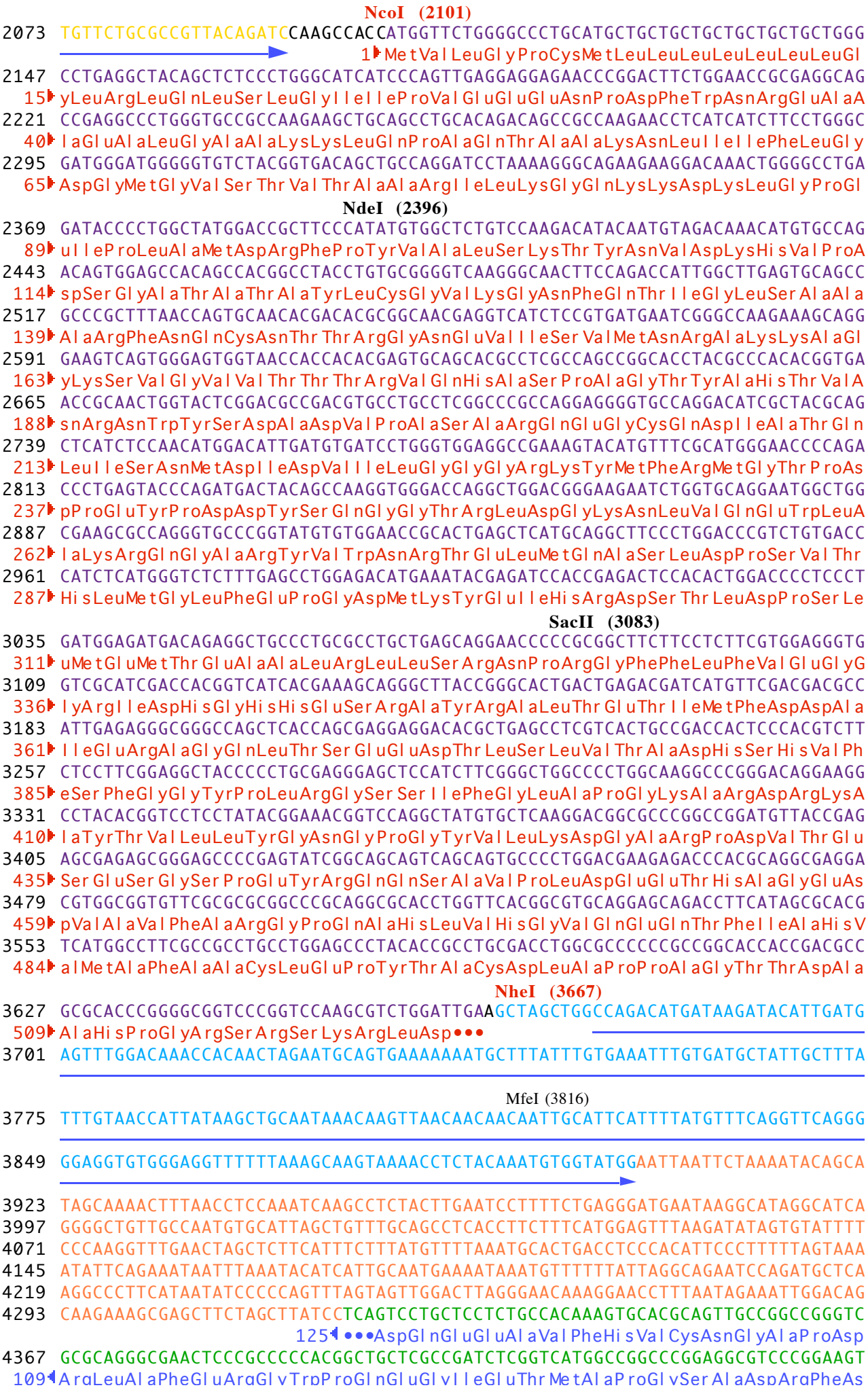

EcoRI (23)
XbaI (19)
SdaI (38)
EcoRV (17)
SpeI (45)
SalI (9)
NotI (2)
ScaI (566)
SdaI (700)
MfeI (4737)
ori pMB1
SgrAI (4574)
EM2K
Zeo
pr. NSE(r)
pDRIVE5s-rNSE
(5571 bp)
XhoI (1555)
SV40 p(A)
R-U5'
MfeI (3816)
HindIII (1816)
NheI (3667)
SEAP
NcoI (2101)
NdeI (2396)
SacII (3083)


EcoRI (23)
EcoRV (17)
NotI (2)
SalI (9)
XbaI (19)
SdaI (38)
SpeI (45)
1 GCGGCCGCGTCGACGATATCTAGAATTCGGATCCTGCAGGGCCCACTAGTAGCTCTGAGCTCCTCCTCTGCTCG
75 CCCAATCCTTCCAACCCCCTATGGTGGTATGGCTGACACAGAAAATGTCTGCTCCTGTATGGGACATTTGCCCC
149 TCTTCTCCAAATATAAGACAGGATGAGGCCTAGCTTTTGCTGCTCCAAAGTTTTAAAAGAACACATTGCACGGC
223 ATTTAGGGACTCTAAAGGGTGGAGGAGGAATGAGGGAATTGCATCATGCCAAGGCTGGTCCTCATCCATCACTG
297 CTTCCAGGGCCCAGAGTGGCTTCCAGGAaGTATTCTTACAAAGGAAGCCCGATCTGTAGCTAACACTCAGAGCC
371 CATTTTCCTGCGTTAACCCCTCCCGACCTCATATACAGGAGTAACATGATCAGTGACCTGGGGGAGCTGGCCAA
445 ACTGCGGGACCTGCCCAAGCTGAGGGCCTTGGTGCTGCTGGACAACCCCTGTGCCGATGAGACTGACTACCGCC
ScaI (566)
519 AGGAGGCCCTGGTGCAGATGGCACACCTAGAGCGCCTAGACAAAGAGTACTATGAGGACGAGGACCGGGCAGAA
593 GCTGAGGAGATCCGACAGAGGCTGAAGGAGGAACAGGAGCAAGAACTCGACCCGGACCAAGACATGGAACCGTA
SdaI (700)
667 CCTCCCGCCAACTTAGTGGCTCCTCTAGCCTGCAGGGACAGTAAAGGTGATGGCAGGAAGGCAGCCCCCGGAGG
741 tCAAAGGCTGGGCACGCGGGAGGAGAGGCCAGAGTCAGAGGCTGCGGGTATCTCAGATATGAAGGAAAGATGAG
815 AGAGGCTCAGGAAGAGGTAAGAAAAGACACAAGAGACCAGAGAAGGGAGAAGAATTAGAGAGGGAGGCAGAGGA
889 CCGCTGTCTCTACAGACATAGCTGGTAGAGACTGGGAGGAAGGGATGAACCCTGAGCGCATGAAGGGAAGGAGG
963 TGGCTGGTGGTATATGGAGGATGTAGCTGGGCCAGGGAAAAGATCCTGCACTaaaaATCTGAAGCTaaaaatAA
1037 CAGGACACGGGGTGGAGAGGCGAAAGGAGGGCAGAGTGAgGCAGAGAGACTGAGagGCCTGGGGATGTGGGCAT
1111 TCCGGTAGGGCACACAGTTCACTTGTCTTCTCTTTTTCCAGGAGGCCAAAGATGCTGACGTCAAGAACTCATAA
1185 TACCCCAGTGGGGACCACCGCATTCATAGCCCTGTTACAAGAAGTGGGAGATGTTCCTTTTTGTCCCAGACTGG
1259 AAATCCgTTACATCCCGAGGCTCAGGTTCTGTGGTGGTCATCTCTGTGTGGCTTGTTCTGTGGGCCTACCTAAA
1333 GTCCTAAGCACAGCTCTCAAGCAGATCCGAGGCGACTAAGATGCTAGTAGGGGTTGTCTGGAGAGAAGAGCCGA
1407 GGAGGTGGGCTGTGATGGATCAGTTCAGCTTTCAAATAAAAAGGCGTTTTTATATTCTGTGTCGAGTTCGTGAA
1481 CCCCTGTGGTGGGCTTCTCCATCTGTCTGGGTTAGTACCTGCCACTATACTGGAATAAGGgGACGCCTGCTTCC
XhoI (1555)
1555 CTCGAGTTGGCTGGACAAGGTTATGAGCATCCGTGTACTTATGGGGTTGCCAGCTTGGTCCTGGATCGCCCGGG
1629 CCCTTCCCCCACCCGTTCGGTTCCCCACCACCACCCGCGCTCGTACGTGCGTCTCCGCCTGCAGCTCTTGACTC
1703 ATCGGGGCCCCCGGGTCACATGCGCTCGCTCGGCTCTATAGGCGCCGCCCCCTGCCCACCCCCCGCCCGCGCTG
HindIII (1816)
1777 GGAGCCGCAGCCGCCGCCACTCCTGCTCTCTCTGCGCCGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGC
1851 CCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCT
1925 GAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGC
1999 CTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTC


NcoI (2101)
NdeI (2396)
SacII (3083)
NheI (3667)
MfeI (3816)


4441 TCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTG
84 nThr Ser Val Val Glu Ser Trp Glu Ala Tyr Leu Glu Asp Leu Gly Arg Val Trp Val Trp Ala Leu Thr Asn A
SgrAI (4574)
4515 TCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTC
59 spPro Val Val Gln Asp Gln Val Ala Ser Ile Phe Leu Thr Val Asp Asp Arg Val Val Gly Ala Phe Asp Asp
4589 CTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGA
35 Glu Val Phe Asp Arg Ser Phe Gly Leu Arg Asp Thr Trp Phe Glu Val Ala Gly Ala Val Asp Arg Ala Thr Le
4663 GCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTA
10 uVal Pro Val Ala Ser Thr Leu Lys Ala Met
MfeI (4737)
4737 CAATTGCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCA
4811 GGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTT
4885 TTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGG
4959 ACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCG
5033 GATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCG
5107 GTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
5181 TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA
5255 GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACA
5329 GTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACA
5403 AACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAG
5477 ATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCT
5551 AGTTAATTAACATTTAAATCA