pDRIVE-hFerH/RU5'

A plasmid with a composite promoter comprised of the human Ferritin Heavy and HTLV 5' UTR

Catalog # pdrive-hferhru5

For research use only

Version # 04J05-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-hFerH/RU5'.
- GT100 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1*.
- 4 pouches of *E. coli* Fast-Media® Zeo

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.
- Promoter activity has been confirmed by transient transfection of 293 cells as well as other selected cell lines.

GENERAL PRODUCT USE

pDRIVE is an expression plasmid containing a native or composite promoter of interest. **pDRIVE** 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' sites include *Sda* I, *Pst* I, and *Spe* I. *Sda* I is compatible with *Nsi* I and *Pst* 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 pDRIVE promoter drives the expression of the *LacZ* reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the *LacZ* gene is flanked by unique restriction sites (*Nco* I and *EcoR* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	FerL	Human	190
5'UTR	HTLV	Viral	267
Intron	-	-	-

hFerH/RU5' promoter

Ferritin is a ubiquitous iron storage protein. Ferritin is a 24 subunit protein composed of two subunit types termed H (heavy) and L (light) which perform complementary functions in the protein. The synthesis of ferritin is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through interaction between a 28-nucleotide iron-responsive element (IRE) located in the 5' UTR of ferritin mRNAs and a cytosolic protein, the iron regulatory protein[1].To eliminate the iron regulation of the ferritin promoter, the 5' UTR of FerH has been replaced by the 5' UTR of the HTLV. This modification makes the FerH promoter ubiquitous, strong and constitutive.

PLASMID FEATURES

- **LacZ gene** encodes β-galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope.
- **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.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- ***Sh ble*** 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 pDRIVE-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 pDRIVE 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.*

References:

1- Eisenstein RS & Munro HN. (1990). Enzyme 44(1-4): 42-58.

TECHNICAL SUPPORT
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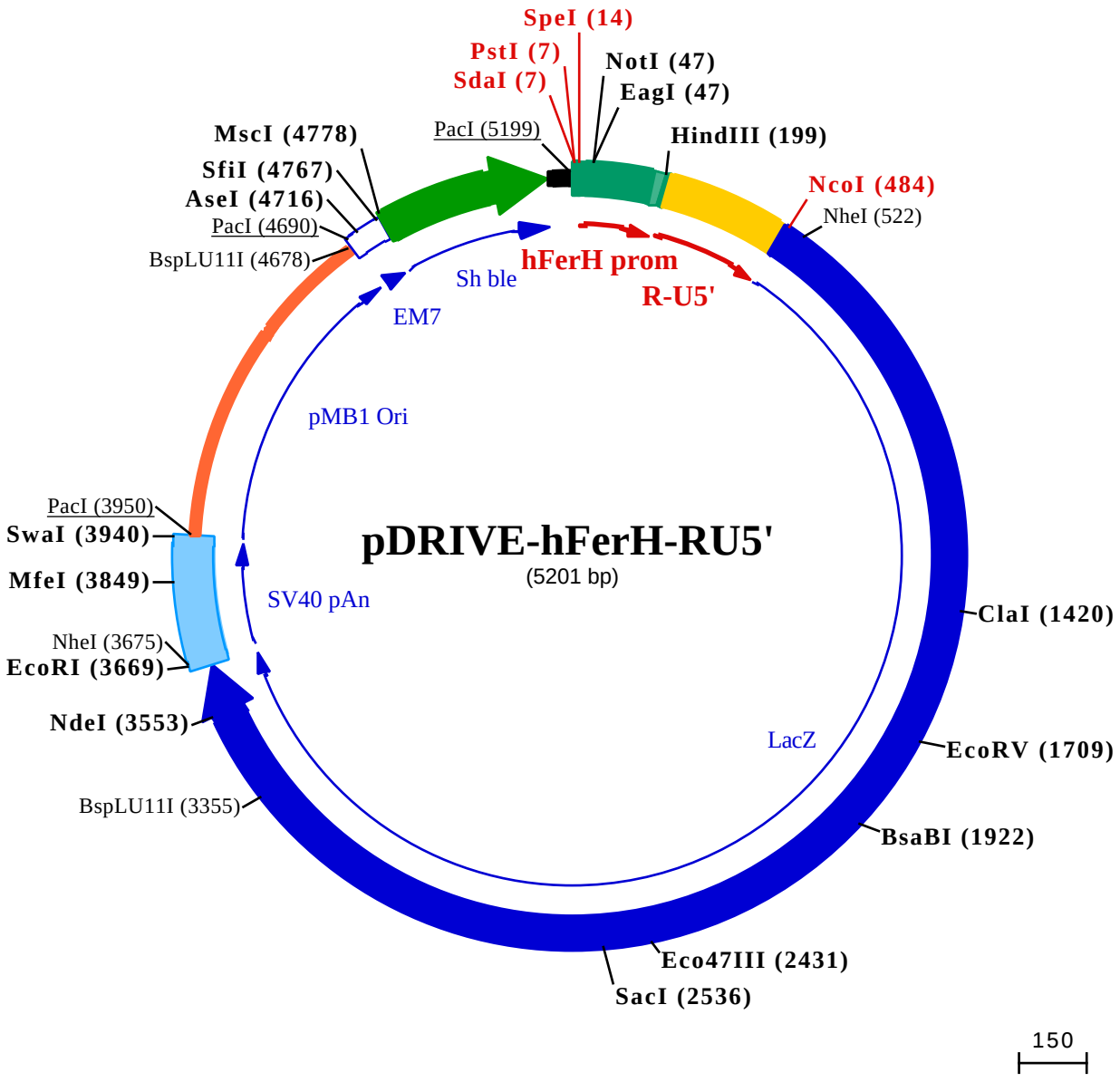
SpeI (14)
PstI (7)
SdaI (7)
NotI (47)
EagI (47)
PacI (5199)
HindIII (199)
MscI (4778)
SfiI (4767)
AseI (4716)
PacI (4690)
BspLU11I (4678)
NcoI (484)
NheI (522)
hFerH prom
R-U5'
Sh ble
EM7
pMB1 Ori
PacI (3950)
SwaI (3940)
MfeI (3849)
NheI (3675)
EcoRI (3669)
NdeI (3553)
BspLU11I (3355)
pDRIVE-hFerH-RU5'
(5201 bp)
SV40 pAn
ClaI (1420)
LacZ
EcoRV (1709)
BsaBI (1922)
Eco47III (2431)
SacI (2536)
150

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      PstI (7)                                   EagI (47)
      SdaI (7)      SpeI (14)                    NotI (47)
   1  CCTGCAGGGCCCACTAGTTCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGGGCGGGGTCCCGCGCCCACCGGAAGGAGCGG

                                                                                                HindIII (199)
 101  GCTCGGGGCGGGCGGCGCTGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCGACCCGCAGGGCCAGACGTTCTTCGCCGAAG

 201  CTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTG

 301  CCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCT

                                                                                          NcoI (484)
 401  CTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCCACCATGGGGGGTTCTCATC
                                                                                    1▶ MetGlyGlySerHisH
                                NheI (522)
 501  ATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGT
   6▶ isHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyArgAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyVa
 601  TGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGC
  39▶ lAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSer
 701  GAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCT
  73▶ GluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerT
 801  GGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCC
 106▶ rpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProI leTyrThrAsnValThrTyrPr
 901  CATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACG
 139▶ ol leThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThr
1001  CGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACC
 173▶ ArgI leI lePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspL
1101  TGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAG
 206▶ euSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSe
1201  CGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTA
 239▶ rGlyI lePheArgAspValSerLeuLeuHisLysProThrThrGlnI leSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaVal
1301  CTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGC
 273▶ LeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaP
                          ClaI (1420)
1401  CTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCC
 306▶ roPheGlyGlyGluI leI leAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluI lePr
1501  GAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAAT
 339▶ oAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuI leGluAlaGluAlaCysAspValGlyPheArgGluValArgI leGluAsn
1601  GGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGG
 373▶ GlyLeuLeuLeuLeuAsnGlyLysProLeuLeuI leArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetV
          EcoRV (1709)
1701  TGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGG
 406▶ alGlnAspI leLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGl
1801  CCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGC
 439▶ yLeuTyrValValAspGluAlaAsnI leGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArg
                          BsaBI (1922)
1901  GTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATC
 473▶ ValThrArgMetValGlnArgAspArgAsnHisProSerVal I leI leTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrA
2001  GCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGT
 506▶ rgTrpI leLysSerValAspProSerArgProValGlnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspI leI leCysProMetTyrAlaArgVa
2101  GGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCC
 539▶ lAspGluAspGlnProPheProAlaValProLysTrpSerI leLysLysTrpLeuSerLeuProGlyGluThrArgProLeuI leLeuCysGluTyrAla
2201  CACGCGATGGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATC
 573▶ HisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspG
2301  AGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCT
 606▶ lnSerLeuI leLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLe
                                  Eco47III (2431)
2401  GGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACC
 639▶ uValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrI leGluValThr
                                  SacI (2536)
2501  AGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCG
 673▶ SerGluTyrLeuPheArgHisSerAspAsnGluLeuLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValA
2601  CTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGAC
 706▶ laProGlnGlyLysGlnLeuI leGluLeuProGluLeuProGlnProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaTh
2701  CGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCAT
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2801  CTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATA
 773▶ LeuThrThrSerGluMetAspPheCysI leGluLeuGlyAsnLysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpI leGlyAspL
2901  AAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTG
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3001  GGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCT
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 873▶ HisAlaTrpGlnHisGlnGlyLysThrLeuPheI leSerArgLysThrTyrArgI leAspGlySerGlyGlnMetAlaI leThrValAspValGluValA
3201  CGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTA
 906▶ laSerAspThrProHisProAlaArgI leGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTy
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BspLU11I (3355)

3301 TCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGC
939 rProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCys
3401 GGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATC
973 GlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisA

NdeI (3553)

3501 GCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGA
1006 rgHisLeuLeuHisAlaGluGluGlyThrTrpLeuAsnIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGl

NheI (3675)

EcoRI (3669)

3601 ATTACAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAATAATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTG
1039 uLeuGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys•••
3701 ATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATT

MfeI (3849)

3801 GCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTT

PacI (3950)

SwaI (3940)

3901 AAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCG
4001 TCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGG
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4201 AGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACC
4301 GGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCG
4401 AACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGA
4501 GCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCA

PacI (4690)

BspLU11I (4678)

4601 GGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAAATTTTTCA

MscI (4778)

AseI (4716) **SfiI (4767)**

4701 AAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCA
1 MetAlaLysLeuThrSerAlaValPro
4801 GTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCA
10 ValLeuThrAlaArgAspValAlaGlyAlaValGluPheTrpThrAspArgLeuGlyPheSerArgAspPheValGluAspAspPheAlaGlyValValA
4901 GAGATGATGTCACCCTGTTCATCTCAGCAGTCCAGGACCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGC
43 rgAspAspValThrLeuPheIleSerAlaValGlnAspGlnValValProAspAsnThrLeuAlaTrpValTrpValArgGlyLeuAspGluLeuTyrAl
5001 TGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGA
76 aGluTrpSerGluValValSerThrAsnPheArgAspAlaSerGlyProAlaMetThrGluIleGlyGluGlnProTrpGlyArgGluPheAlaLeuArg

PacI (5199)

5101 GACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAAACCCCGCCCCGGCGGGGTTTTTTGTTAATTA
110 AspProAlaGlyAsnCysValHisPheValAlaGluGluGlnAsp•••
5201 A