pDRIVE-SV40-hFerH-mEF1

A plasmid with a composite promoter comprised of the SV40 enhancer, human Ferritin Heavy core promoter and mouse EF1 5' UTR

Catalog # pdrive-sv40ferhef1

For research use only

Version # 05E13-MT

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-SV40-hFerH-mEF1.
- 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.

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.

- 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
Enhancer	SV40	Viral	235
Promoter	FerH	Human	179
5' UTR	EF1	Mouse	1002
Intron			944

SV40-hFerH-mEF1 promoter (1423 bp)

Ferritin is an ubiquitous iron storage protein. It 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the iron regulatory protein[1].To eliminate the iron regulation of the ferritin promoter, the 5' UTR of FerL has been replaced by 5'UTR of the chimpanzee elongation factor 1 (EF1) gene. This modification makes the FerL promoter ubiquitous and constitutive. To further increase its activity in a large host range, the SV40 enhancer has been added. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells[2].

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 **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.*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. and Munro HN., 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58

2. Moreau P. *et al.*, 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res. 9(22):6047-68.

TECHNICAL SUPPORT
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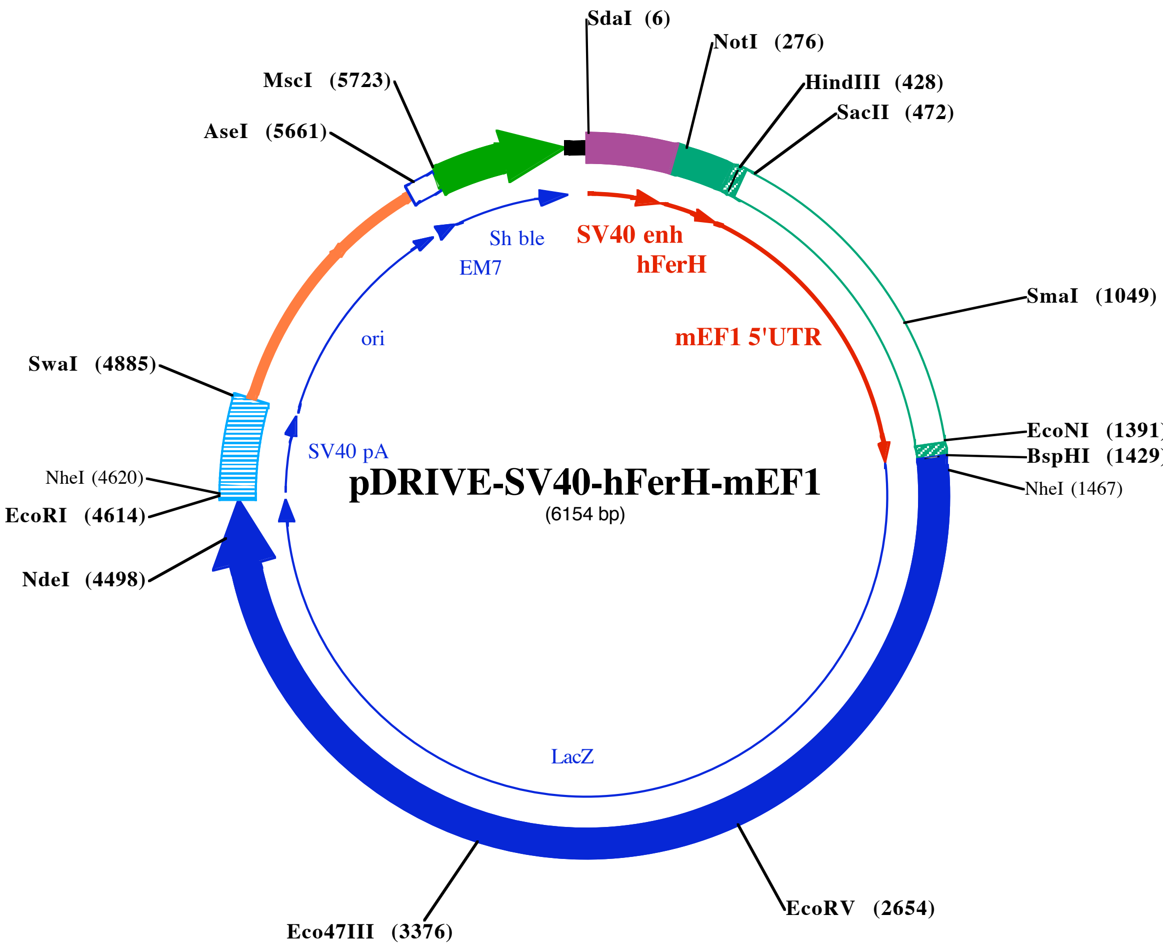

pDRIVE-SV40-hFerH-mEF1
(6154 bp)
SdaI (6)
NotI (276)
HindIII (428)
SacII (472)
SmaI (1049)
EcoNI (1391)
BspHI (1429)
NheI (1467)
EcoRV (2654)
Eco47III (3376)
NdeI (4498)
EcoRI (4614)
NheI (4620)
SwaI (4885)
AseI (5661)
MscI (5723)
SV40 enh
hFerH
mEF1 5'UTR
LacZ
SV40 pA
ori
EM7
Sh ble

SdaI (6)

1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG

NotI (276)

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGG

301 GCGGGGTCCCGCGCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGCTGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCG

HindIII (428) **SacII (472)**

401 ACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGCTTCCGCGGGCCGCCGAGCTGGAGGTCCTGCTCCG

501 AGCGGGCCGGGCCCCGCTGTCGTCGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGCGCGGGAGGCAGAGTGCGAGGCCTAGCGGCAA

601 CCCCGTAGCCTCGCCTCGTGTCCGGCTTGAGGCCTAGCGTGGTGTCCGCGCCGCCGCCGCGTGCTACTCCGGCCGCACTCTGGTCTTTTTTTTTTTTGTT

701 GTTGTTGCCCTGCTGCCTTCGATTGCCGTTCAGCAATAGGGGCTAACAAAGGGAGGGTGCGGGGCTTGCTCGCCCGGAGCCCGGAGAGGTCATGGTTGGG

801 GAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCCGCCTTCGGAGCACATGTCCGACGCCACCTGGATGGGGCGAGGCCTGGGGTTTTCCCGAAG

901 CAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCGGCGCCGCGTTGCCCTGCCTCCCTAACTAGGGTGA

SmaI (1049)

1001 GGCCATCCCGTCCGGCACCAGTTGCGTGCGTGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGAGCTCAAAATGGAGGACGCGGCAGCCCGGTGGAGC

1101 GGGCGGGTGAGTCACCCACACAAAGGAAGAGGGCCTGGTCCCTCACCGGCTGCTGCTTCCTGTGACCCCGTGGTCCTATCGGCCGCAATAGTCACCTCGG

1201 GCTTTTGAGCACGGCTAGTCGCGGCGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGCT

EcoNI (1391)

1301 GGAAGTCATTTTTGGAATTTGTCCCCTTGAGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTTAAACCCTTTTTTAGGTGT

BspHI (1429) NheI (1467)

1401 TGTGAAAACCACCGCTAATTCAAAGCAATCATGAGCGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGG
1 MetSerGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyArgA

1501 ATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACT
24 spLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLe

1601 TAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
57 uAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGlu

1701 TGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGC
91 TrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTrpG

1801 AGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTC
124 lnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSe

1901 GCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGC
157 rLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArg

2001 TGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGTT
191 TrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgT

2101 GGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGA
224 rpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAs

2201 TTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACA
257 pPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThr

2301 GTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACAC
291 ValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyGlyTyrAlaAspArgValThrL

2401 TACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGA
324 euArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuIleGl

2501 AGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCAC
357 uAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuLeuIleArgGlyValAsnArgHis

EcoRV (2654)

2601 GAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGC
391 GluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerH

2701 ATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAA
424 isTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluThrHisGlyMetValProMetAs

2801 TCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCG
457 nArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpSer

2901 CTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAG
491 LeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValGlnTyrGluGlyGlyGlyA

3001 CCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCT
524 laAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGlnProPheProAlaValProLysTrpSerIleLysLysTrpLe

3101 TTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGT
557 uSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArg

3201 CAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTG
591 GlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGlyA

Eco47III (3376)

3301 ATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCA

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 624▸ spPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGl
3401  GCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCG
 657▸ nGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeuLeuHisTrpMetValAla
3501  CTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCG
 691▸ LeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGlnGlyLysGlnLeuIleGluLeuProGluLeuProGlnProGluSerA
3601  CCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGA
 724▸ laGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGl
3701  AAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAA
 757▸ uAsnLeuSerValThrLeuProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCysIleGluLeuGlyAsnLysArgTrpGln
3801  TTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATA
 791▸ PheAsnArgGlnSerGlyPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspA
3901  ACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCA
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4001  GTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGG
 857▸ nCysThrAlaAspThrLeuAlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheIleSerArgLysThrTyrArg
4101  ATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGG
 891▸ IleAspGlySerGlyGlnMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgIleGlyLeuAsnCysGlnLeuAlaGlnV
4201  TAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGA
 924▸ alAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAs
4301  CATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTC
 957▸ pMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPhe
                                                                                          NdeI (44
4401  AACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATA
 991▸ AsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyThrTrpLeuAsnIleAspGlyPheHisM
4501  TGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATTACAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAATA
1024▸ etGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGluLeuGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys••
                           NheI (4620)
                  EcoRI (4614)
4601  ATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTG
1057▸ •
4701  AAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGC

                                                                                     SwaI (4885)
4801  ATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAACT

4901  AGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC

5001  GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCA

5101  GCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAAT

5201  CCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACG

5301  GGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGA

5401  GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG

5501  GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTG

                                                                    AseI (5661)
5601  GCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAAATTTTTCAAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGAC

                      MscI (5723)
5701  TCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTG
                         1▸ MetAlaLysLeuThrSerAlaValProValLeuThrAlaArgAspValAlaGlyAlaValGluPheTrpThrAspArgLeu
5801  GGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTCATCTCAGCAGTCCAGGACCAGGTGGTGCCTGACA
  28▸ GlyPheSerArgAspPheValGluAspAspPheAlaGlyValValArgAspAspValThrLeuPheIleSerAlaValGlnAspGlnValValProAspA
5901  ACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCAT
  61▸ snThrLeuAlaTrpValTrpValArgGlyLeuAspGluLeuTyrAlaGluTrpSerGluValValSerThrAsnPheArgAspAlaSerGlyProAlaMe
6001  GACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAA
  94▸ tThrGluIleGlyGluGlnProTrpGlyArgGluPheAlaLeuArgAspProAlaGlyAsnCysValHisPheValAlaGluGluGlnAsp•••
6101  GAATTGAGTTTCAGAAAAGGGGGCCTGAGTGGCCCCTTTTTTCAACTTAATTAA
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