pDRIVE-rEF1α/RU5'

A plasmid with a composite promoter consisting of the rat Elongation Factor-1 alpha and HTLV 5' UTR

Catalog # pdrive-ref1ru5

For research use only

Version # 05B15-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-rEF1α/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 *Bsp*H I, *Afl* III, *Pci* I and *Sty* 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	EF-1α	Rat	349
5'UTR	HTLV	Viral	267
Enhancer	-	-	-

EF-1α promoter

The EF-1 alpha gene encodes for elongation factor-1 alpha which is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The promoter of this "housekeeping" gene exhibits a strong activity, higher than viral promoters such as SV40 and RSV promoters[1], and on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo[2]. The rat EF-1α promoter shares a 45.05% homology to the human EF-1α promoter.

HTLV R-U5' UTR

The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[3] 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.

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.

Note: *For long-term storage of the pDRIVE-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.*

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- Kim DW *et al.* (1990). Gene. 91(2): 217-23.
2- Guo ZS *et al.* (1996). Gene Ther. 3(9):802-10
3- Takebe *et al* (1988). Mol. Cell Biol. 1: 466-472

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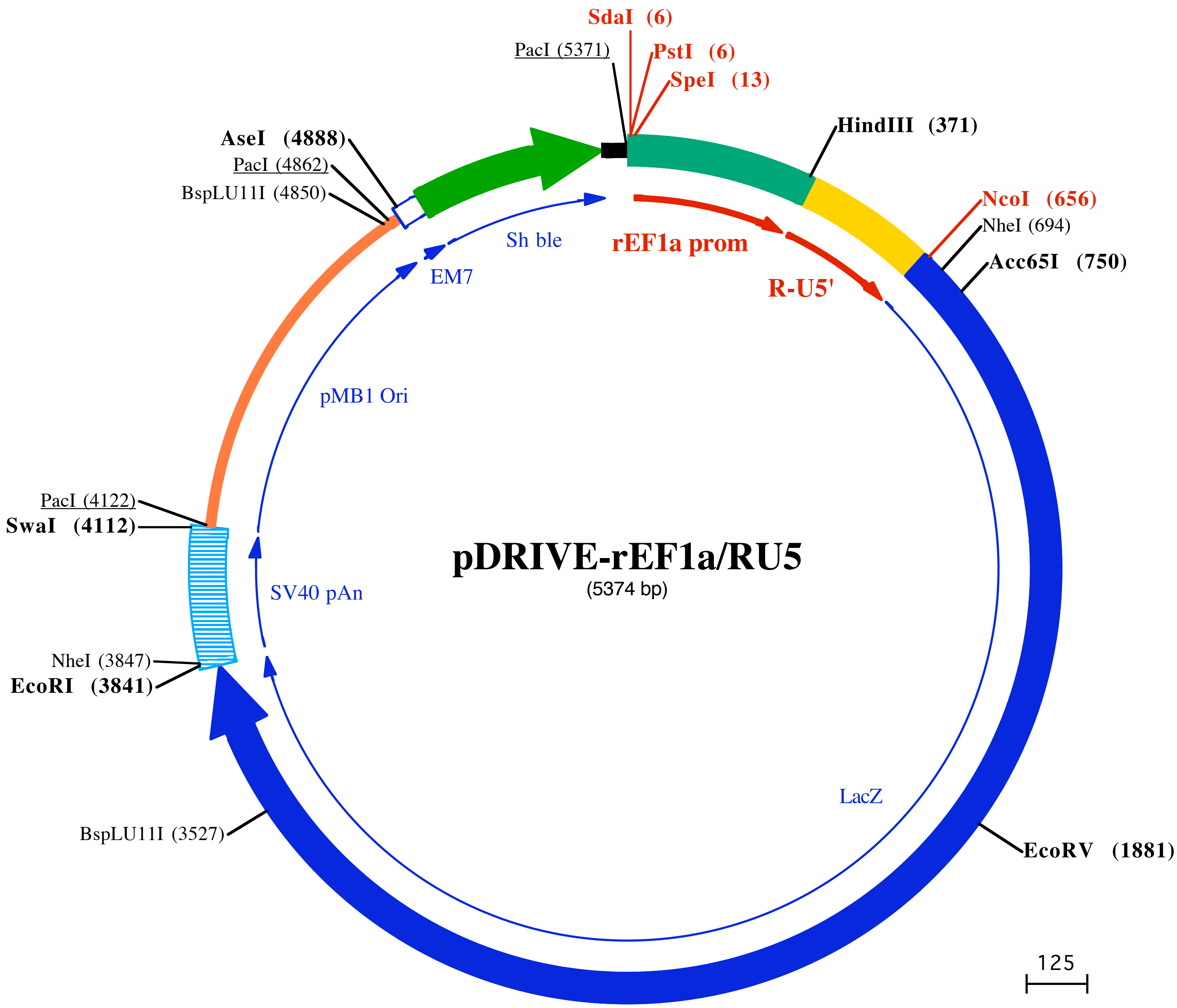
SdaI (6)
PstI (6)
SpeI (13)
PacI (5371)
HindIII (371)
AseI (4888)
PacI (4862)
BspLU11I (4850)
NcoI (656)
NheI (694)
Acc65I (750)
Sh ble
rEF1a prom
EM7
R-U5'
pMB1 Ori
PacI (4122)
SwaI (4112)
pDRIVE-rEF1a/RU5
(5374 bp)
SV40 pAn
NheI (3847)
EcoRI (3841)
LacZ
BspLU11I (3527)
EcoRV (1881)
125

PstI (6)
SdaI (6) SpeI (13)

1 CCTGCAGGGCCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAGAGCCCAGGGACCGTCCCTAAATTCTCACAGAC

101 CCAAATCCCTGTAGCCGCCCCACGACAGCGCGAGGAGCATGCGCCCAGGGCTGAGCGCGGGTAGATCAGAGCACACAAGCTCACAGTCCCCGGCGGTGGG

201 GGGAGGGGCGCGCTGAGCGGGGGCCAGGGAGCTGGCGCGGGGCAAACTGGGAAAGTGGTGTCGTGTGCTGGCTCCGCCCTCTTCCCGAGGGTGGGGGAGA

HindIII (371)

301 ACGGTATATAAGTGCGGTAGTCGCCTTGGACGTTCTTTTTCGCAACGGGTTTGCCGTCAGAACGCAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCA

401 CGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGG

501 TAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGC

NcoI (656) NheI (694)

601 TCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCCACCATGGGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCA
1▸ MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerM

Acc65I (750)

701 TGACTGGTGGACAGCAAATGGGTCGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGA
15▸ etThrGlyGlyGlnGlnMetGlyArgAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAs
801 CTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCC
48▸ pTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSer
901 CAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCG
82▸ GlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaA
1001 ATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCC
115▸ spThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValPr
1101 CACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCG
148▸ oThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSer
1201 GCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAA
182▸ AlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluA
1301 ACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCT
215▸ snArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLe
1401 GCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGC
248▸ uHisLysProThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGly
1501 GAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGC
282▸ GluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluA
1601 GTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACT
315▸ rgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLe
1701 GCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCG
348▸ uHisThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysPro

EcoRV (1881)

1801 TTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGA
382▸ LeuLeuIleArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnA
1901 ACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATAT
415▸ snAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIl
2001 TGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGT
448▸ eGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArg
2101 AATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCC
482▸ AsnHisProSerValIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerA
2201 GCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGT
515▸ rgProValGlnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGlnProPheProAlaVa
2301 GCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGT
548▸ lProLysTrpSerIleLysLysTrpLeuSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGly
2401 TTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACG
582▸ PheAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnG
2501 GCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCC
615▸ lyAsnProTrpSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisPr
2601 AGCGCTGACGGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGAT
648▸ oAlaLeuThrGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAsp
2701 AACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAACAGTTGATTGAAC
682▸ AsnGluLeuLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGlnGlyLysGlnLeuIleGluL
2801 TGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAG
715▸ euProGluLeuProGlnProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSe
2901 CGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGC
748▸ rAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisAlaIleProHisLeuThrThrSerGluMetAspPheCys
3001 ATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCG
782▸ IleGluLeuGlyAsnLysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgA
3101 ATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCA
815▸ spGlnPheThrArgAlaProLeuAspAsnAspIleGlyValSerGluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHi
3201 TTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACC
848▸ sTyrGlnAlaGluAlaAlaLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeuIleThrThrAlaHisAlaTrpGlnHisGlnGlyLysThr
3301 TTATTTATCAGCCGGAAAACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGA
882▸ LeuPheIleSerArgLysThrTyrArgIleAspGlySerGlyGlnMetAlaIleThrValAspValGluValAlaSerAspThrProHisProAlaArgI
3401 TTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTT
915▸ leGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPh

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                                   BspLU11I (3527)
3501 TGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCA
 948 eAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyPro
3601 CACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCA
 982 HisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyT
3701 CATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATTACAGCTGAGCGCCGGTCGCTACCA
1015 hrTrpLeuAsnIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAlaGluLeuGlnLeuSerAlaGlyArgTyrHi
                                                          NheI (3847)
                                                   EcoRI (3841)
3801 TTACCAGTTGGTCTGGTGTCAAAAATAATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAA
1048 sTyrGlnLeuValTrpCysGlnLys•••
3901 TGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGC

4001 AATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTG

                         PacI (4122)
             SwaI (4112)
4101 GTAGATCCATTTAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGA

4201 TCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAA

4301 CTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGC

4401 ACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG

4501 GATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTAT

4601 GAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAA

4701 CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC

                                                                    PacI (4862)
                                                            BspLU11I (4850)
                                                                                         AseI (4888)
4801 AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAAATTTTTCAAAAGTAGTTGACAATTAATCATCGGCA

4901 TAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGA

5001 GCTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTCATCTCAG

5101 CAGTCCAGGACCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAA

5201 CTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTT

                                                                                       PacI (5371)
5301 GTGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAAAACCCCGCCCCGGCGGGGTTTTTTGTTAATTAA
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