

# pDRIVE-chEF1 $\alpha$ /RU5'

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

Catalog # pdrive-chef1ru5

**For research use only**

Version # 04L15-SV

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-chEF1 $\alpha$ /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® is 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 *BspLU11* 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-1 $\alpha$	Chimpanzee	672
5'UTR	HTLV	Viral	267
Intron	-	-	-
Enhancer	-	-	-

### **EF-1 $\alpha$ 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<sup>1</sup>, and on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*<sup>2</sup>. The chimpanzee EF-1 $\alpha$  promoter shares a 98.46% homology to the human EF-1 $\alpha$  promoter.

## 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-1, 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:

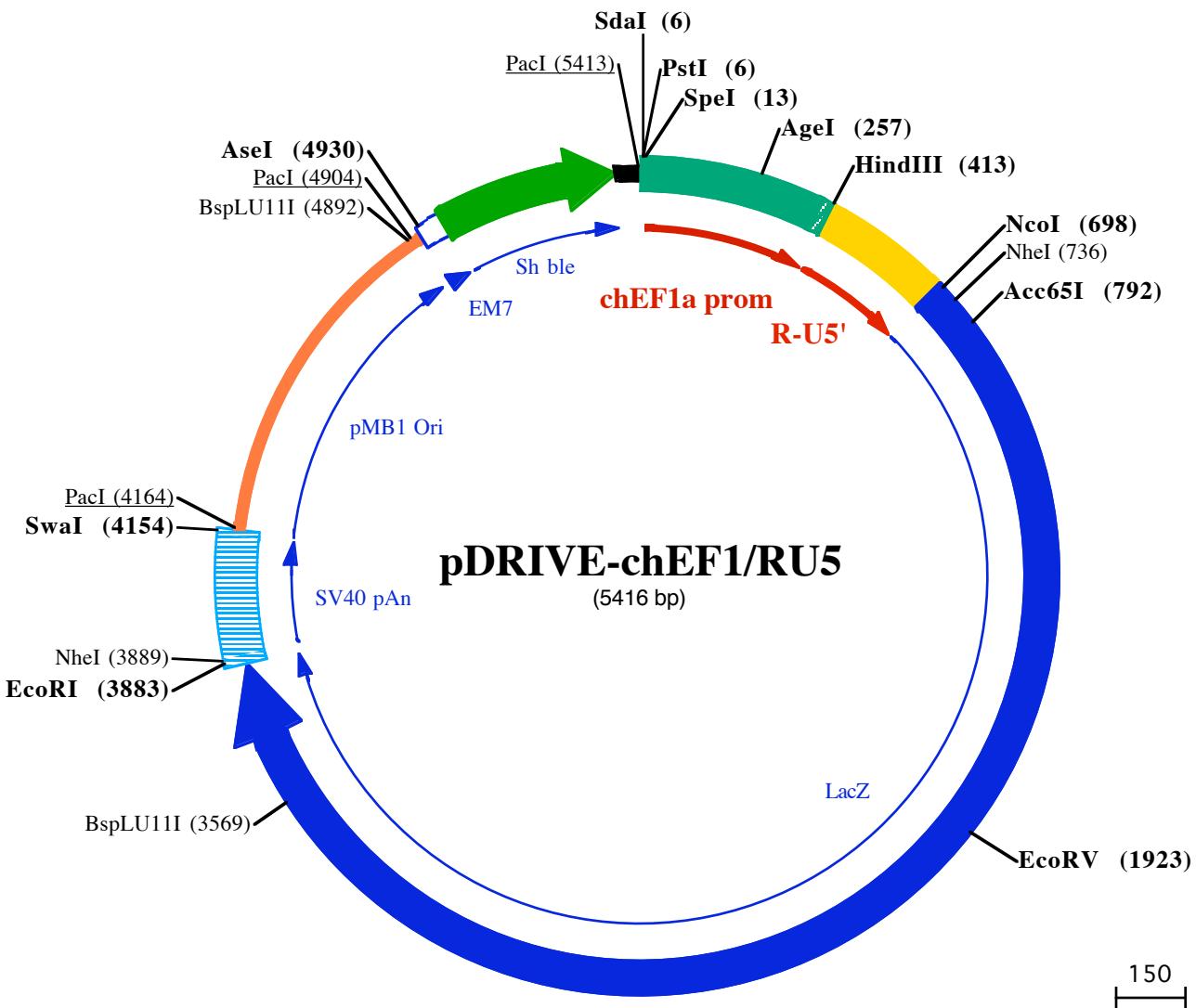
- 1- Kim DW *et al.* (1990). Gene. 91(2): 217-23.
2. Guo ZS *et al.* (1996). Gene Ther. 3(9):802-10

## TECHNICAL SUPPORT

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**PstI (6)**  
**SdaI (6)**  
**SpeI (13)**  
1 CCTCGAGGGCCACTAGTGGAGCCGAGAGTAATTACACAAAGGACTGCCCTGCCTGGGAATCCAGGGACCGTCGTTAACTCCCACTAACGTA  
101 GAACCCAGAGATCGCTGCCCTCGCCCCCTCACCGCCGCTCGTCATCACTGAGGTGGAGAAGAGCATGCGTAGGGCTCGGTTCCCGTCAGTGG  
201 CAGAGCGCACATGCCACAGTCCCACAAGTGGGGAGGGTCGCAATTGAAACCGTGCTAGAGAAGGTGGCGGGTAAACTGGAAAGTGA  
301 TGTCGTGTACTGGCTCCGCTTTCCGAGGGTGGGGAGAACGTATATAAGTCAGTAGTCGCGTGAACGTTCTTCGCAACGGTTGCGTC  
401 AGAACGCACTGAAGCTTCGAGGGCTCGATCTCCTCACGCCGCCGCCACCTGAGGCCCATCCACGCCGGTGGAGTCGCTCTGCCG  
501 CTCCCGCCTGTGGTGCCTCTGAACCTCGTCCGCCGTAGTAAGTTAAAGCTCAGGTCAGACCGGGCTTGTCCGGCCTCCCTGGAGCCTACC  
601 TAGACTCAGCCGGCTCCACGCTTGCCCTGACCTGCTCAACTCACGCTTTGTTCTGCGCCGTTACAGATC  
**NcoI (698)**  
601 CAAGCCACCA  
1 M  
701 TGGGGGGTCTCATCATCATCATCATGGTATGGCTAGCATGACTGGTGAGCAGCAAATGGGCGGATCTGTACGACGATGACGATAAGGTACCTAA  
1 P et Gl y Ser Hi s Hi s Hi s Hi s Gl y Met Al a Ser Met Th r Gl y Gl y Gl n Gl y Met Gl y Arg Asp Leu Tyr Asp Asp Asp Lys Val Pro Ly  
801 GGATCAGCTTGAGATTGATCCCGTCGTTTACAACGTCGTACTGGAAAACCCCTGGCTTACCAACTTAATGCCCTGCAGCACATCCCCCTTCGCC  
34 P s Asp Gl n Leu Gl y Val I Asp P ro V al V al I Leu Gl n Arg Arg Asp Trp Gl u Asn P ro Gl y Val I Th r Gl n Leu Asn Arg Leu Al a Al a Hi s Pro P ro H e Al a  
901 AGCTGGCGTAATAGCGAAGGGCCGACCGATGCCCTCCAAACAGTTGCGCAGCCTGAATGCCATGGCTTACCGCTGGTTCCGGCACCCAGAAG  
68 P Ser Trp Arg Asn Ser Gl u Gl u Al a Arg Th r Asp Arg P ro Ser Gl n Gl n Leu Arg Ser Leu Asn Gl y Gl u Trp Arg P he Al a Trp P he P ro Al a P ro Gl u A  
1001 CGGTGCCGGAAAGCTGGAGTCTCTGAGGCCGATACTGTCGTCGCTCCCTCAAACAGTCAGATGCCAGGTTACGGTACGATGCCCATCTACAC  
101 P l a V al P ro Gl u Ser Trp Leu Gl u Cys Asp Leu P ro Gl u Al a Asp Th r V al V al V al P ro Ser Asn Trp Gl n Met H i s Gl y Ty r Asp Al a P ro I l e Ty r Th  
1101 CAACGTAACCTATCCCATTACGGTAATCCCGCGTTGTTCCCACGGGAATCCGACGGTTTACTCGCTCACATTAACTGTTGATGAAAGCTGGCTA  
134 P r Asn V al Th r Tyr P ro I l e Th r V al Asn P ro P ro H e V al P ro Th r Gl u Asn P ro Th r Gl y Cys Ty r Ser Leu Th r P he Asn V al Asp Gl u Ser Trp Leu  
1201 CAGGAAGGCCAGACCGAATTATTTTATGGCGTAACTCGCGTTCATCTGGTGTGACACGGGCTGGGTCAGGCCAGTCGTTGC  
168 P Gl n Gl u Gl y Gl n Th r Arg I l e l e P he Asp Gl y Val I Asn Ser Al a P he H i s Leu T rp Cys Asn Gl y Arg Trp V al I Gl y Ty r Gl n Asp Ser Arg Leu P  
1301 CGCTGAATTGACCTGAGCCATTTCACGGCCGAGAAAACCCCTCGCGTATGGTGTGCGTTGAGCTTATCTGGAAAGTCAGGCA  
201 P ro Ser Gl u P he Asp Leu Al a P he Leu Arg Al a Gl y Gl u Asn Arg Leu Al a V al I Met V al I Leu Arg Trp Ser Asp Gl y Ser Ty r Leu Gl u Asp Gl n As  
1401 TATGGCGGATGAGCGCATTTCCTGACGTCTGCTGCTGATCAAACGACTACACAAATCAGCATTTCAGTGTGCACTCGCTTTAATGATGAT  
234 P p Me t Trp Arg Met Ser Gl y l l e P he Arg Asp V al Ser Leu Leu H i s Lys Pro Th r Th r Gl n l l e Ser Asp P he H i s V al Al a Th r Arg P he Asn Asp Asp  
1501 TTCAGCCGCGTGTACTGGAGGCTGAAGTTAGATGTGGCGAGTTGCGTACTACCGGTAACAGTTTTATGGCAGGGTAAACCCAGGTC  
268 P he Ser Arg Al a V al Leu Gl u Al a Gl u V al I Gl n Met Cys Gl y Gl u Leu Arg Asp Tyr Leu Arg V al Th r V al Ser Leu T rp Gl n Gl y Gl u Th r Gl n V al I A  
1601 CCAGCGCACCGCCCTTCGGCGTAAATTATCGATGAGCGTGGTTATGCCGATCGCTCACACTACGCTCTGAACGCTGAAAACCCGAAACTGTG  
301 P l a Ser Gl y Th r Al a P ro P he Gl y Gl u l l e l e Asp Gl u Arg Gl y Gl y Ty r Al a Asp Arg V al I Th r Leu Arg Leu Asn V al Gl u Asn P ro Ly Leu Tr  
1701 GAGCGCGGAATCCGAATCTCTATCGCGTGGTTGACTGCACCCGCGACGGCAGCTGATGAGCAGAAGCCTGCGATGTCGGTTCCGGAG  
334 P p Ser Al a Gl u l l e P ro Asn Leu Ty r Arg Al a V al V al Gl u Leu H i s Th r Al a Asp Gl y Th r Leu I l e Gl u Al a Cys Asp V al Gl y P he Arg Gl u  
1801 GTGCGGATTGAAATGGTCTGCTGCTGAACGGCAAGCGTTGATTGAGGCGTTAACCGTCAGCAGCATCTCTGCAATGGTCAGGTATGG  
368 P V al Arg I l e Gl u Asn Gl y Leu Leu Leu Asn Gl y Lys Pro Leu Leu I l e Arg Gl y V al Asn Arg H i s Gl u Hi s H i s Pro Leu H i s Gl y Gl u V al I M e t A  
**EcoRV (1923)**  
1901 ATGAGCAGACGATGGTCAGGATATCTGCTGATGAGCAGAACAACTTTAACGCCGTGCGCTGTTGCAATTATCGAACCATCCGCTGTGGTACACGCT  
401 P sp Gl u Gl n Th r Met V al I Gl n Asp I l e Leu Leu Met Lys Gl n Asn Asn P he Asn Al a V al I Arg Cys Ser H i s Ty r P ro Asn H i s P ro Leu Trp Ty r Th r Le  
2001 GTGCACCGCTACGGCCTGATGTTGGATGAAGCCAATATTGAAACCCACGGCATGGTCCAATGATCGTGCACCGTATCCGCGCTGGCTACCG  
434 P u Cys Asp Arg Tyr Gl y Leu Ty r V al V al Asp Gl u Al a Asn I l e Gl u Th r H i s Gl y Met V al I Pro Met Asn Arg Leu Th r Asp Asp P ro Arg Trp Leu Pro  
2101 GCGATGAGCGAACCGTAACGCGAATGGTCAGCGCAGTCGAATACCCGAGTGTGATCATCTGGTGTGCTGGGAATGAATCAGGCCACGGCGCTAAC  
468 P Al a Met Ser Gl u Arg V al Th r Arg Met V al Gl n Arg Asp Arg Asn H i s P ro Ser V al I l l e l e T rp Ser Leu Gl y Asn Gl u Ser Gl y H i s Gl y Al a Asn H  
2201 ACGACCGCTGATCGCTGGATCAAATCTGTCGATCTCCCGGGTGCAGTATGAAAGCGGGAGCCGACACCACGCCACCGATATTATTCGCC  
501 P i s Asp Al a Leu Ty r Arg Trp I l e Lys Ser V al Asp P ro Ser Arg P ro V al Gl n Ty r Gl u Gl y Gl y Al a Asp Th r H i s Th r Asp I l e Cys P r  
2301 GATGATCGCGCGTGGATGAAGGACGCCCTCCCGCTGCGCAAATGGTCTACAAAAATGGCTTTCGCTACCTGGAGAGACGCCGCGCTGATC  
534 P o Met Ty r Al a Arg V al Asp Gl u Asp Gl n P ro P he P ro Al a V al I Pro Lys Trp Ser I l e Lys Lys Trp Leu Ser Leu P ro Gl y Gl u Th r Arg P ro Leu I l e  
2401 CTTTGCAGATAACGGCCACGGTAAACAGTCTGGCGTTCCGCTAAATCTGGCAGGGTTCGCTAGTACCCGTTACAGGGCGCTCGTCT  
568 P Leu Cys Gl u Ty r Al a H i s Al a M e t Gl y Asn Ser Leu Gl y Gl y P he Al a Lys Ty r Trp Gl n Al a P he Arg Gl n Ty r P ro Arg Leu Gl n Gl y Gl y P he V a I T  
2501 GGGACTGGGTGGATCAGTCGCTGATTAATATGATGAAACCGGCAACCCGTTGCGCTTACGGCGTGATTTGGCGATACGCCGAACGATGCCAGTT  
601 P r P asp Trp V al I Asp Gl n S er Leu I l e Lys Ty r Asp Gl u Asn Gl y Asn P ro Trp Ser Al a Ty r Gl y Gl y Asp P he Gl y Asp Th r P ro Asn Asp Arg Gl n P h  
2601 CTGTATGACCGCTCTGGCTTCCGCGACGCCGACGGCAGTCAGGAAAGCAGAACACCCAGCAGCAGCAGTCTCCAGTCCGGCAA  
634 P e Cys Met A s n Gl y Leu V al P he Al a Asp Arg Th r P ro H i s P ro Al a Leu Th r Gl u Al a Lys H i s Gl n Gl n P he P he Arg Leu Ser Gl y Gl n P he Arg Leu Ser Gl y Gl n  
2701 ACCATCGAAGTGACCGAGAACATCTTCCGCTCATCGATAACGGCTCTGACTGGATGTTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTAG  
668 P Thr I l e Gl u V al Th r Ser Gl u Ty r Leu P he Arg H i s S er Asp Asn Gl u Leu Leu H i s T rp Met V al Al a Leu Asp Gl y Lys Pro Leu Al a S er Gl y Gl u V  
2801 TGCCTCTGGATGTCGCTTCAAGGTAACAGTTGATTGACTGCCTGAACTACCGCAGGGAGAGCGCCGGCAACTCTGGCTCACAGTACCGTAGT  
701 P al P ro Leu Asp V al Al a P ro Gl n Gl y Lys Gl n Leu I l e Gl u Leu P ro Gl u Leu P ro Gl n P ro Gl u S er Al a Gl y Gl n Leu T rp Leu Th r V al Arg V al V a  
2901 GCAACCGAACCGCAGCCATGGTCAGAGGCCACATCAGCGCTGGCAGCAGTGGCTCTGGCGAAAACCTCAGTGTGACGCTCCCGCCGCTC  
734 P I Gl n P ro Asn Al a Th r Al a T rp Ser Gl u Al a Gl y H i s I l l e S er Al a T rp Gl n Gl n T rp Arg Leu Al a Gl u Asn Leu S er V al I Th r Leu P ro Al a Al a S er  
3001 CACGCCATCCCGCATCTGACCACCGGAAATGGATTTTCGATCGAGCTGGTATAAGCGTTGCAATTAAACGCCAGTCAGGCTTCTTCAGA  
768 P H i s Al a l l e P ro H i s Leu Th r Th r Ser Gl u M et T asp P he Cys I l e Gl u Leu Gl y Asn Lys Arg Trp Gl n P he Asn Arg Gl n S er Gl y P he Leu S er Gl n M  
3101 TGTGATTGGCGATAAAAACACTGTCACGCCGCTGCGCAGTCAGTTACCCGTCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCT  
801 P et Trp I l e Gl y Asp Lys Lys Gl n Leu Leu Th r P ro Leu Arg Asp Gl n P he Th r Arg Al a P ro Leu Asp Asn I l e Gl y V al S er Gl u Al a Th r Arg I l  
3201 TGACCCATAACGCCGGTGAACGCTGGAGCGCGGGCATTACAGGCCAGCAGCTGGTGTGAGTCAGTCAGGCCAGTACACTTGCTGATGCCG  
834 P e Asp P ro Asn Al a T rp V al Gl u Arg Trp Lys Al a Al a Gl y H i s Ty r Gl n Al a Gl u Al a l e Leu Gl n Cys Th r Al a Asp Th r Leu Al a Asp Al a V al  
3301 CTGATTACGCCGCTACGCCGAGCATCGGGAAAACCTTATTCAGCGGGAAACCTACCGGATTGATGGTAGTGGTCAAATGGCATTACCG  
868 P Leu I l e Th r Th r Al a H i s Al a T rp Gl n H i s Gl n Gl y Lys Gl n Leu Ty r Leu P he I l e S er Arg Lys Th r Arg I l e Asp Gl y Ser Gl y Gl n Met Al a l l e Th r V  
3401 TTGATGTTGAAGTGGCGAGCGATACCCGATCCGGCGATTGGCCTGAACTGCCAGCTGGCGAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGG  
901 P al Asp V al Gl u V al Al a S er Asp Th r P ro H i s P ro Al a Arg I l e Gl y Leu Asn Cys Gl n Leu Al a Gl n V al Al a Gl u Arg V al Asn Trp Leu Gl y Leu Gl

BspLU1II (3569)

3501 GCCGCAAGAAAATCCGACCGCCTTACTGCCGCTGTTGACCGCTGGGATCTGCCATTGTCAGACATGTATAACCCGTACGTCTCCCGAGCGAA  
934▶ yProGlnGl uAsnTyrProAspArgLeuThrAl aAl aCysPheAspArgTrpAspLeuSerAspMetTyrThrProTyrValPheProSerGlu  
3601 AACGGTCTCGCTCGGGACGGCGAATTGAATTATGGCCCACACCAGTGGCGGGCAGTCCAGTCAACATCAGCCGTACAGTCACAGCAACTGA  
968▶ AsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnLeuM  
3701 TGGAAACCAGCCATGCCCATCTGCTGCACCGGAAAGGACATGGCTGAATATCGACGTTCCATATGGGATTGGTGGCACGACTCTGGAGCCC  
1001▶ etGluThrSerHisArgHisLeuLeuHisAlaGluGlyThrTrpLeuAsnIleAspGlyPheHisMetGlyIleGlyAspAspSerTrpSerPr

NheI (3889)

EcoRI (3883)

3801 GTCAGTATCGCGGAATTACAGCTGAGCGCCGGTGCCTACATTACCACTGGTCTGGTGTAAAAATAATAATCTAGTCGAGAATTGCTAGCTCGACA  
1034▶ oSerValSerAlaGluLeuGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys\*\*\*  
3901 TGATAAGATACATTGATGAGTTGGACAAACACAATAGAATGAGTAAAAAAATGCTTATTGTGAAATTGATGCTATTGCTTATTTGAA

4001 ATTTGTGATGCTATTGCTTATTGTGAAACATTATAAGCTGCAATAAACAAAGTTAACACAATTGCAATTCTATTGTTAGGTTAGGGGAGG

PacI (4164)

SwaI (4154)

4101 TGTGGGAGGTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTAAATGTTAAACTAGCCATGACCAAAATCCCTAACGTGAGTT  
4201 TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTGAGATCCTTTCTGCGCTAATCTGCTGCTGCAAACAAAAAACAC  
4301 CGCTACCAGCGGTGGTTGTTGCCGATCAAGAGCTACCAACTCTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATAACAAACTGTTCTCT  
4401 AGTGTAGCCGTAGTTAGGCCACCACTCAAGAACTCTGAGCACCGCTACATACCTCGCTGCTAATCCTGTTACAGTGGCTGCTGCCAGTGGCAG  
4501 AAGTCGTGCTTACCGGGTGGACTCAAGACGATAGTTACCGATAAGGCCAGCGGTGGCTGAACGGGGGTTCTGCACACAGCCAGCTGGAGC  
4601 GAACGACCTACACCGAACTGAGATACTACAGCGTGAGCTATGAGAAAGGCCAGCGCTCCGAAGGGAGAAAGGCCAGAGTATCCGTAAGCGCAG  
4701 GGTGGAACAGGGAGAGCGCACGAGGGAGCTTCAGGGGAAACGCCCTGGTATCTTATAGTCCTGCGGTTGCCACCTGACTTGAGCGTCGATT

PacI (4904)

BspLU1II (4892)

4801 TTGTGATGCTCGTCAGGGGGCGGAGCTATGGAAAACGCCAGCAACGCCCTTTACGTTCTGGCTTTGCTGGCTTTGCTCACATGTTCT

AseI (4930)

4901 TAATTAATTTTCAAAAGTAGTTGACAATTAAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAAGGGCCATCATGGCCAAGTT  
5001 ACCAGTGTGTCAGTGGCTCACAGCCAGGGATGTTGAGCTGGACTGAGTTCTGGACTGACAGGTTGGGTTCTCCAGAGATTTGTGGAGGATGACT  
5101 TTGCAGGTGTGGTCAGAGATGATGTCACCCGTTCATCTCAGCAGTCAGGACCAACTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGA  
5201 GGATGAGCTGTATGCTGAGTGGAGTGAGGTGGCTCCACCAACTCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGA  
5301 GAGTTGCCCTGAGAGACCCAGCAGGCAACTGTGCACTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAACCCGCCCGGGCGG

PacI (5413)

5401 GTTTTTGTTAATTAA