

pBLAST42-mAngio

An expression vector containing the mouse Angiostatin open reading frame

Catalog # pbla-mangio

For research use only

Version # 05E30-JC

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized plasmid DNA pBLAST42-mAngio
- 4 pouches of *E. coli* Fast-Media® Blas (2 for agar media, 2 for liquid media).

Storage and stability:

- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid DNA was prepared using affinity column and lyophilized.
- Plasmid construct has been confirmed by restriction analysis and ORF sequencing.

GENERAL PRODUCT USE

pBLAST is a ready-made expression vector containing a gene of interest from the angiostatic, angiogenic, growth factor, or differentiation inhibitor family.

pBLAST may be used for:

Obtaining a gene to subclone into another vector. Two restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Stable gene expression in mammalian cells. pBLAST plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pBLAST plasmids contain the blasticidin resistance gene (*bsr*) driven by the SV40 promoter in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli* AND the selection of stable clones in mammalian cells.

pBLAST allows a high level of expression and secretion of the gene product: genes coding for secreted proteins are cloned with their native signal sequence. However, proteins that lack a natural signal sequence (e.g. angiostatin) are engineered for secretion by addition of the hIL-2 signal sequence.

mAngio gene may be cut out by using SgrAI and NheI enzymes. Age I is compatible with Xma I, BspE I, NgoM IV and SgrA I. SgrA I is compatible with Xma I, BspE I, NgoM IV, and Age I. Nco I is compatible with BspH I and BspLU11 I. BspH I is compatible with Nco I and BspLU11 I. Nhe I is compatible with Xba I, Spe I, and Avr II.

PLASMID FEATURES

• **EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter¹ and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a "house keeping" gene. The promoter is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat² 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.

• mAngiostatin gene (with hIL2 signal sequence):

Intronless ORF from the ATG to the stop codon.

ORF Size (bp): 1152

Cloning fragment size (bp): 1171

• **SV40 polyA:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

• **SpAn:** A synthetic polyadenylation site and strong pause site, placed downstream of the pMB1 Ori, to limit transcriptional interference between both transcription units. The synthetic polyA site is based on the highly efficient polyA signal from the rabbit β-globin gene⁴.

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

• **SV40 promoter:** The Simian Virus 40 promoter allows the expression of the blasticidin resistance gene in mammalian cells.

• **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. The *bsr* gene is driven by the SV40 promoter in tandem with the bacterial EM7 promoter. Therefore each pBLAST plasmid can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **bGh polyA:** The bovine growth hormone (bGh) polyadenylation (pAn) signal and a transcriptional pause are placed 3' of the blasticidin gene. The bGh pAn has been shown to be as efficient as SV40 and HSV1tk polyadenylation signals in many different cell types⁵. The use of bGH pAn minimizes interference and possible recombination events with the SV40 polyadenylation signal. The pause site prevents transcriptional interference and read-through.

References

- 1- Kim et al (1990). *Gene* 2: 217-223.
- 2- Takebe et al (1988). *Mol. Cell Biol.* 1: 466-472.
- 3- Carswell et al (1989). *Mol. Cell Biol.* 10: 4248-4258.
- 4- Levitt et al. (1989). *Genes Dev.* 7: 1019-1025
- 5- Goodwin et al. (1992). *J. Biol. Chem.* 23: 16330-16334

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile water. Store resuspended plasmid at -20°C.

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

E. coli Fast-Media® Blas 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® Blas is a TB (liquid) or LB (solid) based medium with blasticidin, and contains stabilizers. *E. coli* Fast-Media® Blas can be ordered separately (catalog code # fas-bl-1, fas-bl-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

Toll free (US): 888-457-5873

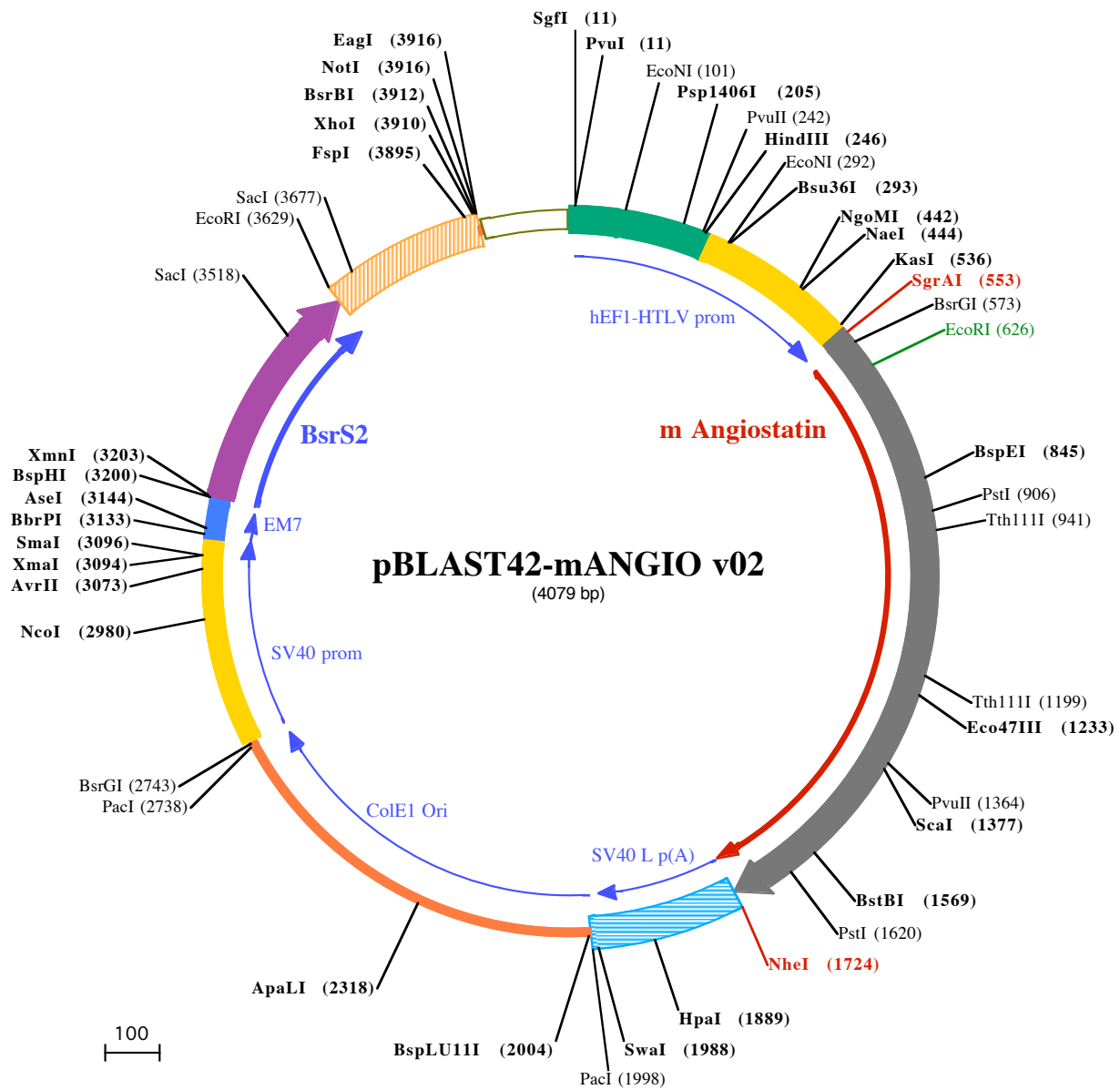
Outside US: (+1) 858-457-5873

E-mail: info@invivogen.com

Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA



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Toll free (US): 888-457-5873
 Outside US: (+1) 858-457-5873
 E-mail: info@invivogen.com
 Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite A
 San Diego, CA92121 - USA

PvuI (11)
SgfI (11)
EcoNI (101)

1 GGATCTGGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGGGGTAACGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

Psp1406I (205)
HindIII (246)
Bsu36I (293)

201 GTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCTTACAGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMI (442)
NaeI (444)

401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCCTACCTAGACTACGCGGCTCTCCACGCTTTGCTGACCCTGCTTCTCAACTCTACGTCTTTGTTTCGTTT

KasI (536)
SgrAI (553)
BsrGI (573)

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCCCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA

1▶MetTyrArgMetGlnLeuLeuSerCysI

EcoRI (626)

601 TTGCACTAAGTCTTGCACTGTGCACGAATTCGGTGTATCTGTGACAGATTAAGACCGGCATCGGCAACGGCTACAGAGAACCATGTCCAGGACAAAGAG

701 TGGTGTTCCTGTCAAAGTGGGTGCCACGTTCCCCACGTACCAACTACTCTCCAGTACACATCCAATGAGGGACTAGAAGAGAACTACTGTAGG

801 AACCCAGACAATGATGAACAAGGCCTTGGTGTACACTACAGATCCGGACAAGAGATATGACTACTGCAACATTCCTGAATGTGAAGAGGAATGCATGT

901 ACTGCAGTGGAGAAAAGTATGAGGGCAAATCTCAAGACCATGTCTGGACTTACTGCCAGGCCTGGATTCTCAGAGCCCACATGCTCATGGATACAT

1001 CCTTGCCAAATTTCCAAGCAAGAACCTGAAGATGAATTTGCCGCAACCCTGACGGGGAGCCAAGGCCCTGGTCTTACAACAGACCCCAACCAACGC

1101 TGGGAATACTGTGACATCCCCCGCTGCACAACCCCCGCCCCACCCAGCCCAACCTACCAATGTCTGAAAGGAAGAGGTGAAAATTACCGAGGGACCG

1201 TGTCTGTACCGTGTCTGGGAAAACCTGTCAGCGCTGGAGTGAGCAAACCCCTCATAGGCACAACAGGACACCAGAAAATTTCCCTGCAAAAATCTGGA

1301 AGAGAATACTGCGGAAACCCAGATGGAGAAAAGTCTCCCTGGTGTATACCCTGACAGCCAGCTGAGGTGGGAGTACTGTGAGATTCATCTCGCAG

1401 TCCTCAGCATCACCAGACAGTCAAGTCTCAGTTCACCAGAGGAGCAAACCTGTGGTCCAGGAATGCTACCAGAGCGATGGGCAGAGCTATCGGG

1501 GTACATCGTCCACTACCATCACAGGGAAGAAGTCCAGTCTGGGCGAGTATGTTCCACACAGGCATTGCAAGACCCAGAGAACTTCCAGATGCTGG

1601 CTTGGAGATGAACACTGCGGAACCCGGATGGTGACAAGGGCCCTTGGTGTACACCACTGACCCGAGCGTCAGGTGGGAATACTGCAACCTGAAGCGG

1701 TGCTCAGAGACAGGAGGAGTTAGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTAAAAAATGCTTTATT

1801 TGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACA

Eco47III (1233)

1201 TGTCTGTACCGTGTCTGGGAAAACCTGTCAGCGCTGGAGTGAGCAAACCCCTCATAGGCACAACAGGACACCAGAAAATTTCCCTGCAAAAATCTGGA

1301 AGAGAATACTGCGGAAACCCAGATGGAGAAAAGTCTCCCTGGTGTATACCCTGACAGCCAGCTGAGGTGGGAGTACTGTGAGATTCATCTCGCAG

1401 TCCTCAGCATCACCAGACAGTCAAGTCTCAGTTCACCAGAGGAGCAAACCTGTGGTCCAGGAATGCTACCAGAGCGATGGGCAGAGCTATCGGG

1501 GTACATCGTCCACTACCATCACAGGGAAGAAGTCCAGTCTGGGCGAGTATGTTCCACACAGGCATTGCAAGACCCAGAGAACTTCCAGATGCTGG

1601 CTTGGAGATGAACACTGCGGAACCCGGATGGTGACAAGGGCCCTTGGTGTACACCACTGACCCGAGCGTCAGGTGGGAATACTGCAACCTGAAGCGG

1701 TGCTCAGAGACAGGAGGAGTTAGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTAAAAAATGCTTTATT

1801 TGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACA

BstBI (1569)

1501 GTACATCGTCCACTACCATCACAGGGAAGAAGTCCAGTCTGGGCGAGTATGTTCCACACAGGCATTGCAAGACCCAGAGAACTTCCAGATGCTGG

1601 CTTGGAGATGAACACTGCGGAACCCGGATGGTGACAAGGGCCCTTGGTGTACACCACTGACCCGAGCGTCAGGTGGGAATACTGCAACCTGAAGCGG

1701 TGCTCAGAGACAGGAGGAGTTAGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTAAAAAATGCTTTATT

1801 TGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACA

NheI (1724)

1701 TGCTCAGAGACAGGAGGAGTTAGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTAAAAAATGCTTTATT

1801 TGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACA

HpaI (1889)

1801 TGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACA

PacI (1998)
SwaI (1988)

1901 TTGCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCATTAAATGTTAATTA

BspLU11I (2004)

2001 AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAA

2101 AAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAAGCTCCCTCGTGCCTCTCTGTTCCGACC

2201 CTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT

ApaLI (2318)

2301 GCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAAGACACGACTT

2401 ATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACT

2501 AGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACCAACCCGCTGGTAGCG

2601 GTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGA

2701 **PacI (2738) BsrGI (2743)**
 A A A C T C A C G T T A A G G G A T T T T G G T C A T G G C T A G T T A A T T A A G C T G T A C A C T G T G G A A T G T G T C A G T T A G G T G T G G A A A G T C C C C A G G C T C C C C A G C A
 2801
 G G C A G A A G T A T G C A A A G C A T G C A T C T C A A T T A G T C A G C A A C C A G G T G T G G A A A G T C C C C A G G C T C C C C A G C A G G C A G A A G T A T G C A A A G C A T G C A T C T C A

2901 **NcoI (2980)**
 A T T A G T C A G C A A C C A T A G T C C C G C C C T A A C T C C G C C C A T C C C G C C C T A A C T C C G C C C A G T T C C G C C C A T T C C G C C C A T G G C T G A C T A A T T T T T T T

3001 **AvrII (3073) XmaI (3094) SmaI (3096)**
 T A T T T A T G C A G A G G C C G A G G C C G C C T C T G C C T C T G A G C T A T T C C A G A A G T A G T G A G G A G G C T T T T T T G G A G G C C T A G G C T T T T G C A A A A A G C T C C C G G G A

3101 **BbrPI (3133) AseI (3144) BspHI**
 G C T T G T A T A T C C A T T T T C G G A T C T G A T c a g C A C G T G T T G A C A A T T A A T C A T C G G C A T A G T A T A T C G G C A T A G T A T A A T A C G A C A A G G T G A G G A C T A A A T

3201 **XmnI (3203)**
 C A T G A A G A C T T C A A C A T C T C T C A G C A G G A T C T G G A G C T G G T G G A G G T C G C C A C T G A G A A G A T C A C C A T G C T C T A T G A G G A C A A C A A G C A C C A T G T C G G G

3301 **1** ▶ Met Lys Thr Phe Asn I l e Ser Gl n Gl n Asp Leu Gl u Leu Val Gl u Val Al a Thr Gl u Lys I l e Thr Met Leu Tyr Gl u Asp Asn Lys Hi s Hi s Val Gl y
 G C G G C C A T C A G G A C C A A G A C T G G G G A G A T C A T C T C T G C T G T C C A C A T T G A G G C C T A C A T T G G C A G G G T C A C T G T C T G T G C T G A A G C C A T T G C C A T T G G G T

3401 **34** ▶ Al a Al a l l e Arg Thr Lys Thr Gl y Gl u l l e l l e Ser Al a Val Hi s l l e Gl u Al a Tyr l l e Gl y Arg Val Thr Val Cys Al a Gl u Al a l l e Al a l l e Gl y S
 C T G C T G T G A G C A A C G G G C A G A A G G A C T T T G A C A C C A T T G T G G C T G T C A G G C A C C C T A C T C T G A T G A G G T G G A C A G A T C C A T C A G G G T G G T C A G C C C C T G

3501 **67** ▶ er Al a Val Ser Asn Gl y Gl n Lys Asp Phe Asp Thr l l e Val Al a Val A Arg Hi s P ro Tyr Ser Asp Gl u Val Asp Arg Ser l l e Arg Val Val Ser Pro Cy
 T G G C A T G T G C A G A G A G C T A T C T C T G A C T A T G C T C T G A C T G C T T T G T G C T A T T G A G A T G A A T G G C A A G C T G G T C A A A C C A C C A T T G A G G A A C T A T C

3601 **100** ▶ s Gl y Met Cys Arg Gl u Leu l l e Ser Asp Tyr Al a P ro Asp Cys Phe Val Leu l l e Gl u Met Asn Gl y Lys Leu Val Lys Thr Thr l l e Gl u Gl u Leu l l e
 C C C C T C A A G T A C A C C A G G A A C T A A A C C T G A A T T C G C T A G A G G G C C C T A T T C T A T A G T G T C A C C T A A A T G C T A G A G C T C G C T G A T C A G C C T C G A C T G T G C C

3701 **134** ▶ Pro Leu Lys Tyr Thr Arg Asn •••
 T T C T A G T T G C C A G C C A T C T G T T G T T T G C C C C T C C C C G T G C C T T C C T T G A C C C T G G A A G G T G C C A C T C C C A C T G T C C T T T C T A A T A A A A T G A G G A A A T T

3801 **FspI (3895)**
 G C A T C G C A T T G T C T G A G T A G G T G T C A T T C T A T T C T G G G G G T G G G T G G G C A G G A C A G C A A G G G G A G G A T T G G G A A G A C A A T A G C A G G C A T G C G C A G G

3901 **EagI (3916) NotI (3916) BsrBI (3912) XhoI (3910)**
 G C C C A A T T G C T C G A G C G G C C G C A A T A A A A T A T C T T T A T T T C A T T A C A T C T G T G T T G G T T T T T T G T G T G A A T C G T A A C T A A C A T A C G C T C C C A T C A A

4001 A A C A A A A C G A A A C A A A C A A A C T A G C A A A A T A G G C T G T C C C C A G T G C A A G T G C A G G T G C C A G A A C A T T T C T C T A T C G A A