

pBLAST hAT3

An anti-angiogenic vector expressing hAT3

Catalog # pbla-hat3

For research use only

Version# 01F22-GC

PRODUCT INFORMATION

Content

- 20µg of lyophilized pBLAST hAT3 plasmid DNA.
- 4 pouches of *E. coli* FastMedia™ Blast1 (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* FastMedia™ Amp at room temperature. FastMedia™ is stable 18 months when stored properly.

Quality control

- Plasmid DNA was prepared using affinity column and lyophilized. Endotoxin levels were determined to be < 0.5 EU/µg plasmid DNA (1 ng LPS=1.8 EU). Therefore, this DNA can be used directly for transfection in eukaryotic cells or for animal injections
- Plasmid construct has been confirmed by restriction analysis sequencing.
- Expression has been confirmed by transfection and selection using murine melanoma B16 cells as well as selected other cell lines

GENERAL PRODUCT USE

Product use and advantages:

Anti-angiogenic proteins (also called angiostatic) are utilized in cancer therapy to inhibit tumor angiogenesis, i.e. the formation of blood vessels that feed tumor cells. However, angiostatic proteins are difficult to obtain and purify. Therefore, the use of vectors such as pBLAST expressing human or murine angiostatic genes should simplify the production of angiostatic proteins *in vitro* and *in vivo*.

pBLASTs are **ready-made plasmids** expressing an angiostatic protein directly transfectable into cells for:

- **convenient production of angiostatic proteins *in vitro***
- **anti-angiogenic gene therapy**

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.

The new pBLAST backbone with a single antibiotic resistance gene, blasticidin, allows very rapid and convenient selection of both bacteria and mammalian cell transformants: mammalian stable transfectants are selected in only a few days.

Other pBLAST vectors: pBLAST is a family of blasticidin-expressing vectors containing angiostatic genes. The pBLAST family offers:

The largest collection of angiostatic genes allowing you to test the effect of different angiostatic molecules in your system.

Unique angiostatic fusions designed by InvivoGen to provide synergistic effects and equimolar expression of angiostatic proteins. The proteins are fused with a polypeptide linker.

Fusions available:

- hEndo::Angio, a fusion of human endostatin and angiostatin
- hEndo::Kringle, a fusion of human endostatin and kringle-5
- hEndo::PEX, a fusion of human endostatin and PEX
- mMig::IP-10, a fusion of the angiostatic chemokines murine Mig and IP-10.

PLASMID FEATURES

● **EF-1α / HTLV hybrid promoter** is a composite promoter comprised from Elongation Factor-1α (EF-1α)⁽¹⁾ promoter 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 in all cell cycles except G0. The promoter is also non-tissue specific; it is highly expressed in all cell types. The promoter has been modified to enhance stability of DNA and RNA using the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁽²⁾. The modification not only increases steady state transcription and also significantly increases translation efficiency possibly through mRNA stabilization.

● **Angiostatic gene : hAT3 gene**

Size: 1397 bp

● **SV40 polyA** : InvivoGen uses the Simian Virus 40 Late polyadenylation signal for the second transcriptional unit. The efficiency of this signal was first described by Carswell *et al*⁽³⁾. The signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

● **Ori colE1** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

● **SV40 promoter:**

The SV40 promoter allows the expression of the blasticidin resistance gene in mammalian cells.

● **EM7:** The EM7 promoter enables expression of the blasticidin resistance gene in *E. coli*.

● **Bsr2 gene:**

Blasticidin, a nucleoside isolated from *Streptomyces griseochromogenes*, is a potent translational inhibitor. The blasticidin resistance (blast^r) gene from *Bacillus cereus* which codes for a blasticidin deaminase confers resistance to blasticidin. InvivoGen has developed an optimized blasticidin resistance gene, bsrS2.

Blasticidin S is used as a selective agent that allows the positive selection of cells carrying the blast^r gene. Because blasticidin is active against both prokaryotic and eukaryotic cells, plasmids carrying the blast^r gene only require blasticidin for selection in both cell types. Typically, bacteria are sensitive to blasticidin concentrations of 50µg/ml, while mammalian cells are sensitive to 1-5µg/ml blasticidin. After blasticidin treatment, cell death occurs rapidly, thus allowing the **selection of mammalian cell transfectants in as little as 7 days post-transfection.**

● **bGh pAn:**

The bovine growth hormone (bGh) polyadenylation (pAn) signal and a transcriptional pause is placed 3' of the blasticidin gene. Efficient polyadenylation is facilitated by inserting the flanking sequence of the bGh gene 3' to the standard AAUAAA polyadenylation sequence. The bGh pAn has been shown to be as efficient as SV40 and *HSV1tk* polyadenylation signals in many different cell types⁽⁴⁾. 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.

● **Pause site:**

InvivoGen uses a pause site derived from the 3' flanking region of the alpha2 globin gene⁽⁵⁾ to stop transcription of the first transcriptional unit. When the pause site is positioned immediately downstream from a strong poly (A) signal, significant levels of transcriptional termination take place. The pause site is essential for termination when genes with strong promoters are placed close together to prevent transcriptional interference.

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- Goodwin *et al* (1992). J. Biol. Chem. 23: 16330-16334.
- 5- Enriquez-Harris *et al*. (1991). EMBO J. 7: 1833-1842.

TECHNICAL SUPPORT

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METHODS

Selection of bacteria with *E. coli* FastMedia™ Blas

E. coli FastMedia™ Amp is a new, fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Amp is a TB (liquid) or LB (solid) based medium with ampicillin, and contains stabilizers.

E. coli FastMedia™ Amp can be ordered separately (catalog code # fas-am-1, fas-am-s, fas-am-x).

Method

- 1- Pour the content of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200ml 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 FastMedia™.**
- 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.

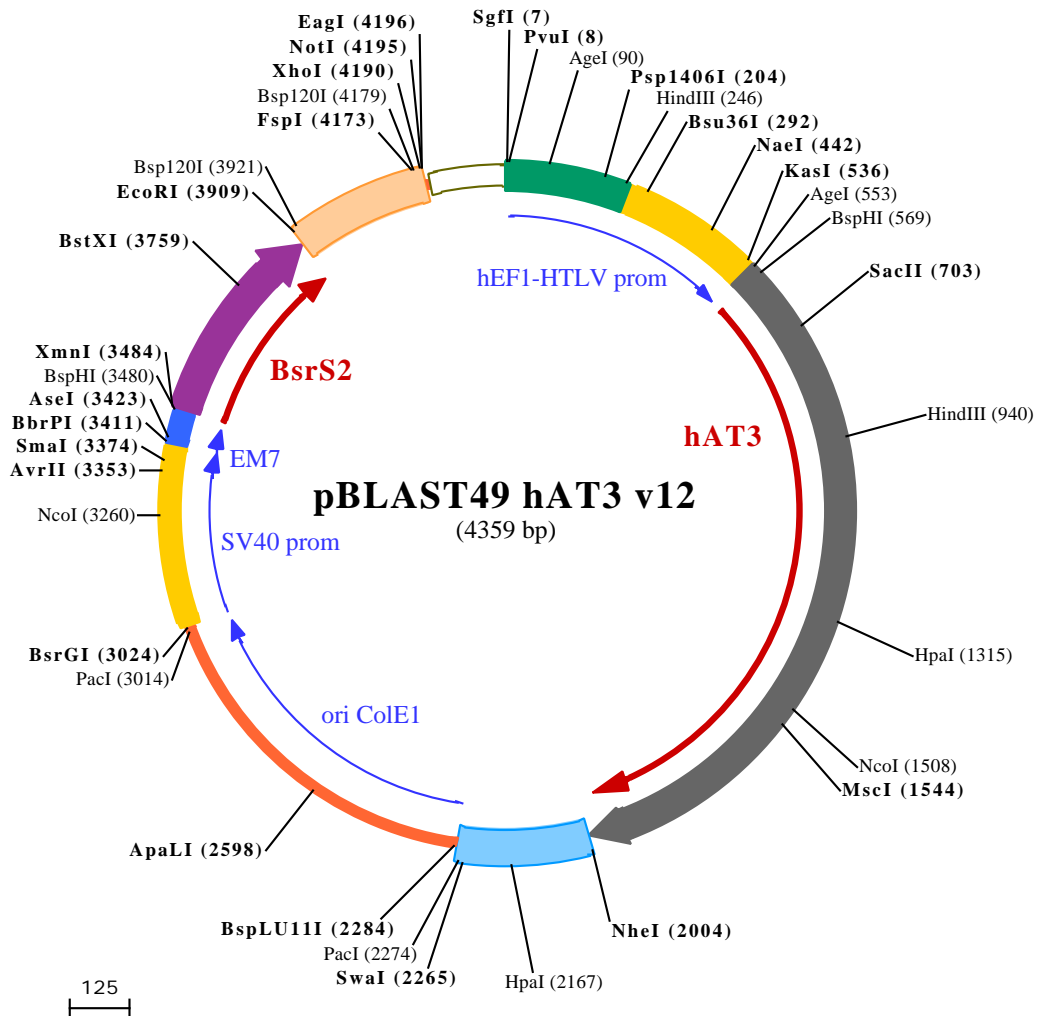
Note: Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.

Selection of stable transfectants with blasticidin S:

We recommend the use of InvivoGen's Blasticidin S. Blasticidin S is normally used at 3 to 10µg/ml to select mammalian stable transfectants carrying a plasmid with the blasti^r gene.

Method:

- 1- Transfect the cells of your choice with pBLAST containing the gene of interest (several methods for cell transfection are available).
- 2- 24 hours after transfection, remove medium, wash cells, and add fresh medium
- 3- 48 hours after transfection, split the cells 1:10 and add blasticidin at a concentrations ranging from 3 to 10µg/ml. Lower the concentration of blasticidin if all the cells die.
- 4- Change the medium of the cells after 3 days
- 5 -Evaluate the formation of foci after 3-7 days of selection. Foci may need several more days to develop.



PvuI (8)
SgfI (7)
GGATCTGCGATCGCTCCGGTGCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA
GAGAAGGTGGCGGGGTAAACTGGAAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (204) **HindIII (246)** **Bsu36I (292)**
GTGAACGTTCTTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCGACCTGAGGCC
GCCATCCACGCGGTTGAGTCCGGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTCCGCTCGCCGCTCTAGGTAAGTTAAAGCTCAGGTCGAGACC

NaeI (442)
GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

KasI (536) **AgeI (553)** **BspHI (569)**
TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTAGGAGGGCCATCATGATTTATTCCAATGTGATAGGAAGTGT
AACCTCTGGAAAAAGGAAGGTTTATCTTTTGTCTTGTGCTCATTGGCTTCTGGACTGCGTGACCTGTACGGGAGCCCTGTGGACATCTGCACAGCC

▶ **I**ThrSerGlyLysArgLysValTyrLeuLeuSerLeuLeuLeuIeGlyPheTrpAspCysValThrCysHisGlySerProValAspIeCysThrAla
SacII (703)
AAGCCGCGGGACATTTCCCATGAATCCCATGTGCATTTACCGCTCCCGGAGAAGAAGGCAACTGAGGATGAGGGCTCAGAACAGAAGATCCCGGAGGCCA
▶ **LysProArgAspIeProMetAsnProMetCysIeTyrArgSerProGluLysLysAlaThrGluAspGluGlySerGluGlnLysIeProGluAlaT**
CCAACCGCGTGTCTGGAACTGTCCAAGGCCAATCCCGCTTTGCTACCCTTCTATCAGCACCTGGCAGATTCCAAGAATGACAATGATAACATTTT
▶ **hrAsnArgArgValTrpGluLeuSerLysAlaAsnSerArgPheAlaThrPheTyrGlnHisLeuAlaAspSerLysAsnAspAsnAspAsnIePh**

HindIII (940)
CCTGTACCCCTGAGTATCTCCACGGCTTTTGCTATGACCAAGCTTGGTGCCTGTAATGACACCCCTCCAGCAACTGATGGAGGTATTTAAGTTTGACACC
▶ **eLeuSerProLeuSerIeSerThrAlaPheAlaMetThrlLysLeuGlyAlaCysAsnAspThrLeuGlnGlnLeuMetGluValPheLysPheAspThr**
ATATCTGAGAAAAATCTGATCAGATCCACTTCTTTGCCAACTGAACTGCCACTCTATCGAAAAGCCAACAAATCCTCCAAGTTAGTATCAGCCA
▶ **I**leSerGluLysThrSerAspGlnIeHisPhePhePheAlaLysLeuAsnCysArgLeuTyrArgLysAlaAsnLysSerSerLysLeuValSerAlaA
ATCGCCTTTTTGGAGACAAATCCCTTACCTTCAATGAGACCTACCAGGACATCAGTGAGTTGGTATATGGAGCCAAGCTCCAGCCCTGGACTTCAAGGA
▶ **snArgLeuPheGlyAspLysSerLeuThrPheAsnGluThrTyrGlnAspIeSerGluLeuValTyrGlyAlaLysLeuGlnProLeuAspPheLysGI**
AAATGCAGAGCAATCCAGAGCGCCATCAACAAATGGGTGTCCAATAAGACCGAAGGCCGAATCACCGATGTCATTCCTCGGAAGCCATCAATGAGCTC
▶ **uAsnAlaGluGlnSerArgAlaAlaIeAsnLysTrpValSerAsnLysThrGluGlyArgIeThrAspValIeProSerGluAlaIeAsnGluLeu**

HpaI (1315)
ACTGTTCTGGTGTGTTAACACCATTTACTTCAAGGGCTGTGGAAGTCAAAGTTCAGCCCTGAGAACAAGGAAGGAACTGTTCTACAAGGCTGATG
▶ **ThrValLeuValLeuValAsnThrIeTyrPheLysGlyLeuTrpLysSerLysPheSerProGluAsnThrArgLysGluLeuPheTyrLysAlaAspG**
GAGAGTCGTGTTACAGATCTATGATGTACCAGGAAGCAAGTTCGGTATCGCGCGTGGCTGAAGGCACCCAGGTGCTTGGTTGCCCTTCAAAGGTGA
▶ **lyGluSerCysSerAlaSerMetMetTyrGlnGluGlyLysPheArgTyrArgValAlaGluGlyThrGlnValLeuGluLeuProPheLysGlyAs**

NcoI (1508) **MscI (1544)**
TGACATACCATTGGTCTCATCTTGCCCAAGCCTGAGAAGAGCCTGGCCAAGTGTAGAGAAGGAAGTCAACCCAGAGGTGCTGCAAGAGTGGCTGGATGAA
▶ **pAspIeThrMetValLeuIeLeuProLysProGluLysSerLeuAlaLysVaIGluLysGluLeuThrProGluValLeuGlnGluTrpLeuAspGlu**
TTGGAGAGATGATGCTGGTGGTCCACATGCCCGCTTCCGCTTGGAGACGGCTTCAAGTTGAAGGAGCAGCTGCAAGACATGGGCCTTGTCCGATCTGT
▶ **LeuGluGluMetMetLeuValValHisMetProArgPheArgIeGluAspGlyPheSerLeuLysGluGlnLeuGlnLeuGlnLeuGlnLeuGlnLeuP**
TCAGCCCTGAAAAGTCCAACTCCAGGTATTGTTGCAGAAGGCCGAGATGACCTCTATGTCTCAGATGCATTCCATAAGGCATTTCTTGAGGTAATGA
▶ **heSerProGluLysSerLysLeuProGlyIeValAlaGluGlyArgAspAspLeuTyrValSerAspAlaPheHisLysAlaPheLeuGluValAsnGI**
AGAAGGCAGTGAAGCAGCTGCAAGTACCCTGTTGTGATGTGTCGCTTCCGCTTAAACCCCAACAGGGTACTTCAAGGCCAACAGGCCTTTCCTGGTT
▶ **uGluGlySerGluAlaAlaAlaSerThrAlaValValIeAlaGlyArgSerLeuAsnProAsnArgValThrPheLysAlaAsnArgProPheLeuVal**
TTTATAAGAGAAGTTCCCTGAACTATTATCTCATGGCCAGAGTAGCCAAACCCTTGTGTTAAGTAAATGTTCTTATTCTTTGCACCTCTTCTCTATT
▶ **PheIeArgGluValProLeuAsnThrIeIeIePheMetGlyArgValAlaAsnProCysValLys•••**

NheI (2004)
TTTGGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACAACCTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA
HpaI (2167)
TTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAAACAACAATTCGATTCAATTTATGTTT

PacI (2274)
CAGGTTACAGGGGAGGTGTGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAGATCATTAAATGTTAATTAAAGCATGTGAGCAAAGG
CCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTGTGCGGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG
AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCGGCTTACCGGATACC

ApaLI (2598)
TGTCGCCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCCGTTGAGGTCGTTCCGCTCCAAGCTGGGCTGTGT
GCACGAACCCCGCTTACGCCCACCGCTGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCC
ACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGTATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTGTTTGGAA
GCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATT

PacI (3014) **BsrGI (3024)**
TTGGTCATGGCTAGTTAATTAAGCTGTACACTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCAAGGCTCCCAAGCAGGCAGAAGTATGCAAAGCA
TGCATCTCAATTAGTCAGCAACCGGTTGTGAAAGTCCCAAGGCTCCCAAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGT

NcoI (3260)
CCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCGCCCATTTCTCCGCCATGGCTGACTAATTTTTTTTATTTATGAGAGGCCGAG

AvrII (3353)

SmaI (3374)

GCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCG

BbrPI (3411) AseI (3423)

GATCTGATcagCACGTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAATCATGAAGACCTTCAACATC

TCTCAGCAGGATCTGGAGCTGGTGGAGGTGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGA

▶ SerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysT

CTGGGGAGATCATCTCTGCTGCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGCA

▶ hrGlyGluI leI leSerAlaValHisI leGluAlaTyrI leGlyArgValThrValCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGI

BstXI (3759)

GAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCCGTGGCATGTGCAGAGAGCTC

▶ nLysAspPheAspThrI leValAlaValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeu

ATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGGAACTCATCCCCCTCAAGTACACCAGGA

▶ I leSerAspTyrAlaProAspCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgA

EcoRI (3909) Bsp120I (3921)

ACTAAACCTGAATTCGCTAGAGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCT

▶ sn•••

GTTGTTTGCCCTCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCACTGTCTTTTCTAATAAAATGAGGAAATGCATCGCATTGTCTGAGTA

EagI (4196)

Bsp120I (4179)

NotI (4195)

FspI (4173)

XhoI (4190)

GGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCGCAGGGCCCAATTGCTCGAGCGGC

CGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACA

AACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA