

pBLAST2-mEndo XVIII::Angio

A plasmid expressing the mouse Endostatin 18::Angiostatin fusion gene

Catalog # pbla2-mendoangio

For research use only

Version # 10B10-MM

PRODUCT INFORMATION

Contents:

- 20 µg of lyophilized pBLAST2-mEndo-Angio plasmid DNA.
- 4 pouches of *E. coli* Fast-Media® Blast (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 sequencing.

GENERAL PRODUCT USE

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 pBLAST, a family of plasmids expressing human or murine angiostatic genes should simplify the production of angiostatic proteins *in vitro* and *in vivo*.

pBLASTs may be used 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.

pBLAST carries a single antibiotic resistance gene, blasticidin, which allows very rapid and convenient selection of both bacteria and mammalian cell transformants.

PLASMID FEATURES

- **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². EF-1α is a 'housekeeping' gene ubiquitously expressed in eukaryotic cells. The EF-1α promoter exhibits a strong activity, higher than viral promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of DNA and RNA.
- **mEndo XVIII::Angio**
Intronless ORF from the ATG to the stop codon
ORF size (bp): 1731
Cloning fragment size (bp): 1751
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr (blasticidin resistance gene):** The *bsr* gene from *B. 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 to allow blasticidin selection in both mammalian cells and *E. coli* bacteria.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription³.

References

1. Kim *et al.* (1990). *Gene* 2: 217-223.
2. Takebe *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. 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 H₂O. 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-l, 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.

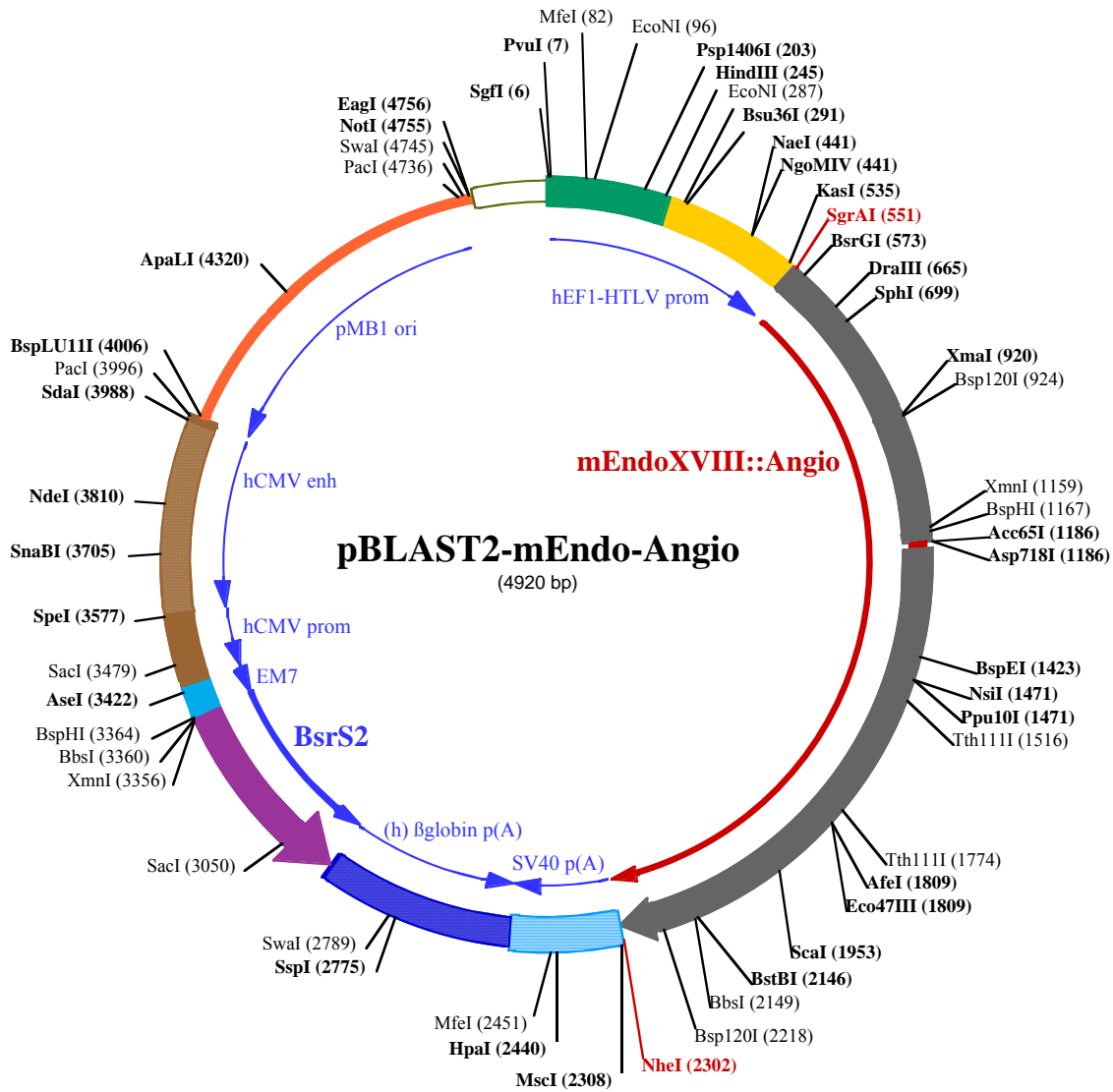
Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

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125

PvuI (7)
SgfI (6) MfeI (82) EcoNI (96)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTAAGTCTGGCTCCGCTTTTCCCGAGGGTGGGGGAGAACCGGTATATAAGTGCAGTAGTCGGC

Bsu36I (291)

Psp1406I (203) **HindIII (245)** EcoNI (287)

201 GTGAACGTTCTTTTCGCAACGGGTTTGCCTCCAGAACACACAGCTGAAGCTTCGAGGSGCTCGCATCTCTCCTTACCGCGCCCGCCCTACCTGAGGCC

301 GCCATCCACGCGGTTGAGTTCGCGTCTCTCCGCTCCCGCTGTGGTGCCTCTGAAGTGCCTGCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMIV (441)
NaeI (441)

401 GGGCCTTTGTCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

KasI (535) **SgrAI (551)** **BsrGI (573)**

501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTGTGCA

1 M Y R M Q L L S C

DraIII (665) **SphI (699)**

601 TTGCACTAAGTCTTGCACACTTGTACGAAATTCGGCTCATACTCATCAGGACTTTTCAGCCAGTGTCTCCACTGGTGGCACTGAACACCCCTGTCTGGAGG

10 I A L S L A L V T N S A H T H Q D F Q P V L H L V A L N T P L S G G

701 CATGCGTGGTATCCGTGGAGCAGATTTCCAGTGTCTCCAGCAAGCCGAGCCGAGCGGGCTGTCCGGCACCTTCCGGGCTTCTCTGTCTCTAGGCTGCAG

43 M R G I R G A D F Q C F Q Q A R R V G L S G T F R A F L S S R L Q

801 GATCTCTATAGCATCGTGCCTGTGTGACCGGGGTTCTGTGCCCATCGTCAACCTGAAGGACGAGGTGTATCTCCAGCTGGGACTCCCTGTTTCTGT

77 D L Y S I V R R A D R G S V P I V N L K D E V L S P S W D S L F S

Bsp120I (924)
XmaI (920)

901 GCTCCCGAGGTCACACTGCAACCCGGGCGCCGATCTTTTCTTTTGAAGCGCAGAGATGTCTGAGACACCCAGCCTGGCCGAGAGAGCGTATGGCACGG

110 G S Q G Q L Q P G A R I F S F D G R D V L R H P A W P Q K S V W H G

1001 CTCGGACCCAGTGGCGGAGGCTGATGGAGAGTTACTGTGAGACATGGCGAACTGAAACTACTGGGCTACAGGTGAGGCTCCTCCTGTCTGTGAGGC

143 S D P S G R R L M E S Y C E T W R T E T T G A T G Q A S S L L S G

BspHI (1167) **Asp718I (1186)**
XmnI (1159) **Acc65I (1186)**

1101 AGGCTCTGGAACAGAAAGCTGCGAGCTGCCACAACAGCTACATCGTCTGTGCATTGAGAATAGCTTCATGACCTCTTCTCCAAAGTACCCGGAGTAG

177 R L L E Q K A A S C H N S Y I V L C I E N S F M T S F S K V P G V

1201 GTACGAATTCGGTGTATCTGTCAAGATTAAGACCGGCATCGGCAACCGCTACAGAGGAACCATGTCCAGGACAAAGAGTGGTGTTCCTGTCAAAGTGT

210 G T N S V Y L S E C K T G I G N G Y R G T M S R T K S G V A C Q K W

1301 GGGTGCACAGTTCCTCCACGTCACCAACTACTCTCCAGTACACATCCCAATGAGGGACTAGAAGAGAACTACTGTAGGAACCCAGACAATGATGAACAA

243 G A T F P H V P N Y S P S T H P N E G L E E N Y C R N P D N D E Q

Ppu10I (1471)
BspEI (1423) **NsiI (1471)**

1401 GGGCCTTGGTGTACTACTACAGATCCGGACAAGAGATATGACTACTGCAACATTCCTGAATGTGAAGAGGAATGCATGTACTGCAGTGGAGAAAAGTATG

277 G P W C Y T T D P D K R Y D Y C N I P E C E E E C M Y C S G E K Y

Tth1111 (1516)

1501 AGGGCAAATCTCCAAGACCATGTCTGGACTTACTGTCAGGCGCTGGGATTTCTCAGAGCCACATGCTCATGGATACATCCCTGCCAAATTTCCAAGCAA

310 E G K I S K T M S G L D C Q A W D S Q S P H A H G Y I P A K F P S K

1601 GAACCTGAAGATGAATATTGCGCAACCTGACGGGGAGCAAGGCCCTGGTGTTCACAAACAGACCCCAACAACTGGGAATACTGTGACATCCCTCC

343 N L K M N Y C R N P D G E P R P W C F T T D P T K R W E Y C D I P

Tth1111 (1774)

1701 CGCTGCACAACACCCCGCCCGCCAGCCCAACCTACCAATGTCTGAAAGAGAGGTGAAAATTACCGAGGACCGTGTCTGTACCGTGTCTGGGA

377 R C T T P P P P P S P T Y Q C L K G R G E N Y R G T V S V T V S G

Eco47III (1809)
AfeI (1809)

1801 AAACCTGTACAGCTGGAGTGGAGCAACCCCTCATAGGCACAAACAGGACACCAGAAAATTTCCCTGCAAAAATCTGGAAGAGAACTACTGCCGGAACCC

410 K T C Q R W S E Q T P H R H N R T P E N F P C K N L E E N Y C R N P

ScaI (1953)

1901 AGATGGAGAAACTGCTCCCTGGTGTATACCACTGACAGCCAGCTGAGGTGGGAGTACTGTGAGATTCATCCTGCGAGTCCCTCAGCATCACCAGACCAG

443 D G E T A P W C Y T T D S Q L R W E Y C E I P S C E S S A S P D Q

2001 TCAGATTCTCAGTTCACCCAGAGGACAAACACCTGTGGTCCAGGAATGCTACCAGAGCGATGGGCAGAGTATCCGGGTACATCGTCCACTACCATCA

477 S D S S V P P E E Q T P V V Q E C Y Q S D G Q S Y R G T S S T T I

BbsI (2149)
BstBI (2146)

2101 CAGGGAAGAAGTGCAGCTCTGGGCAGCTATGTTTCCACACAGGCATTTCGAAAGACCCAGAGAACTTCCAGATGCTGGCTTGGAGATGAACACTGCAG

510 T G K K C Q S W A A M F P H R H S K T P E N F P D A G L E M N Y C R

Bsp120I (2218)

2201 GAACCCGGATGGTGACAAGGGCCCTTGGTGTACACCACTGACCCGAGCGTCAAGTGGGAATACTGCAACCTGAAGCGGTGCTCAGAGAAGGAGGAGT

543 N P D G D K G P W C Y T T D P S V R W E Y C N L K R C S E T G G S

MscI (2308)
NheI (2302)

2301 TAGCTAGCTGGCCAGACATGATAAGATACATTTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTTATTGTAATTTGTGATGC

577 •

HpaI (2440) MfeI (2451)

2401 TATTGCTTTTATTGTAACCATATAAGCTGCAATAAACAAGTTAAACAACAACAATTCGATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTT

2501 TTTTAAAGCAAGTAAACCTCTACAATGTGGTATGCAATTTCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTT

2601 CTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATT

SspI (2775) SwaI (2789)

2701 TTCCCAAGGTTTGAAGTCTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTGTAGTAAAATATTCAGAAATAATTTAAATACA

2801 TCATTGCAATGAAAATAAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAAACAAAG

2901 GAACCTTTAATAGAAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTTGAGGGGGATGAGTTCCCTCAATGGTGGTTTTGACCAGC

141 ◀ • N R T Y K L P I L E E I T T K V L

SacI (3050)

3001 TTGCCATTTCATCTCAATGAGCACAAAGCAGTCCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTGCCA

122 ◀ K G N M E I L V F C D P A Y D S I L E R C M G C P S V V R I S R D V

3101 CCTCATCAGAGTAGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCCAATGGCAATGGCTTCAGCACAGACAGT

89 ◀ E D S Y P H R V A V I T D F D K Q G N S V A S G I A I A E A C V T

3201 GACCTTCCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCTCTGATGGCCGCCCGACATGGTGTCTTGTCTCATAGAGC

56 ◀ V R G I Y A E I H V A S I I E G T K T R I A A G V H H K N D E Y L

BspHI (3364)

BbsI (3360)

XmnI (3356)

3301 ATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGCTTTCATGATGGCCCTCCTATAGTGGAGTCTATTATACT

22 ◀ M T I K E T A V E V L E L D Q Q S I N F T K M

AseI (3422)

SacI (3479)

3401 ATGCCGATATACTATGCCGATGATTAATTTGTCAAACACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGACCT

SpeI (3577)

3501 CCCACCGTACACGCCCTACCGCCCAATTTGCGTCAATGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAACCAAACCTCCCAAT

3601 GACGTCATGGGGTGGAGACTTGGAAATCCCGTGGTCAAACCGCTATCCACGCCATTGATGTAAGTACTGCCAAAACCGCATCATCATGGTAATAGCGATG

SnaBI (3705)

3701 ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTCAATGTAAGTGGGCGGATAATGCCAGGCGGGCCATTTACCGTCAATGACGTCATAGGGG

NdeI (3810)

3801 GCGTACTTGGCATATGATACACTTGTATGTAAGTGGGCGGAGTTTACCGTAAATACTCCACCCATTGACGTCATGAAAGTCCCTATTGGCGTTAC

PacI (3996)

SdaI (3988)

3901 TATGGGAACATACGTCAATTTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCCTGCAGGTTAA

BspLU11I (4006)

4001 TTAAGAACATGTGAGCAAAAGGCAGCAAAAGGCAGGAAACCGTAAAAAGGCAGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCA

4101 CAAAAATCGACGCTCAAGTCAAGTGGGCGAAACCGCAGGACTATAAAGATACCAGGCGTTTCCCGTGGAAAGCTCCCTCGTGGCTCTCTCTGTTCCG

4201 ACCCTGCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGG

ApaLI (4320)

4301 TTCGTCCTCAAGCTGGGCTGTGTGACGAACCCCGTTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGGTCCAACCCGGTAAGACACGA

4401 CTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTTGAAGTGGTGGCCTAACTACGGCTAC

4501 ACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTGTAGCTCTTGATCCGGCAACAAACACCGCTGGTA

4601 GCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA

EagI (4756)

PacI (4736) SwaI (4745) NotI (4755)

4701 CGAAAACCTCACGTTAAGGGATTTTGGTCAATGGCTAGTTAAATTAACATTTAAATCAGCGGCCGcaataaaatctttatctttcattacatctgtgtgttg

4801 gttttttgtgtgaatcgtaactaacatacgtctccatcaaaacaaaacgaaacaaaacaaactagcaaaataggctgtccccagtgcaggtgcaggtgc

4901 cagaacatttctctatcgaa