

# pBLAST45-hCALR

An anti-angiogenic plasmid expressing the human Calreticulin gene

Catalog # pbla-hcalr

For research use only

Version # 02H23-SV

## PRODUCT INFORMATION

### Content:

- 20 µg of lyophilized pBLAST-hCALR plasmid DNA.
- 4 pouches of *E. coli* FastMedia™ 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* FastMedia™ Blas at room temperature. FastMedia™ 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

- **EF-1α / eIF4g hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter<sup>1</sup> and the 5' untranslated region of the translation initiation factor eIF4g. 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 eIF4g 5'UTR has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This 5'UTR acts as a translational enhancer when placed upstream of a gene, due to its ability to direct internal initiation<sup>2</sup>.
- **SV40 polyA:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.<sup>3</sup>
- **SpAn:** A synthetic polyadenylation site and a strong pause site are placed downstream of the pMB1 Ori to limit transcriptional interference between both transcriptional units. The synthetic polyA site is based on the highly efficient polyA signal of the rabbit β-globin gene<sup>4</sup>.

### • **Angiostatic hCALR gene**

Size: 1254 bp

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

• **SV40 prom:** 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 pAn:** 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 types<sup>5</sup>. 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- Gan, W., and Rhoads, R.E. (1996). J. Biol. Chem. 271: 623-626.
- 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 H<sub>2</sub>O. Store resuspended plasmid at -20°C.

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

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

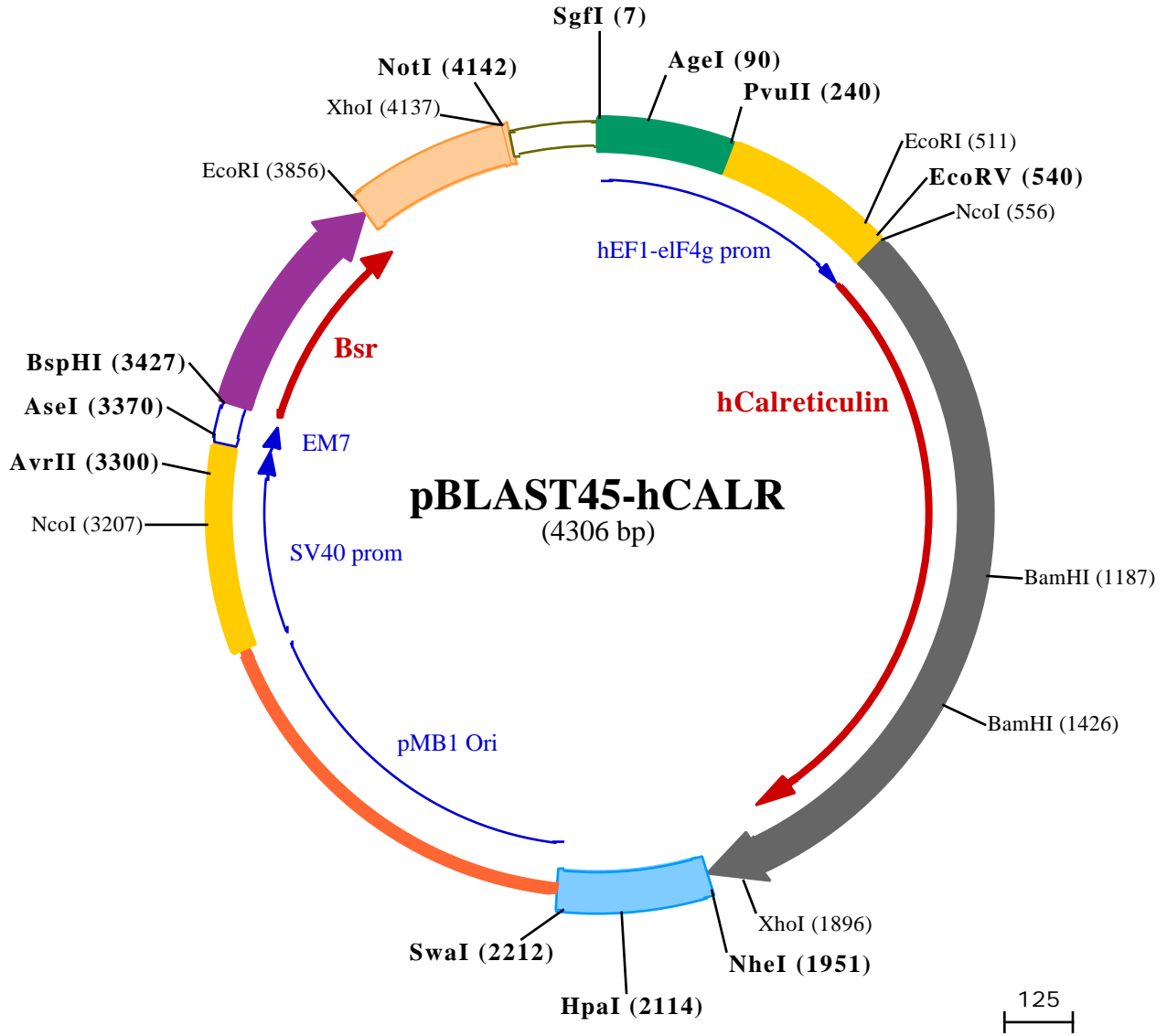
**Note:** Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.

### TECHNICAL SUPPORT

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**SgfI (7)** **AgeI (90)**

1 GGATCTGCATCGCTCCGGTCCCGCTCAGTGGGACAGGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA

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101 GAGAAGGTGGCGCGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCGC

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**PvuII (240)**

201 GTGAACGTTCTTTTTTCGCAACGGGTTTGGCCGACAACACAGCTGGTGGGTAGGGATGAGGGAGGGAGGGGCATTGTGATGTACAGGGCTGCTCTGTGAG

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301 ATCAAGGCTCTCTTAAGGTGGGAGCTGGGGCAGGACTACGAGAGCAGCCAGATGGGCTGAAAGTGAAGTCAAGGGGTTTCTGGCACCTACCTACCTG

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401 CTTCCCGCTGGGGGTGGGGAGTTGGCCAGAGCTTAAAGATTGGGGCAGGGTGGAGAGGTGGGCTCTCTGCTTCCCACTCATCTTATAGCTTTCTTT

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**EcoRI (511)** **EcoRV (540)** **NcoI (556)**

501 CCCCAGATCCGAATTCGAGATCCAAACCAAGGAGGAAAGGATATCACAGAGGAGACCATGGCTCTGCTATCCGTGCGCTGCTGCTCGGCCCTCTCGGCC

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601 TGGCCGTCGCGAGCCTTCCGCTCTACTTCAAGGAGCAGTTTCTGGACGGAGACGGGTGGACTTCCCGCTGGATCGAATCCAAACACAAGTCAGATTTTGG

615 ▶ euAlaValAlaGluProAlaValTyrPheLysGluGlnPheLeuAspGlyAspGlyTrpThrSerArgTrpI leGluSerLysHisLysSerAspPheG

701 CAAATTCGTTCTCAGTTCCGGCAAGTCTACGGTGACGAGGAGAAAGATAAAGGTTTGACAGACAAGCCAGGATGCACGCTTTTATGCTCTGTGCGCCAGT

48 ▶ yLysPheValLeuSerSerGlyLysPheTyrGlyAspGluGluLysAspLysGlyLeuGlnThrSerGlnAspAlaArgPheTyrAlaLeuSerAlaSer

801 TTCGAGCCTTTCAGCAACAAAGGCCAGACGCTGGTGGTGACGTTACGCGTGAACATGAGCAGAACATCGACTGTGGGGGCGGCTATGGAAGCTGTTTC

82 ▶ PheGluProPheSerAsnLysGlyGlnThrLeuValValGlnPheThrValLysHisGluGlnAsnI leAspCysGlyGlyGlyTyrValLysLeuPheP

901 CTAATAGTTTGGACCAGACAGACATGCAGGAGACTCAGAATAACAACATCATGTTTGGCCGACATCTGTGGCCCTGGCAACGAAGGTTTCATGTCAT

115 ▶ roAsnSerLeuAspGlnThrAspMetHisGlyAspSerGluTyrAsnI leMetPheGlyProAspI leCysGlyProGlyThrLysLysValHisVal I I

1001 CTTCAACTACAAGGGCAAGAAGCTGCTGATCAACAAGGACATCCGTTGCAAGGATGATGAGTTTACACACCTGTACACACTGATTGTGCGGCCAGACAAC

148 ▶ ePheAsnTyrLysGlyLysAsnValLeul leAsnLysAspI leArgCysLysAspAspGluPheThrHisLeuTyrThrLeul leValArgProAspAsn

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**BamHI (1187)**

1101 ACCTATGAGGTGAAGATTGACAACAGCCAGGTGGAGTCCGGCTCCTTGAAGACGATTGGGACTTCTGCCACCCAAGAAGATAAAGGATCCTGATGCTT

182 ▶ ThrTyrGluValLysI leAspAsnSerGlnValGluSerGlySerLeuGluAspAspTrpAspPheLeuProProLysLysI leLysAspProAspAlaS

1201 CAAAACCGAAGACTGGGATGAGCGGGCAAGATCGATGATCCACAGACTCCAAGCCTGAGGACTGGGACAAGCCCGAGCATATCCCTGACCCTGATG

215 ▶ erLysProGluAspTrpAspGluArgAlaLysI leAspAspProThrAspSerLysProGluAspTrpAspLysProGluHisI leProAspProAspAl

1301 TAAGAAGCCCGAGGACTGGGATGAAGAGATGGACGGAGAGTGGGAACCCCAAGTATTGAGAACCCCTGAGTACAAGGGTGAAGCCCGGCAGATC

248 ▶ aLysLysProGluAspTrpAspGluGluMetAspGlyGluTrpGluProProVal I leGlnAsnProGluTyrLysGlyGluTrpLysProArgGlnI le

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**BamHI (1426)**

1401 GACAACCCAGATTACAAGGCACTTGGATCCACCCAGAAATTGACAACCCCGAGTATTCTCCGATCCAGTATCTATGCTATGATAACTTTGGCGGTGC

282 ▶ AspAsnProAspTyrLysGlyThrTrpI leHisProGluI leAspAsnProGluTyrSerProAspProSerI leTyrAlaTyrAspAsnPheGlyValI

1501 TGGCCCTGACCTCTGGCAGTCAAGTCTGGCACCATCTTTGACAACCTTCCTATCACCACAGATGAGGCATACGCTGAGGAGTTTGGCAACGAGACGTG

315 ▶ euGlyLeuAspLeuTrpGlnValLysSerGlyThrI lePheAspAsnPheLeuI leThrAsnAspGluAlaTyrAlaGluGluPheGlyAsnGluThrTr

1601 GGGCGTAAACAAGGCACAGACAAATGAAGGACAAACAGGACGAGGACAGAGGCTTAAGGAGGAGGAAGAAGACAAGAAACCAAGAGAGGAGGAG

348 ▶ pGlyValThrLysAlaAlaGluLysGlnMetLysAspLysGlnAspGluGluGlnArgLeuLysGluGluGluGluAspLysLysArgLysGluGluGlu

1701 GAGGCAGAGACAAGGAGGATGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGATGAGGAGGAAGATGCCCCGGCCAGGCCA

382 ▶ GluAlaGluAspLysGluAspAspGluAspLysAspGluAspGluGluAspGluGluAspLysGluGluAspGluGluAspValProGlyGlnAlaI

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**XhoI (1896)**

1801 AGGACGAGCTGTAGAGAGGCCCTGCCTCCAGGGCTGGACTGAGGCTGAGCGCTCCTGCCGAGAGCTTCCCGCCCAATAATGTCTGTGAGACTCGA

415 ▶ ysAspGluLeu•••

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**NheI (1951)**

1901 GAACTTTCATTTTTTCCAGGCTGGTTCGGATTTGGGGTGGATTTTGGTTGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAC

2001 AGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAG

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**HpaI (2114)**

2101 CTGCAATAAACAAGTTAAACAACAATTGCATTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAA

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**SwaI (2212)**

2201 TGTGGTAGATCATTTAAATGTTAATTAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAG

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2301 GCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGC

---

2401 TCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTCGGGAAGCGTGGCGCTTCTCAATGCTCACGCTGTA

---

2501 GGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCAGAACCCCGCTTACGCCGACCGCTGCGCTTATCCGTAACCTATCGTCT

---

2601 TGAGTCCAACCCGTAAGACAGCACTTATCGCCACTGCCAGCAGCCGCTGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTG

---

2701 AAGTGGTGGCTAACTAGGGCTACACTAGAAGAACAGTATTTGGTATCTGTGCTGTGTAAGCCAGTTACTTTCGAAAAAGAGTTGGTAGCTCTTGAT

---

2801 CCGGCAACAAACCACCGCTGTGAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC

---

2901 TACGGGCTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGATTTTGGTCATGGCTAGTTAATTAAGCTGTACACTGTGGAATGTGTGTACGTTAGGG

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3001 TGTGAAAAGTCCCCAGGCTCCCCAGCAGGCAAGTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAAGTCCCCAGGCTCCCCAGCAG

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3101 GCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCGCCCATTC

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**NcoI (3207)** **AvrII (3300)**

3201 TCCGCCCATGGCTGACTAATTTTTTTTTATTTATGTCAGAGGCCGAGGCCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGC

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**AseI (3370)**

3301 CTAGGCTTTTGCAAAAGCTCCCGGAGCTTGTATATCCATTTTCGGATCTGATCAGCACGTGTGACAATTAATCATCGGCATAGTATATCGGCATAGT

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**BspHI (3427)**

3401 ATAATACGACAAGGTGAGGAATAATCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTC

▶ 1 ▶ MetLysThrPheAsnI leSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysI leThrMetLeu

3501 TATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTG  
25 ▶ TyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI leSerAlaValHisI leGluAlaTyrI leGlyArgValThrV  
3601 TCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGA  
58 ▶ alCysAlaGluAlaI leAlaI leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrI leValAlaValArgHisProTyrSerAspGluValAs  
3701 CAGATCCATCAGGGTGGTCAGCCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTG  
91 ▶ pArgSerI leArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyrAlaProAspCysPheValLeuI leGluMetAsnGlyLysLeu  
EcoRI (3856)  
3801 GTCAAAACCACCATTGAGGAACTCATCCCCCAAGTACACCAGGAACTAAACCTGAATTCGCTAGAGGGCCCTATTCTATAGTGTACCTAAATGCTAG  
125 ▶ ValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgAsn•••  
3901 AGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCTTTGACCCTGGAAGGTGCCACTCCCCT  
4001 GTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATT  
NotI (4142)  
XhoI (4137)  
4101 GGGGAGACAATAGCAGGCATGCGCAGGGCCCAATTGCTCGAGCGGCCCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAA  
4201 TCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTCTCT  
4301 ATCGAA