

# pBOOST3-mTBK1

New DNA vaccine adjuvant of the pBOOST3 plasmids expressing the mouse TBK1 gene

Catalog # pbst3-mtbk1

For research use only

Version 20K16-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of lyophilized pBOOST3-mTBK1 plasmid expressing the mouse TBK1 gene
- 1 ml of Zeocin™ (100 mg/ml)

### Shipping and storage:

Products are shipped at room temperature.

Lyophilized DNA is stable for 12 months when stored at -20°C. Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles.

Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

### Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing.

Plasmid DNA was purified by ion exchange chromatography and lyophilized.

## GENERAL PRODUCT USE

The pBOOST3 plasmid was developed as a genetic adjuvant for DNA vaccines to potentiate the immune response to a specific antigen. The plasmid contains the mouse TANK-binding kinase 1 (mTBK1) gene. TBK1, a non-canonical IκB kinase, was shown to mediate the adjuvant effect of DNA vaccines<sup>1</sup>. Administration of DNA vaccines induces the production of type I interferons and inflammatory cytokines in a CpG-independent manner but in TBK1-dependent manner<sup>1</sup>.

The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) response<sup>2</sup>. A DNA vaccine incorporated with genetic adjuvant such as the MyD88 or the TRIF gene has been shown to enhance immune responses<sup>3</sup>. As TBK1 has been shown to play a crucial role in humoral responses, coadministration of a TBK1-expressing plasmid is expected to further boost DNA vaccine-induced immunogenicity.

## PLASMID FEATURES

### • mTBK1

TBK1 signaling is thought to be critical for optimal humoral response as well as for helper T (Th)1 cytokine production after DNA vaccination<sup>1</sup>.

• **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter<sup>4</sup> and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat<sup>5</sup>. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

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

- **Ori pMB1** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Sh ble** : The *Sh ble* gene from *Streptoalloteichus hindustanus* encodes a small protein that confers resistance to Zeocin™ by binding to the antibiotic.

### References:

1. Ishii KJ. *et al.*, 2008. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. *Nature*. 451:725-729.
2. Robinson HL., 1999. DNA vaccines: basic mechanism and immune responses (Review). *Int J Mol Med*. 4(5):549-55.
3. Takeshita F. *et al.*, 2006. Toll-like receptor adaptor molecules enhance DNA-raised adaptive immune responses against influenza and tumors through activation of innate immunity. *J. Virol*. 80:6218-6224.
4. Kim, D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223.
5. Takebe, Y. *et al.*, 1988. R alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol*. 1: 466-472.

## METHODS

### **Plasmid resuspension**

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H<sub>2</sub>O. Store resuspended plasmid at -20 °C.

### **Plasmid amplification and cloning**

Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

### **Zeocin™ usage**

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

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## **Intramuscular inoculation**

### Plasmid DNA solution

- Prepare the vaccine plasmid solution by resuspending 10 µg of the vaccine plasmid DNA in 50 µl saline solution.
- Prepare the pBOOST3 solution by mixing 10 µg of pBOOST3-mTBK1 and 90 µg of the mock plasmid pBOOST3-null in 50 µl saline solution for low dose, or 100 µg of pBOOST3-mTBK1 in 50 µl saline solution for high dose.
- Combine both solutions to obtain a total of 110 µg DNA in 100 µl saline solution.

*Note: The quantities are per mouse.*

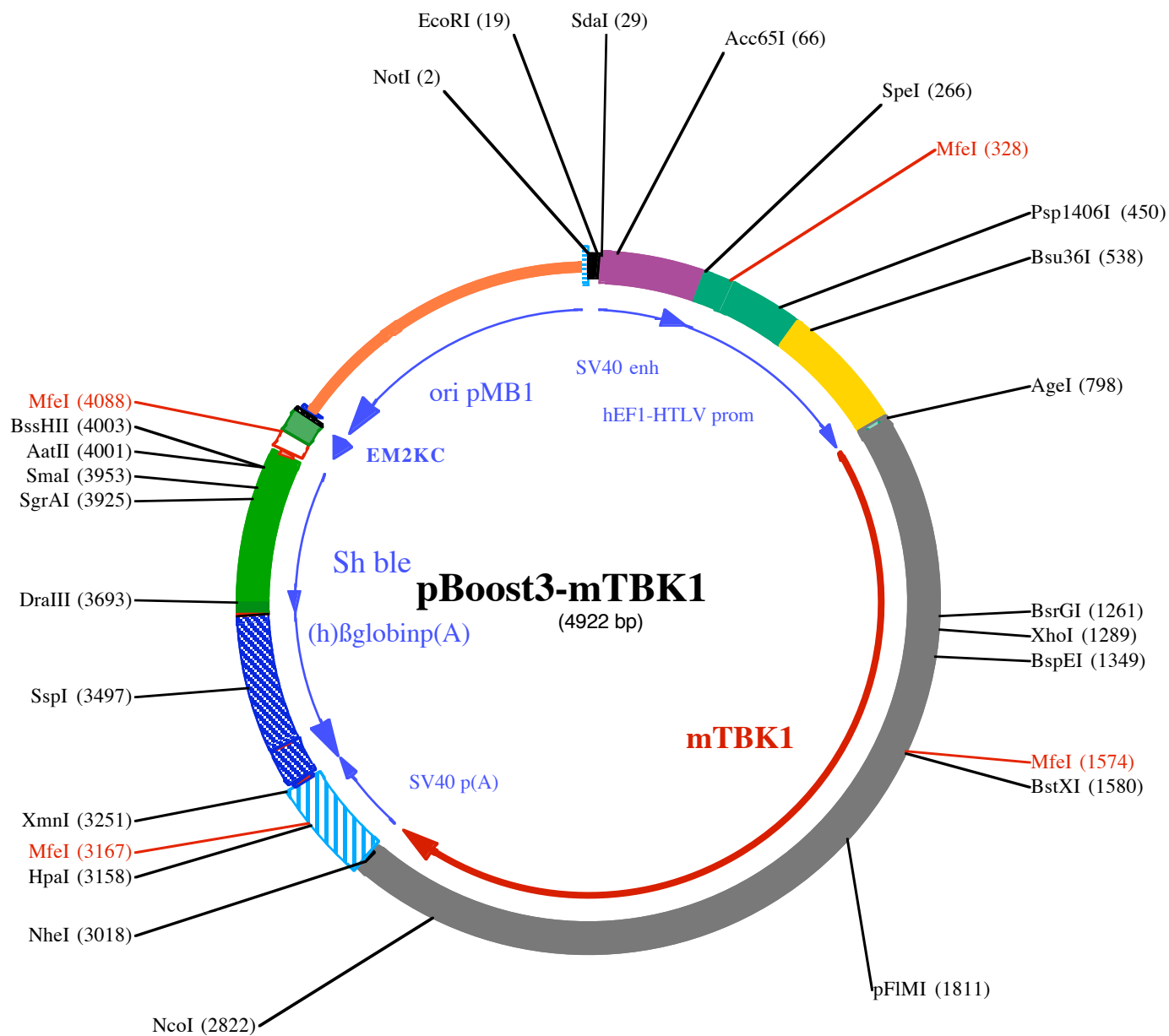
### Intramuscular injections

- Inoculate 6 to 8-week old female BALB/c mice with 100 µl plasmid DNA solution (described above) into the quadriceps at 0 and 4 weeks.
- Collect sera and analyze for antibodies at 8 weeks.

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NotI (2) SdaI (29) EcoRI (19) Acc65I (66)  
GCGGCCGCTATGCATCTAGAATTCCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAG

GCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGC  
AAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCA

SpeI (266)  
TGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTT  
GGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAAGTGTATGTCGTGTACTG

Psp1406I (450)  
GCTCCGCTTTTTCCCAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGG

Bsu36I (538)  
GTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCGCTACCTGAGGCCG  
CCATCCACGCCGGTTGAGTCGCGTTTGTCCGCCTCCCGCTGTGGTGCCTCCTGAACTGCGTCCGCGCTTAGGTAAG  
TTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCT  
TTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

AgeI (798)  
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GGGGCCACTGCAAATGTCTTCCGAGGAAGGCATAAGAAAACCTGGTGATCTCTATGCTGTCAAAGTATTTAATAACATA  
▶ Gl yAl aThr Al aAsnVal PheArgGl yA rgHi sLysLysThr Gl yAspLeuTyrAl aVal LysVal PheAsnAsnI l e  
AGCTTCCTTCGCCAGTGGATGTTCAAATGAGAGAATTTGAAGTGTTAAAAAACTCAATCACAAAAACATTGTCAAG  
▶ Ser PheLeuArgP roVal AspVal Gl nMetArgGl uPheGl uVal LeuLysLysLeuAsnHi sLysAsnI l eVal Lys  
TTATTTGCTATTGAAGAGGAGACAACAACAAGACATAAAGTGCTTATTATGGAGTTTTGTCCCTGTGGGAGTTTATAC  
▶ LeuPheAl a l eGl uGl uGl uThr Thr Thr ArgHi sLysVal Leu l eMe tGl uPheCysP roCysGl ySer LeuTyr  
ACTGTTCTAGAGGAGCCGTCGAATGCGTATGGACTTCCAGAATCAGAATTTCTCATTGTCTTACGAGATGTGGTGGGC  
▶ Thr Val LeuGl uGl uP roSerAsnAl aTyrGl yLeuP roGl uSer Gl uPheLeu l eVal l eLeuArgAspVal Val Gl y  
GGGATGAATCATCTCCGAGAGAACGGCATAGTGCACCGAGATATCAAGCCAGGCAACATCATGCGCGTCATAGGGGAG  
▶ Gl yMetAsnHi sLeuArgGl uAsnGl y l eVal Hi sArgAsp l eLysP roGl yAsn l eMe tArgVal l eGl yGl u

BsrGI (1261) XhoI (1289)  
GACGGCCAGTCTGTGTACAACTCACGGATTTTCGGCGCCGCTCGAGAGCTGGAGGACGATGAGCAGTTTGTGTCTCTG  
▶ AspGl yGl nSer Val TyrLysLeuThrAspPheGl yAl aAl aArgGl uLeuGl uAspAspGl uGl nPheVal Ser Leu

BspEI (1349)  
TACGGCACAGAAGAGTACCTGCATCCGGACATGTATGAAAGGGCAGTGCTAAGAAAGGACCATCAGAAGAAGTACGGG  
▶ TyrGl yThr Gl uGl uTyrLeuHi sProAspMetTyrGl uArgAl aVal LeuArgLysAspHi sGl nLysLysTyrGl y  
GCTACCGTTGATCTGTGGAGTGTGGAGTGACATTCTACCATGCAGCCACGGGTGCTGCCGTTTAGACCCTTCGAG  
▶ Al aThr Val AspLeuTrpSer Val Gl yVal Thr PheTyrHi sAl aAl aThr Gl ySer LeuP roPheArgP roPheGl u  
GGGCCACGGAGGAACAAAGAAGTAATGTATAAAATAATCACTGGGAAGCCGTCTGGTGCAATATCTGGAGTACAGAAA  
▶ Gl yProArgArgAsnLysGl uValMetTyrLys l e l eThr Gl yLysP roSer Gl yAl a l eSer Gl yVal Gl nLys

BstXI (1580)  
GCAGAAAACGGACCAATTGACTGGAGTGGAGACATGCCTCTCTCCTGTAGTCTTTCTCAGGGTCTTCAGGCACTGCTT  
▶ Al aGl uAsnGl yPro l eAspTrpSer Gl yAspMetP roLeuSer CysSer LeuSer Gl nGl yLeuGl nAl aLeuLeu  
ACCCAGTTCTTGCAAACATACTTGAAGCTGATCAGGAGAAGTGCTGGGGTTTTGACCAGTTCTTTGCAGAGACCAGT  
▶ Thr ProVal LeuAl aAsn l eLeuGl uAl aAspGl nGl uLysCysTrpGl yPheAspGl nPhePheAl aGl uThr Ser  
GATGTGCTTACCGAATGGTGATCCATGTCTTCTCGCTACAACACATGACGGCGCATAAGATTTACATTACAGCTAT  
▶ AspVal LeuHi sArgMetVal l eHi sVal PheSer LeuGl nHi sMe tThr Al aHi sLys l eTyr l eHi sSer Tyr

pFIMI (1811)  
AACACTGCTGCTGTGTTCCATGAACTGGTCTATAAAACAAACCAAGATTGTTTCTCAAATCAAGAACTTATCTACGAA  
▶ AsnThrAl aAl aVal PheHi sGl uLeuVal TyrLysGl nThr Lys l eVal Ser SerAsnGl nGl uLeu l eTyrGl u  
GGACGACGCTTAGTCTAGAACTCGGACGACTAGCCAGCATTTCCTAAAACACAGAGGAAAAATCCTATCTTTGTC  
▶ Gl yA rgArgLeuVal LeuGl uLeuGl yA rgLeuAl aGl nHi sPheP roLysThr Thr Gl uGl uAsnP ro l ePheVal  
ACGAGCCGGGAACAACTCAATACCGTAGGACTGAGATATGAAAAATTTCCCTCCCTAAAATACATCCACGCTATGAT  
▶ Thr SerArgGl uGl nLeuAsnThr Val Gl yLeuArgTyrGl uLys l eSer LeuP roLys l eHi sP roArgTyrAsp

CTGGATGGGGACGCCAGCATGGCCAAGGCAGTGACGGGGGTTGTGTGCTACGCTGCAGAAGTCCAGTACCCTGCTG  
▶ LeuAspGlyAspAlaSerMetAlaLysAlaValThrGlyValValCysTyrAlaCysArgThrAlaSerThrLeuLeu  
CTCTATCAAGAATTAATGCGAAAGGGGTACGGTGGCTGGTTGAACTGGTTAAGGATGATTACAACGAGACCGTCCAC  
▶ LeuTyrGlyNgluLeuMetArgLysGlyValArgTrpLeuValGlyLeuValLysAspAspTyrAsnGlyuThrValHis  
AAGAAGACGGAGGTAGTGATCACACTGGATTTCTGCATCAGGAACATTGAGAAGACTGTGAAAGTGTATGAGAAGTTG  
▶ LysLysThrGlyValValIleThrLeuAspPheCysIleArgAsnIleGlyLysThrValLysValTyrGlyLysLeu  
ATGAAGGTCAACCTGGAAGCCGAGAGCTGGGTGAGATTTAGACATACACACCAAGCTGCTGAGACTTTCCAGTTCT  
▶ MetLysValAsnLeuGlyAlaAlaGlyLeuGlyGlyIleSerAspIleHisThrLysLeuLeuArgLeuSerSerSer  
CAGGGAACAATAGAAAGCAGTCTTCAGGACATCAGCAGCAGGCTGTCTCCAGGGGGCTTGGTGGCCGACACCTGGGCA  
▶ GlyNglYthrIleGlyuSerSerLeuGlyAspIleSerSerArgLeuSerProGlyGlyLeuLeuAlaAspThrTrpAla  
CATCAAGAAGGCACGCATCCAAGAGACAGGAATGTAGAAAACTGCAGGTCCTGTTGAACTGCATCACAGAGATTTAC  
▶ HisGlyNgluGlyThrHisProArgAspArgAsnValGlyLysLeuGlyNvalLeuLeuAsnCysIleThrGlyIleTyr  
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▶ TyrGlyNpheLysLysAspLysAlaGlyuArgArgLeuAlaTyrAsnGlyuGlyNleHisLysPheAspLysGlyNlys  
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▶ LeuTyrTyrHisAlaThrLysAlaMetSerHisPheSerGlyuGlyCysValArgLysTyrGlyAlaPheLysAspLys  
TCGGAAGAGTGGATGAGAAAGATGCTTCATCTTAGGAAGCAGCTGTTATCGCTAACTAATCAGTGTTCGATATCGAA  
▶ SerGlyuGlyuTrpMetArgLysMetLeuHisLeuArgLysGlyNleuLeuSerLeuThrAsnGlyNcysPheAspIleGlyu  
GAGGAAGTGTCCAAGTATCAAGACTATACTAACGAGTTACAAGAACTCTGCCTCAGAAAATGCTCGCAGCCTCCGGC  
▶ GlyuGlyValSerLysTyrGlyNaspTyrThrAsnGlyuLeuGlyNgluThrLeuProGlyNlysMetLeuAlaAlaSerGly

NcoI (2822)

GGCGTCAAGCACGCCATGGCCCCGATCTACCCAGCTCTAACACCTTAGTGGAGATGACTCTTGGTATGAAGAAGTTA  
▶ GlyValLysHisAlaMetAlaProIleTyrProSerSerAsnThrLeuValGlyuMetThrLeuGlyMetLysLysLeu  
AAGGAGGAGATGGAAGGCGTGGTTAAGGAGCTGGCCGAGAACAATCATATTTTAGAAAGGTTTGGGTCTTTAAACAATG  
▶ LysGlyuGlyuMetGlyuGlyValLysGlyuLeuAlaGlyuAsnAsnHisIleLeuGlyuArgPheGlySerLeuThrMet

NheI (3018)

GATGGTGGCCTTCGCAATGTGGACTGTCTTTAGCTTCTAGGGAGTCTGGGAAGCTAGCTGGCCAGACATGATAAGAT  
▶ AspGlyGlyLeuArgAsnValAspCysLeu●●●  
ACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTG

HpaI (3158)

CTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAACAACAAATTGCATTCATTTTATGTTTTCAGGTTTCAGG

XmnI (3251)

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TTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTTATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTG

SspI (3497)

AACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAAATAAT  
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DraIII (3693)

TCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTGCGCAGGGCGAACTCCCGCCCCACGG  
▶ ●●●AspGlyNgluGlyAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGlyuArgGlyTrpPro  
CTGCTCGCCGATCTCGGTCTGCGCCGCGCCGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTA  
▶ GlyNgluGlyIleGlyuThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGlyuSerTrpGlyAlaTyr  
CAGCTCGTCCAGGCCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGCTGATGAA  
▶ LeuGlyuAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValGlyNaspGlyNvalAlaSerIlePhe

SgrAI (3925)

SmaI (3953)

CAGGGTCACGTCGTCCCGGACCACCGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGTCCA  
▶ LeuThrValAspAspArgValValGlyAlaPheAspAspGlyuValPheAspArgSerPheGlyLeuArgAspThrTrp

BssHII (4003)

AatII (4001)

GAACTCGACCGCTCCGGCGACGTCGCGCGGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCC  
▶ PheGlyuValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet  
tgtcaggagaggaagagaagaaggtttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCA

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