

pBOOST2-sahIRF7/3

New DNA vaccine adjuvant of the pVAC plasmids expressing a super-activated IRF7/3 chimeric gene

Catalog # pbst2-sahirf73

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBOOST2-sahIRF73 plasmid expressing a human super-activated IRF7/3 chimeric gene
- 1 ml of Zeocin™ (100 mg/ml)

Shipping and storage:

Products are shipped at room temperature.

Lyophilized DNA should be resuspended upon receipt and stored at -20°C.

Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year 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

pBOOST2 plasmids were developed as genetic adjuvants for DNA vaccines to potentiate the immune response to a specific antigen. They feature different genes from the interferon regulatory factor family (IRF). IRFs are transcriptional activators for IFN-α, IFN-β and IFN-stimulated genes. In particular IRF-1, IRF-3 and IRF-7 act as direct transducers of virus-mediated signaling pathways activating IFN-α and IFN-β in infected cells. Recently, IRF-1, IRF-3 and IRF-7 were shown to be able to bias T cells towards type 1 or type 2 immune responses, leading to the activation of cytotoxic T cells and/or the production of antibodies. The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) or type 2 (antibody) response¹. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination^{2,3}.

PLASMID FEATURES

- **sahIRF73** (super-activated human IRF7/3 chimeric gene)
IRF-3 and IRF-7 increase both Th1 T-cell and Th2 antibody responses by transactivating different target promoters². To exploit the biological features of both IRFs, a chimeric form of IRF-7 and IRF-3 was generated by combining the DNA binding specificity of IRF-7 with the strong transactivation capacity of super-activated IRF-3. IRF-7/3 chimera provides >10-fold greater induction of IFN-α and IFN-β promoters than super-activated IRF-3 alone³.
- **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁵. 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** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Sh-ΔCpG (Synthetic Zeocin[®] gene):** The *Sh ble* gene from *Streptoalloteichus hindustanus* encodes a small protein that confers resistance to Zeocin™ by binding to the antibiotic. To reduce the amount of CpG motifs that may skew the raised antigen-specific immune response, pBOOST2 contains a CpG-free allele of the Zeo[®] gene. All CpGs from the wild-type gene (50) were removed by synthesizing a new allele that contains no CpGs but encodes the exact same protein sequence.

References:

1. Robinson HL., 1999. DNA vaccines: basic mechanism and immune responses (Review). *Int J Mol Med.* 4(5):549-55.
2. Sasaki S. *et al.*, 2002. Regulation of DNA-raised immune responses by cotransfected interferon regulatory factors. *J Virol.* 76(13):6652-9.
3. Bramson JL. *et al.*, 2003. Super-activated interferon-regulatory factors can enhance plasmid immunization. *Vaccine.* 21(13-14):1363-70.
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 H₂O. 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

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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 pBOOST2 solution by mixing 10 μg of pBOOST2-sahIRF73 and 90 μg of the mock plasmid pBOOST2-null in 50 μl saline solution for low dose, or 100 μg of pBOOST2-sahIRF73 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.

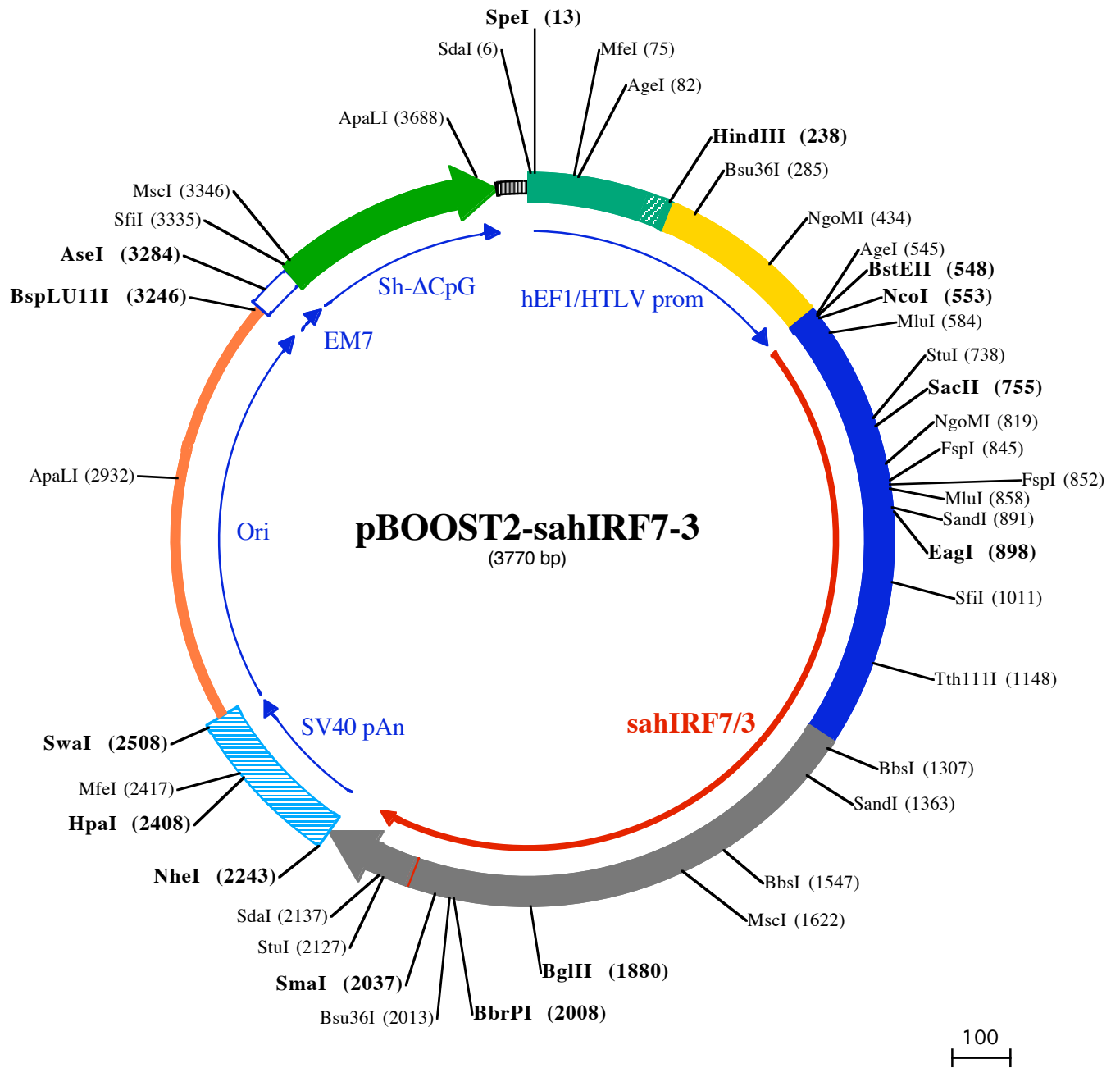
Intramuscular injections

- 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.

Note: For more information see the article by Sasaki S. et al.¹

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SdaI (6) SpeI (13) MfeI (75) AgeI (82)
1 CCTGCAGGGCCCACTAGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGTGCCTAGAGAAGGT
101 GGC CGGGGTAAACTGGAAAGTGATGTCGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGT
HindIII (238) Bsu36I (285)
201 TCTTTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACCGGCCCGCCCTACCTGAGGCCGCATCCA
301 CGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTCCGCTCGCCGCTAGGTAAAGTTAAAGCTCAGGTCGAGACCGGGCCTTT
NgoMI (434)
401 GTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGCTTTTGTTCGTTTTCTGTTCT
NcoI (553)
BstEII (548) MluI (584)
501 GCGCCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCAGTACCATTGGCTTGGCTCCTGAGAGGGACGCCACGCTGCTTCCGGAG
1MetAl aLeuAl aProAl uArgAl aAl aProArgVal LeuPheGl yG
601 AGTGGCTCCTTGAGAGATCAGCAGCGGCTGCTATGAGGGGCTGCAGTGGCTGGACGAGGCCCGCACCTGTTCCGCGTGCCTGGAAGCACTTCGCGCG
16I uTrpLeuLeuGl yGl uI eSer Ser Gl yCysTyrGl uGl yLeuGl nTrpLeuAspGl uAl aArgThr CysPheArgVal P roT rpLysHi sPheAl aAr
StuI (738) SacII (755)
701 CAAGGACCTGAGCGAGGCCGACGCGCATCTTCAAGGCTGGCTGTGGCCGCGGAGGTGGCCGCTAGCAGCAGGGGAGGTGGCCGCCCCCGAG
49I gLysAspLeuSer Gl uAl aAspAl aArgI l ePheLysAl aTrpAl aVal Al aArgGl yArgT rpP roP roSer Ser ArgGl yGl yI yProP roP roGl u
EagI (898)
NgoMI (819) FspI (845) FspI (852) MluI (858) SandI (891)
801 GCTGAGACTGCGGAGCGCGCGGCTGGAAAACCACTTCCGCTGCGCACTGCGCAGCAGCGCTCGCTTCGTGATGCTGCGA GATAACTCGGGGACCCGG
83I aGl uThr Al aGl uArgAl aGl yTrpLysThr AsnPheArgCysAl aLeuArgSer Thr ArgArgPheVal MetLeuArgAspAsnSer Gl yAspProA
901 CCGACCCGACAAGGTGTACGCGCTCAGCCGGAGCTGTGCTGGCGAGAAGGCCAGGCAGGCAGACTGAGGCAGAGGCCCGCAGCTGTCCACC
116I aAspP roHi sLysVal I TyrAl aLeuSer ArgGl uLeuCysT rpArgGl uGl yProGl yThr AspGl nThr Gl uAl aGl uAl aProAl aAl aVal P roP r
SfiI (1011)
1001 ACCACAGGGTGGGCCCCAGGGCCATTCTGGCACACACATGCTGGACTCCAAGCCCCAGGCCCTCCCTGCCCCAGCTGGTGACAAGGGGACCTC
149I oProGl nGl yGl yProP roGl yProPheLeuAl aHi sThr Hi sAl aGl yLeuGl nAl aProGl yProLeuP roAl aProAl aGl yAspLysGl yAspLeu
Tth111I (1148)
1101 CTGCTCAGGCAGTGAACAGAGCTGCCTGGCAGACATCTGCTGACAGCGTCATGGGGGAGATCCAGTCCCAACCAAGGCTCTGGAGAGGGACAAG
183I LeuLeuGl nAl aVal Gl nGl nSer CysLeuAl aAspHi sLeuLeuThr Al aSer TrpGl yAl aAspP roVal P roThr LysAl aP roGl yGl uGl yGl nG
1201 AAGGCTTCCCTGACTGGGCTGTGCTGGAGGCCAGGGCTCCCTGCTGGGAGCTGTACGGTGGCAGTAGAGACGCCCGCAGCCCACTTCTGA
216I uGl yLeuP roLeuThr Gl yAl aCysAl aGl yGl yProGl yLeuP roAl aGl yGl uLeuTyrGl yTrpAl aVal I Gl uThr Thr P roSer P roThr Ser As
BbsI (1307) SandI (1363)
1301 TACCCAGGAAGACATTCTGGATGAGTACTGGTAAATGGTGTGGCCCACTCCAGATCCGGGACCCCAAGCCCTGGCTGTAGCCCTGAGCCCTGC
249I pThr Gl nGl uAspI l eLeuAspGl uLeuLeuGl yAsnMeTVal LeuAl aP roLeuP roAspP roGl yProP roSer LeuAl aVal Al aP roGl uP roCys
1401 CCTCAGCCCCCTGGGAGCCCACTGGACAACTCCCACTCCCTTCCAAACCTGGGGCCCTCTGAGAACCCTGAAGCGGCTGTGGTGGCGGGGGAAG
283I P roGl nP roLeuArgSer P roSer LeuAspAsnP roThr P roPheP roAsnLeuGl yProSer Gl uAsnP roLeuLysArgLeuLeuVal P roGl yGl uG
BbsI (1547)
1501 AGTGGGAGTTCAGGTGACAGCCTTCTACCGGGGCCCAAGTCTCCAGCAGACCATCTCTGCCCGGAGGGCTGCGGCTGGTGGGGTCCGAAGTGGG
316I l uTrpGl uPheGl uVal I Thr Al aPheTyrArgGl yArgGl nVal I LeuGl nGl nThr I l eSer CysP roGl uGl yLeuArgLeuVal Gl ySer Gl uVal I Gl
MscI (1622)
1601 AGACAGGACGCTGCCTGGATGGCCAGTCACTGCCAGACCTGGCATGTCCCTGACAGACAGGGGAGTGATGAGCTACGTGAGGCATGTGCTGAGCTGC
349I yAspArgThr LeuP roGl yTrpP roVal I Thr LeuP roAspP roGl yMetSer LeuThr AspArgGl yVal MetSer TyrVal I r gHi sVal I LeuSer Cys
1701 CTGGTGGGGGACTGGCTCTCTGGCGGGCCGGCAGTGGCTCTGGGCCAGCGGCTGGGGCACTGCCACATACTGGCAGTGAGCGAGGAGCTGCTCC
383I LeuGl yGl yGl yLeuAl aLeuT rpArgAl aGl yGl nTrpLeuT rpAl aGl nArgLeuGl yHi sCysHi sThr TyrT rpAl aVal I Ser Gl uGl uLeuLeuP
BglIII (1880)
1801 CCAACAGCGGCATGGCCCTGATGGCGAGTCCCAAGGACAAGGAAGGAGCGTGTGGACCTGGGGCCCTCATTGTAGATCTGATTACCTTACCGGA
416I r oAsnSer Gl yHi sGl yProAspGl yGl uVal P roLysAspLysGl uGl yGl yVal I PheAspLeuGl yProPheI l eVal I AspLeuI l eThr PheThr Gl
1901 AGGAAGCGGACGCTACCCAGCTATGCCCTCTGGTCTGTGGGGAGTCACTGGCCCAAGGACAGCCGCTGGACCAAGAGGCTCGTGATGGTCAAGGTT
449I uGl ySer Gl yArgSer P roArgTyrAl aLeuT rpPheCysVal Gl yGl uSer TrpP roGl nAspGl nP roT rpThr LysArgLeuVal I MetVal I LysVal
Bsu36I (2013)
BbrPI (2008) SmaI (2037)
2001 GTGCCACGTGCTCAGGCCTTGGTAGAAATGGCCCGGTAGGGGTCCTCCTCCCTGGAGAATACTGTGGACCTGCACATTGACAACGCCACCCAC
483I Val P roThr CysLeuArgAl aLeuVal Gl uMeTAl aArgVal I Gl yGl yAl aSer Ser LeuGl uAsnThr Val AspLeuHi sI l eAspAsnSer Hi sP roL
SdaI (2137)
StuI (2127)
2101 TCTCCCTCACCTCCGACCAGTACAAGGCCTACCTGCAGGACTTGGTGGAGGGCATGGATTTCCAGGGCCCTGGGGAGACTGAGCCCTCGCTCCTCATGG
516I euSer LeuThr Ser AspGl nTyrLysAl aTyrLeuGl nAspLeuVal I Gl uGl yMetAspPheGl nGl yProGl yGl uThr ●●●
NheI (2243)
2201 TGTGCTCCAACCCCTGTTCCCAACCTCAACCAATAAGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCA
2301 GTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATA
HpaI (2408) MfeI (2417)
2401 AACAAGTTAACAACAACAATTGCATTATTATGTTTCAGGTTTCAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTTACAATGTGGTAG
SwaI (2508)
2501 ATCCATTTAAATGTTAATTAAGTACGATGACCAAAATCCCTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTT
2601 CTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGAACAAAAAACCACCGCTACAGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCT
2701 TTTTCCGAAGGTAAGTGGCTTACGACAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCG

2801 CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATA

2901 AGGCGCAGCGGTTCGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
 ApaLI (2932)

3001 AAGCGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCC

3101 TGGTATCTTTATAGTCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCA

3201 ACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCTCACATGTTCTTAATTAATTTTTCAAAGTAGTTGACAATTAATCATCGGCATAGT
 BspLU11I (3246) AseI (3284)

3301 ATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAAGTGTCCAGTGTCTCACAGCCAGGGATGTGGCTGGAGCTG
 SfiI (3335) MscI (3346)

3401 TTGAGTTCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTTCATCTCAGCAGT
 1▶MetAl aLysLeuThr SerAl aVal ProVal LeuThrAl aArgAspValAl aGl yAl aV

3501 CCAGGACCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCACTTC
 20▶a l Gl uPheTrpThrAspArgLeuGl yPheSerArgAspPheVal Gl uAspAspPheAl aGl yVal ValA r gAspAspVal Thr LeuPhe l eSerAl aVa
 53▶l Gl nAspGl nVal Val ProAspAsnThr LeuAl aTrpVal l TrpVal A r gGl yLeuAspGl uLeuTyrAl aGl uTrpSer Gl uVal Val Ser ThrAsnPhe

3601 AGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACCTTTGTGG
 87▶A r gAspAl aSer Gl yProAl aMe tThr Gl ul eGl yGl uGl nP roTrpGl yA r gGl uPheAl aLeuArgAspP roAl aGl yAsnCysVal l Hi sPheVal A

3701 CAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAACCCCGCCCGCGGGTTTTTTGTTAATTAA
 120▶l aGl uGl uGl nAsp●●●
 ApaLI (3688)