pBOOST2-mcs

Negative control plasmid for pBOOST2 DNA vaccine adjuvant plasmids

Catalog # pbst2-mcs

For research use only

Version 20K16-MM

PRODUCT INFORMATION

Content:

• 20 µg of lyophilized pBOOST2-mcs plasmid
• 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 6 months when stored at -20˚C.
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-ß 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-ß 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) response[1]. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination[2, 3].

PLASMID FEATURES

• **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[4] 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[5]. 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*-Δ*CpG* (Synthetic Zeocin^r 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^R 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 H_2O. 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
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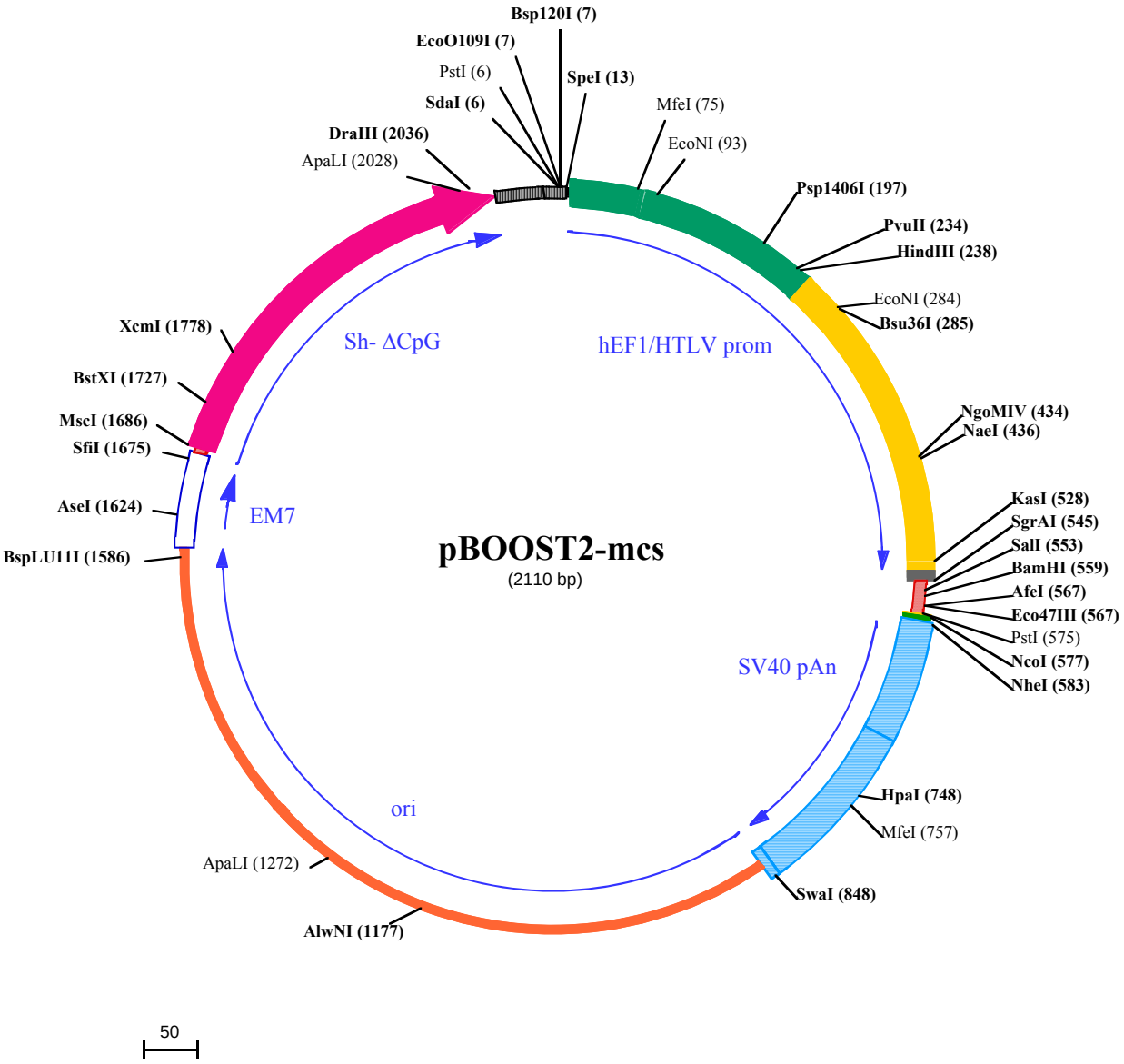
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EcoO109I (7)
PstI (6)
SpeI (13)
SdaI (6)
MfeI (75)
DraIII (2036)
EcoNI (93)
ApaLI (2028)
Psp1406I (197)
PvuII (234)
HindIII (238)
EcoNI (284)
Bsu36I (285)
XcmI (1778)
Sh- ΔCpG
hEF1/HTLV prom
BstXI (1727)
NgoMIV (434)
NaeI (436)
MscI (1686)
SfiI (1675)
KasI (528)
SgrAI (545)
SalI (553)
AseI (1624)
EM7
BamHI (559)
AfeI (567)
BspLU11I (1586)
pBOOST2-mcs
(2110 bp)
Eco47III (567)
PstI (575)
SV40 pAn
NcoI (577)
NheI (583)
HpaI (748)
ori
MfeI (757)
ApaLI (1272)
SwaI (848)
AlwNI (1177)
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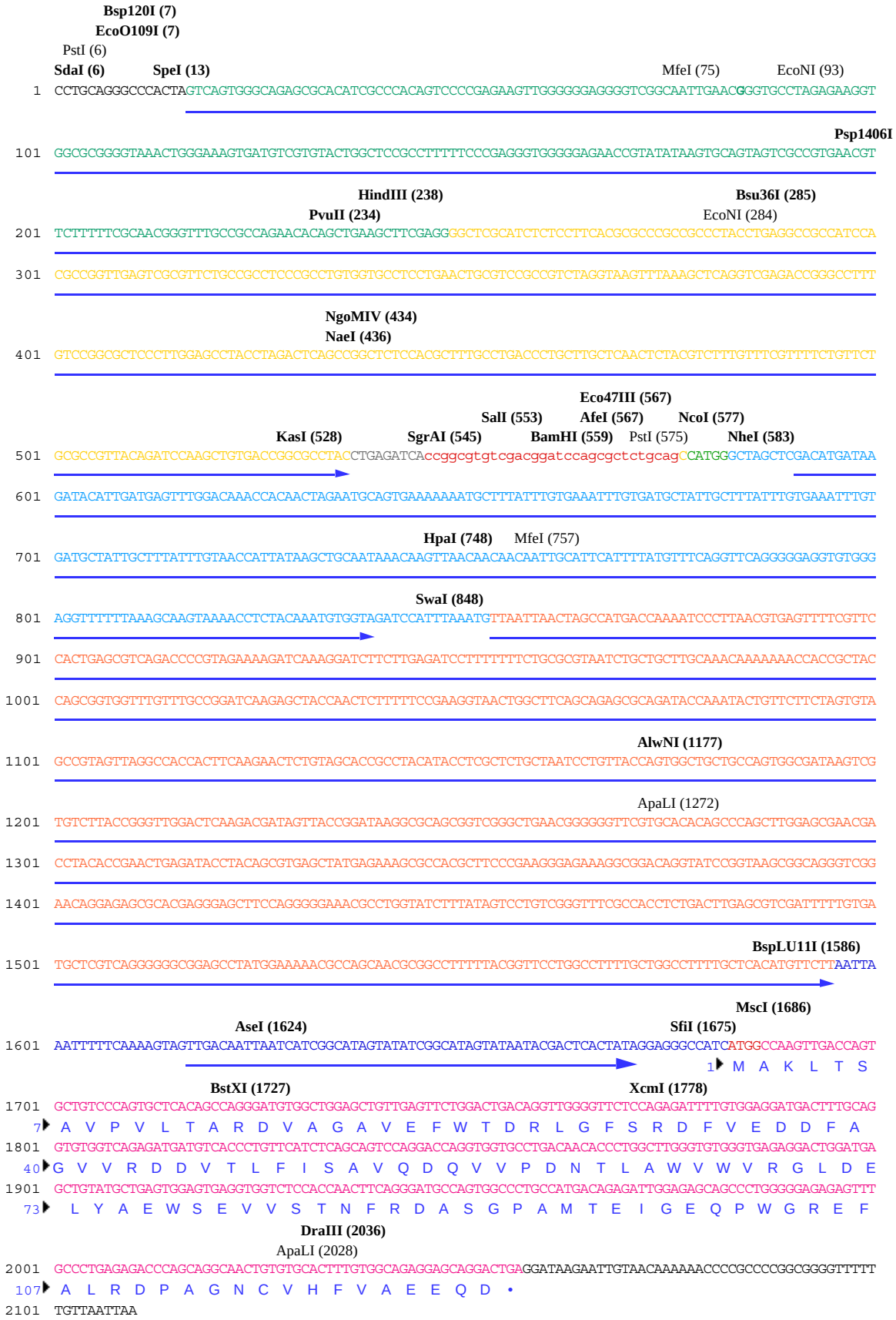
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PstI (6)
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Psp1406I
101 GGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGT
HindIII (238)
Bsu36I (285)
PvuII (234)
EcoNI (284)
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SalI (553)
AfeI (567)
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KasI (528)
SgrAI (545)
BamHI (559)
PstI (575)
NheI (583)
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HpaI (748)
MfeI (757)
701 GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGG
SwaI (848)
801 AGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTC
901 CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTAC
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AlwNI (1177)
1101 GCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCG
ApaLI (1272)
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BspLU11I (1586)
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MscI (1686)
AseI (1624)
SfiI (1675)
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1 M A K L T S
BstXI (1727)
XcmI (1778)
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7 A V P V L T A R D V A G A V E F W T D R L G F S R D F V E D D F A
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40 G V V R D D V T L F I S A V Q D Q V V P D N T L A W V W V R G L D E
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DraIII (2036)
ApaLI (2028)
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2101 TGTTAATTAA