pDUO-mMD2/TLR4

A plasmid coexpressing the murine MD2 and TLR4 genes

Catalog code: pduo-mmd2tlr4

https://www.invivogen.com/pduo-md2-tlr4

For research use only

Version 20H26-MM

PRODUCT INFORMATION

Contents

- 20 µg of pDUO-mMD2/TLR4 provided as DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store blasticidin at 4°C or -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

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors, homologues of the Drosophila Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharides (LPS), peptidoglycans and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade that involves a number of proteins, such as MyD88 and IRAK. This signaling cascade leads to the activation of the transcription factor NF-κB which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

To date ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. In many instances, TLRs require the presence of a co-receptor to initiate the signaling cascade. One example is TLR4 which interacts with MD2 and CD14 to induce NF-κB in response to LPS stimulation.

pDUO is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other.

The genes cloned into pDUO comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

- **Murine MD2** (480 bp) / **Murine TLR4** (2505 bp)

TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionaly interact with LPS[1,2]. TLR4 physically associates with MD2, and together with a third protein called CD14, this complex is responsible for LPS recognition and signaling[3].

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein[4]. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[5].
- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer[6].
- **SV40 pAn:** 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.*[7]
- **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression[8].

TECHNICAL SUPPORT
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• **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 *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.
• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

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

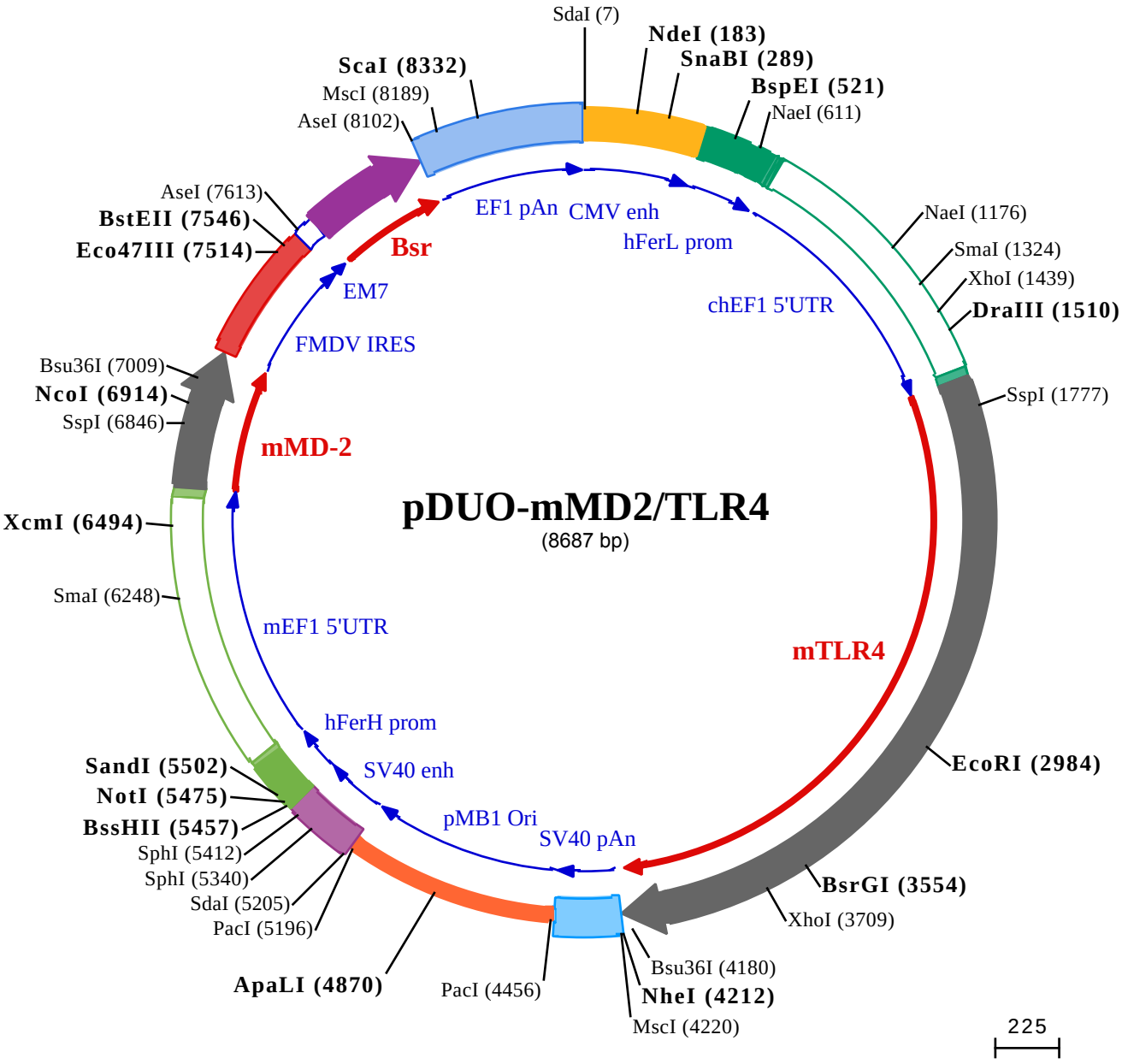
Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

References

1. **Shimazu R. *et al.*, 1999.** MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med, 189(11):1777-82.
2. **Nagai Y. *et al.*, 2002.** Essential role of MD-2 in LPS responsiveness and TLR4 distribution. Nat Immunol. 3(7):667-72.
3. **da Silva Correia J. *et al.*, 2001.** Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex transfer from CD14 to TLR4 and MD-2. J Biol Chem. 276(24):21129-35.
4. **Eisenstein RS. & Munro HN. 1990.** Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58.
5. **Dean DA. *et al.*, 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22.
6. **Boshart M. *et al.*, 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 141(2):521-30.
7. **Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258.
8. **Ramesh N *et al.*, 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700.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SdaI (7)
NdeI (183)
SnaBI (289)
BspEI (521)
NaeI (611)
ScaI (8332)
MscI (8189)
AseI (8102)
AseI (7613)
BstEII (7546)
Eco47III (7514)
Bsr
EF1 pAn
CMV enh
hFerL prom
EM7
chEF1 5'UTR
FMDV IRES
NaeI (1176)
SmaI (1324)
XhoI (1439)
DraIII (1510)
Bsu36I (7009)
NcoI (6914)
SspI (6846)
SspI (1777)
mMD-2
XcmI (6494)
SmaI (6248)
mEF1 5'UTR
mTLR4
hFerH prom
SandI (5502)
NotI (5475)
BssHII (5457)
SV40 enh
pMB1 Ori
SV40 pAn
EcoRI (2984)
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NheI (4212)
MscI (4220)
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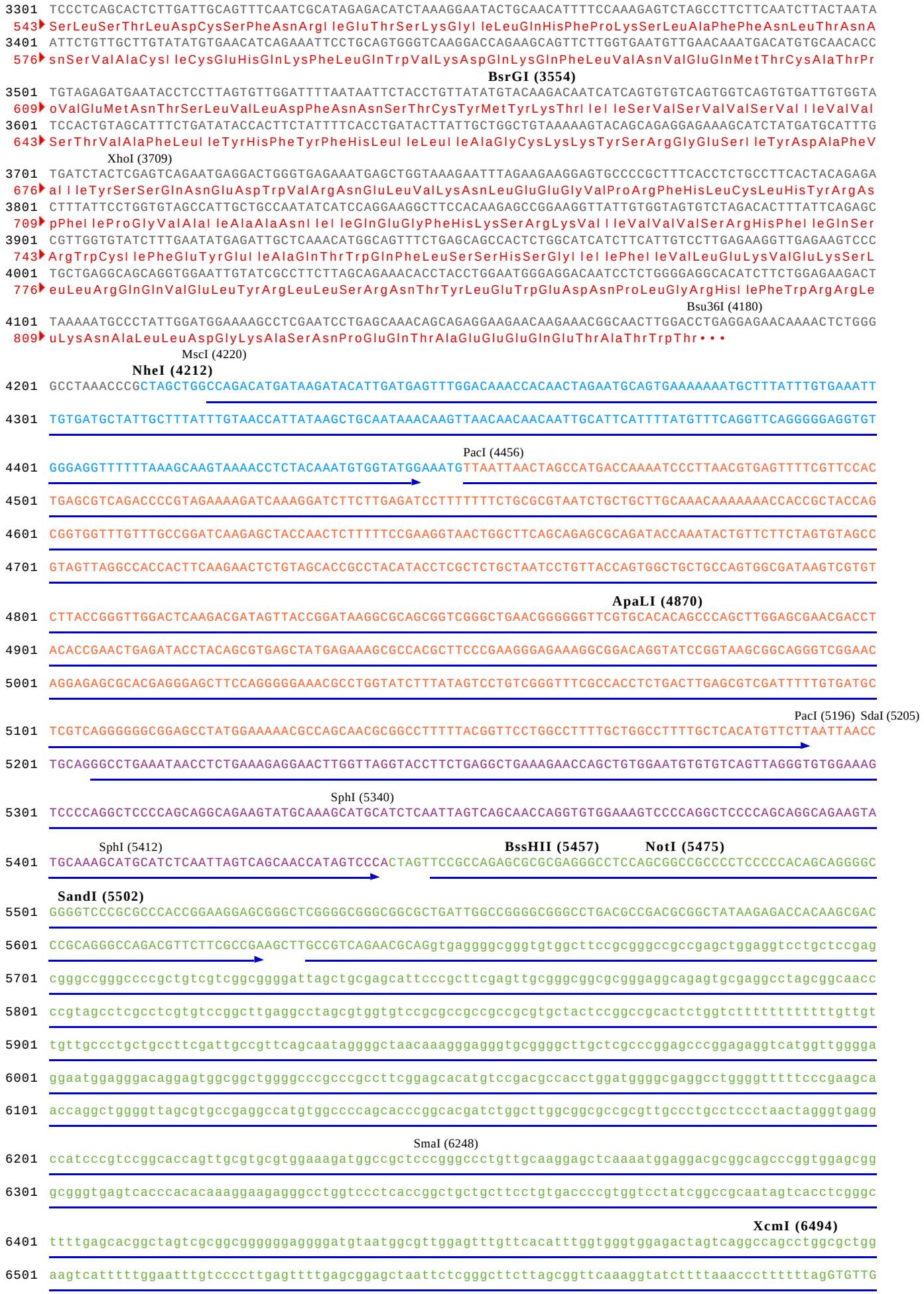
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SdaI (5205)
SphI (5340)
SphI (5412)
BssHII (5457)
NotI (5475)
SandI (5502)
SmaI (6248)
XcmI (6494)


6601 TGAAAACCACCGCTAATTCAAAGCAATCATGTTGCCATTTATTCTCTTTTCGACGCTGCTTTCTCCCATATTGACTGAATCTGAGAAGCAACAGTGGTTC
1 MetLeuProPhe leLeuPheSerThrLeuLeuSerPro leLeuThrGluSerGluLysGlnGlnTrpPhe
6701 TGCAACTCCTCCGATGCAATTATTTCCTACAGTTATTGTGATCACTTGAAATTCCCTATTTCAATTAGTTCTGAACCCTGCATAAGACTGAGGGGAACCA
25 CysAsnSerSerAspAlaI leI leSerTyrSerTyrCysAspHisLeuLysPhePro leSerI leSerSerGluProCysI leArgLeuArgGlyThrA
SspI (6846)
6801 ATGGATTTGTGCATGTTGAGTTCATTCCAAGAGGAAACTTAAAATATTTATATTTCAACCTATTCATCAGTGTCAACTCCATAGAGTTGCCGAAGCGTAA
58 snGlyPheValHisValGluPhe leProArgGlyAsnLeuLysTyrLeuTyrPheAsnLeuPhe leSerValAsnSerI leGluLeuProLysArgLy
NcoI (6914)
6901 GGAAGTTCTGTGCCATGGACATGATGATGACTATTCTTTTTGCAGAGCTCTGAAAGGAGAGACTGTGAATACATCAATACCATTCTCTTTCGAGGGAATA
91 sGluValLeuCysHisGlyHisAspAspAspTyrSerPheCysArgAlaLeuLysGlyGluThrValAsnThrSerI leProPheSerPheGluGlyI le
Bsu36I (7009)
7001 CTATTTCCTAAGGGCCATTACAGATGTGTTGCAGAAGCTATTGCTGGGGATACTGAAGAAAAGCTCTTCTGTTTGAATTTCACCATCATTCACCGCCGTG
125 LeuPheProLysGlyHisTyrArgCysValAlaGluAlaI leAlaGlyAspThrGluGluLysLeuPheCysLeuAsnPheThrI leI leHisArgArgA
7101 ATGTCAATTAGAATATGCTGAGCTAGGAGCAGGTTTCCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAAC
158 spValAsn•••
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BstEII (7546)
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AseI (7613)
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11 LeuGluLeuValGluValAlaThrGluLysI leThrMetLeuTyrGluAspAsnLysHisHisValGlyAlaAlaI leArgThrLysThrGlyGluI leI
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7901 CACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTAT
77 pThrI leValAlaValArgHisProTyrSerAspGluValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeuI leSerAspTyr
AseI (8102)
8001 GCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGGAACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAA
111 AlaProAspCysPheValLeuI leGluMetAsnGlyLysLeuValLysThrThrI leGluGluLeuI leProLeuLysTyrThrArgAsn•••
MscI (8189)
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8201 AGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGG
ScaI (8332)
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