

pTRIOZ-mIgG2a

Plasmid for high yield production of recombinant murine IgG2a kappa mAbs

Catalog code: ptrioz-migg2a

<https://www.invivogen.com/ptrioz-migg2a>

For research use only

Version 21E28-ED

PRODUCT INFORMATION

Contents

- 20 µg of pTRIOZ-mIgG2a plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability

- pTRIOZ-mIgG2a is provided as a lyophilized powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Store resuspended product at -20 °C. Resuspended product is stable for at least 1 year when properly stored.
- Avoid repeated freeze-thaw cycles.
- Store Zeocin™ 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.

PRODUCT DESCRIPTION

The pTRIOZ plasmid collection has been designed specifically for high yield production of whole recombinant monoclonal antibodies (mAbs).

The pTRIOZ plasmids contain three distinct cassettes for the expression of the heavy and light chain of the mAb as well as antibiotic selection with Zeocin™ in both bacterial (such as *E. coli*) and mammalian (such as CHO) cells. Each cassette is under the control of unique composite promoters for optimal expression (see *Plasmid features for more details*). For successful mAb production, a precise expression ratio of the heavy to light chain is required¹. In the pTRIOZ plasmids this important ratio is under the control of the human ferritin heavy (FerH) and light (FerL) chain promoters, which natively drive the successful co-expression of the two ferritin subunits². Additionally, the pTRIOZ plasmids contain unique multiple cloning sites (MCS) upstream of both the heavy and light chain constant (CH and CL) regions. This enables the cloning of variable (VH and VL) regions of any given antibody.

Majority of mAbs are produced by recombinant DNA technology in mammalian cells, either through transient or stable gene expression. The pTRIOZ plasmid collection has been designed to be used for either method. Transient or stable transfection of mammalian cell lines, such as CHO cells, with a recombinant pTRIOZ plasmid results in high-yield production of a IgG mAb that can be purified from the supernatant using an appropriate Protein A or Protein G affinity chromatography method.

pTRIOZ-mIgG2a expresses the constant region of the heavy (CH) chain from murine IgG2a, and the constant region of the murine kappa light chain (CL). pTRIOZ-mIgG2a is selectable in both bacterial and mammalian cells with Zeocin™.

PLASMID FEATURES

CASSETTE 1: mAb HEAVY CHAIN

- **AldA enh/ hFerH:** This composite promoter combines the human aldehyde dehydrogenase (aldA) enhancer and the core promoter of the human ferritin heavy chain gene.
- **MCS1:** To facilitate cloning of the variable heavy (VH) chain, the multiple cloning site contains the following restriction sites that are compatible with many different enzymes, 5'- *AgeI, MluI, EcoRV, NheI, and Eco47III* -3'.
- **mIgG2a:** The constant region of the murine immunoglobulin IgG2a heavy chain.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

CASSETTE 2: mAb LIGHT CHAIN

- **hCMV enh / hFerL prom:** This composite promoter combines the human cytomegalovirus (CMV) immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene.
- **MCS2:** To facilitate cloning of the variable light (VL) chain, the multiple cloning site contains the following restriction sites that are compatible with many different enzymes, 5'- *SgrAI, AscI, PmeI, and NcoI* -3'.
- **Murine κ light chain:** The constant region of the murine kappa light chain.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

CASSETTE 3: Zeocin™ SELECTION

- **mCMV/hEF1-HTLV prom:** This composite promoter combines mouse cytomegalovirus (mCMV) immediate-early gene 1 enhancer, the elongation Factor-1α (EF-1α) core promoter, as well as the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) type 1 long terminal repeat. 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.
- **EM7 prom:** This is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM7 is located within an intron and is spliced out in mammalian cells.
- **Sh Ble gene:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same gene confers resistance in both mammalian cells and *E. coli*.
- **hEF-1alpha pAn:** This provides a strong polyadenylation signal. InvivoGen uses a sequence that starts after the stop codon of the EF1 cDNA and finishes after a bent structure rich in GT.

GENERAL FEATURES: pTRIOZ-mIgG2a

- **5' UTR:** The 5' UTR enhances mRNA stability and protein translation.
- **Ori:** A minimal *E. coli* origin of replication.

TECHNICAL SUPPORT

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PLASMID RESUSPENSION

- Centrifuge the tube containing the lyophilized pTRIOZ-mIgG2a plasmid to pellet the DNA.
- To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile endotoxin-free H₂O.
- Store resuspended plasmid at -20°C.

GENERAL METHODS

Obtaining the VH and VL sequences

To obtain the cDNA sequence of the variable heavy (VH) and light (VL) regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse-transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. The resulting amplicons must be sequenced.

Additionally, the VH and VL chains of the mAb can be commercially synthesised. This allows for codon optimization, both for the expression system, as well as ensuring that restriction sites in the MCS are avoided. Furthermore, the 5' and 3' cloning ends for both the VH and VL chain regions can be added.

Cloning mAb variable regions into pTRIOZ

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory strains such as DH5α. For selection in *E. coli*, Zeocin™ is commonly used at 25 µg/ml in liquid or solid media

- Variable Heavy (VH) chain

In pTRIOZ-mIgG2a, the constant region of the murine IgG2a heavy chain is preceded by a MCS containing five restriction sites: AgeI, MluI, EcoRV, NheI, and Eco47III. We recommend using the AgeI restriction site for insertion of the 5' end of the mAb VH chain (including the native signal sequence).

In pTRIOZ-mIgG2a, Eco47III must be used for insertion of the 3' end of the VH chain to maintain the integrity of the constant region. Therefore, we recommend to introduce an Eco47III site at the 3' end of the variable region, in frame with the constant region of the murine IgG2a heavy chain. This ensures that no additional amino acids are introduced into the mAb sequence.

- Variable Light (VL) chain

In pTRIOZ-mIgG2a, the constant region of the murine kappa light chain is preceded by a MCS containing four unique restriction sites: SgrAI, AscI, PmeI, and NcoI. Additionally, this MCS contains a BstAPI restriction site, but it is not unique in this plasmid. We recommend using the SgrAI restriction site for insertion of the 5' end of the mAb VL chain (including the native signal sequence).

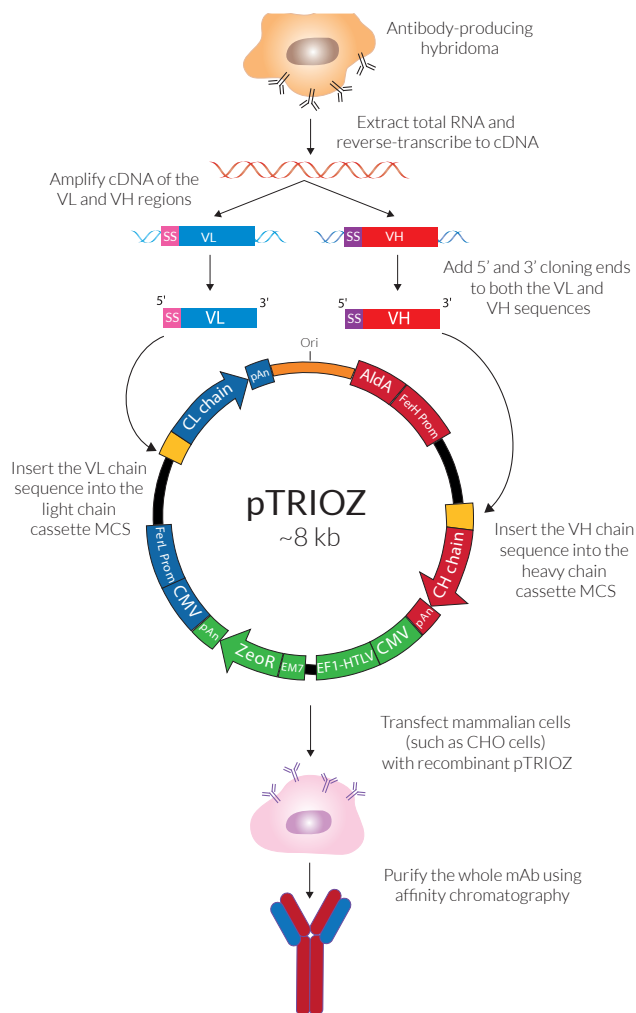
In pTRIOZ-mIgG2a, BstAPI must be used for insertion of the 3' end of the VL chain to maintain the integrity of the constant region. Therefore, we recommend to introduce an BstAPI site at the 3' end of the VL chain, in frame with the constant region of the murine kappa light chain. This ensures that no additional amino acids are introduced into the mAb sequence. **Please note there are additional BstAPI sites present in the plasmid, take care when cloning.**

Antibody production

The pTRIOZ plasmid collection is designed for mAb production in transient-expressing CHO and HEK cells as well as for establishing stable-expressing cell lines. Specifically for stable-expressing cell lines, 72 hours after transfection, cells should be placed into fresh medium containing 50-200 µg/ml of the selection antibiotic Zeocin™.

Note: The optimal Zeocin™ concentration for selection should be calculated by seeding native CHO cells with different concentrations of Zeocin™ and monitoring both cell growth and viability.

Antibody production using pTRIOZ



The selection medium should be changed every 2-3 days until cell viability and growth both become stable. Zeocin™-resistant stable cell pools are obtained typically between 7 - 10 days after selection. The selected stable cell pools can be used for bioproduction of mAbs in batch, fed batch or perfusion process modes.

Antibody purification

The resulting mAb can be purified from the supernatant using the appropriate Protein A or Protein G affinity chromatography.

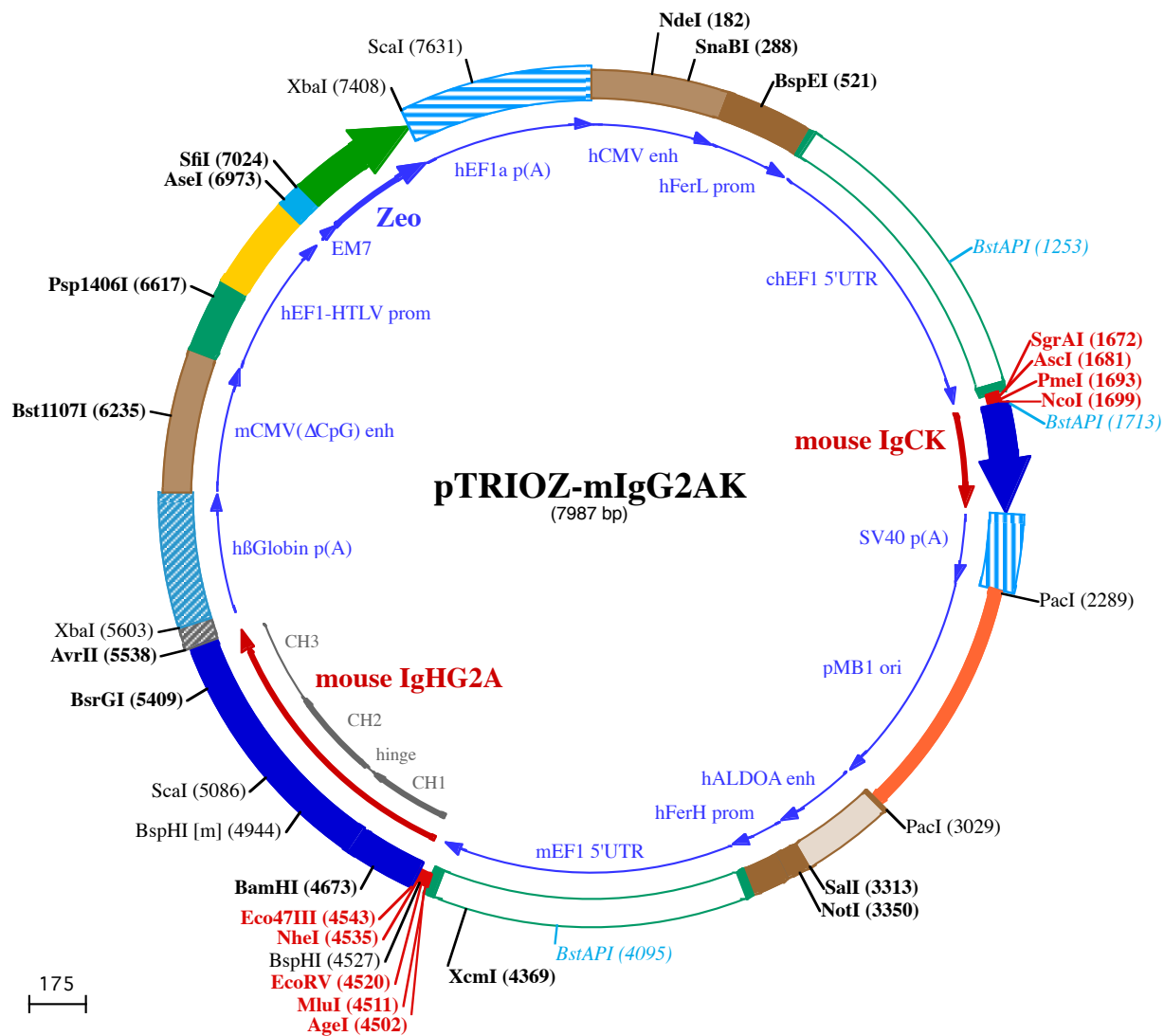
1. Prentice, H.L. *et al.*, 2007. High level expression of proteins using sequences from the ferritin heavy chain gene locus. *J Biotech.* 128:50-60.
2. Rita costa, A. *et al.*, 2010. Guidelines to cell engineering for monoclonal antibody production. *Eur J Pharm Biopharm.* 74(2):127-138.

RELATED PRODUCTS

Product	Catalog Code
ChemiComp GT116	gt116-11
LyoVec™	lyec-12
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC

201 TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC

301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTCCAAGTCTCCACCCCAATTGACGTCAATG

401 GGAGTTTGTGTTGACTAGTCAGGGCCCCAACCCCCCAAGCCCCATTTACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGG

501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCTGCC

601 TCCGAGGGCCGGCGCACCATAAAAGAAGCCGCCTAGCCACGTCCCCTCGAGTTCGGCGGTCCCGCGGTCTGTCTCAAGCTTGCCGCAGAACACAGg

701 taagtgcggtgtgtggttcccgcgggcctggcctctttacgggttatggccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattc

801 ttgatcccagccttccgggttgaagtggtggagagttcagggccttgcgttaaggagcccttcgctcgtgcttgagttgaggcctggcttgggcg

901 ctggggccgcgctgtaactctggtggcaccttcgcgctgtctcgtgcttgcctaagtctctagccatttaaaatgataaccagctgcgacg

1001 cttttttctggcgagatagcttctaagtgcgggaccagatctgcacactggatcttgcgttttggggccgggcgggcgagggccctgctgctcc

1101 agcgcacatgctcggcgaggcgggctgcgagcggccaccgagaatcgagcggggtagtctcaactggcggcctgctggtgctgctgctgc

1201 gccgctgtatgccccgcctgggcggaaggtggcccggtcggcaccagttgctgagcggaaagatggccgcttcccgccctgctgcagggagc

1301 tcaaatggaggacgcgccgggagagcgggagggtgagtcaccacagaagggaaaaggccttcttctcatccgtcgcttcatgtgactcca

1401 cggagtaccggcgccgctccaggcacctcgattagttgctgagcttttgagtagctcgtcttttaggttgggggaggggttttatgcatggagttcc

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1601 tcaagcctcagacagtggttcaagttttttcttccatttcagGTGTCGTGAAAACCTACCCCTAAAAGCCACCGGCGAGGCGCCCAAGTTAAACACC

1701 **ATGGAAGC**AGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAATTCT

1801 ACCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGTCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTA

1901 CAGCATGAGCAGCACCCCTCAGTTGACCAAGGACGATGATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAAGACATCAACTCACCCATTGTC

2001 AAGAGCTTCAACAGGAATGAGTGTAGAGACAAAGTCTGAGACCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAACCACAAGT

2101 AATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGC

2201 ATTCATTTTATGTTTCAGGTTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATGTTAATTAAGTACCCAT

2301 GACCAAAATCCCTAACGTGAGTTTTTCTTCCACTGAGCGTCAGACCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGGTAATC

2401 TGCTGCTTGAACAACAAAAACACCGCTACCAGCGGTGTTTGTGCGGATCAAGAGCTACCAACTCTTTTTCCGAGGTAAGTGGCTTACGAGAG

2501 CGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGTAATCCTGTT

2601 ACCAGTGGCTGCTGCCAGTGGCATAAGTCGTGCTTACCGGTTGGACTCAAGACGATAGTTACCGATAAGGCGCAGCGGTGGGCTGAACGGGGGT

2701 TCGTGACACAGCCAGCTTGGAGCGAACGACTACCCGAAGTACCTACAGCGTGTGATGAGAAAAGCGCCAGCTTCCGAAAGGAGAAAAGG

2801 CGGACAGTATCCGGTAAGCGGAGGTCGGAACAGGAGCGCACAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGCTGTGCGGTTTTCG

2901 CCACCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACCGGCTTTTTACGGTCTGCGCTTT

3001 TGCTGGCCTTTTGTCTACATGTTCTTAATTAACCTGCaggcgaactcagcttccttcgctttccgacttttccatccgcgctcctccacttcccgttccg
PacI (3029)
3101 ccctccccattgccaacattctggctgagtcacggcgccccagagcgcgccaggtgggggaaaggagcagaagggagggccctagcgaccgcgggat
3201 gtggtccgagtcacgtccgaggggggtggggagggatcgtgttctcggcgcccccttccctagcgcgccctctgggtgcgctctcggggcgggcc

3301 gtagccagtcgctcgACTAGTTCCGCCAGAGCGCGGAGGGCTCCAGCGGCCCTCCCCACAGCAGGGGCGGGTCCCGCGCCACCCGGAAGGA
SalI (3313) NotI (3350)
3401 GCGGGCTCGGGGCGGGCGGCTGATTGGCCGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCAGCCGAGGGCCAGACGTTCTTCGCC
3501 GAAGCTTGCCGTCAACGCAGgtgagggcggggtgtggttcgcgggcccgcgagctggaggtcctgctccgagcgggcccggcccgctgctcgg
3601 cggggattagctcgagcattcccgttcgagttgcgggcccgcgggagcagagtcgagggcctagcggcaaccccgtagcctcgcctcgtgctcggc
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4001 ggccatgtggcccagcaccggcacgatctggcttggcggcgcgcttgccctgcctcctaactagggtaggcatcccgctccggcaccagttgcg
BstAPI (4095)
4101 tgcgtggaagatggccgctcccggccctgttgaaggagctcaaatggaggacgcggcagcccggtaggagcgggagggtgagtcacccacacaaagg
4201 aagagggcctggtccctcaccggctgctgcttctgtgaccccgtggtcctatcgccgcaatagtcacctcgggcttttagcacggctagtcgaggcg

4301 gggggaggggatgtaatggcgttggagtttggcactttggtgggtggagactagtcaggccagcctggcgctggaagtcattttggaaattgtcccc
XcmI (4369)
4401 ttgagttttgagcggagctaattctcgggcttcttagcggttcaaaggtatcttttaaaccttttttagTGTTGTGAAAACCACCGTAATTCAAAGC

4501 AACCGGTCGCACGCGTAGATATCACGTCATGAAAGCTAGCAGCGCTAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTGTGGAGATACAAC
EcoRV (4520) NheI (4535) AgeI (4502) MluI (4511) BspHI (4527) Eco47III (4543)
1▶ A K T T A P S V Y P L A P V C G D T T

4601 GGCTCTCGGTGACTCTAGGATGCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGATCCCTGTCCAGTGGTGTGCACACCT
20▶ G S S V T L G C L V K G Y F P E P V T L T W N S G S L S S G V H T
BamHI (4673)
4701 TCCCAGCTGTCTGCAGTCTGACCTCTACACCCTCAGCAGCTCAGTACTGTAACATCGAGCACCTGGCCAGCCAGTCCATCACCTGCAATGTGGCCA
53▶ F P A V L Q S D L Y T L S S S V T V T S S T W P S Q S I T C N V A H
4801 CCCGCAAGCAGCACCAAGTGGACAAGAAAATTGAGCCAGAGGGCCACAATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAACCTCTTGGGT
86▶ P A S S T K V D K K I E P R G P T I K P C P P C K C P A P N L L G

4901 GGACCATCGTCTTCATCTTCCCTCAAAGATCAAGGATGACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATG
120▶ G P S V F I F P P K I K D V L M I S L S P I V T C V V V D V S E D
BspHI [m] (4944)
5001 ACCCAGATGTCCAGATCAGTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGTGGT
153▶ D P D V Q I S W F V N N V E V H T A Q T Q T H R E D Y N S T L R V V
ScaI (5086)
5101 CAGTCCCTCCCATCCAGCACCAGACTGGATGAGTGGAAGGAGTCAAATGCAAGTCAACAACAAGACCTCCAGCGCCATCGAGAGAACCATC
186▶ S A L P I Q H Q D W M S G K E F K C K V N N K D L P A P I E R T I
5201 TCAAAACCAAAGGTGAGTAAGAGCTCACAGGTATATGTCTTCCCTCACCAGAAGAAGAGATGACTAAGAACAGGTCACTCTGACCTGCATGGTCA
220▶ S K P K G S V R A P Q V Y V L P P P E E E M T K K Q V T L T C M V
5301 CAGACTTCATGCTGAAGACATTTACGTGGAGTGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCTGGACTCTGATGGTTC
253▶ T D F M P E D I Y V E W T N N G K T E L N Y K N T E P V L D S D G S
BsrGI (5409)
5401 TTACTTCATGTACAGCAAGCTGAGAGTGAAAAAGAAGAACTGGGTGAAAAGAAATAGCTACTCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCAC
286▶ Y F M Y S K L R V E K K N W V E R N S Y S C S V V H E G L H N H H

AvrII (5538)

5501 ACGACTAAGAGCTTCTCCGGACTCCGGGTAATAAAACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACTTGAACCTCCGCTGGTCTTTCCA
320▶ T T K S F S R T P G K •

XbaI (5603)

5601 GGTCTAGAAGCTCGCTTCTTGTCTGCAATTTCTATTAAGGTTCTTTGTTCCCTAAGTCCAACACTACTAACTGGGGGATATTATGAAGGGCCTTGAG
5701 CATCTGGATTCTGCTAATAAAAAACATTTATTTTCATTGCAATGATGATTTAAATTATTTCTGAATATTTTACTAAAAAGGAATGTGGGAGGTCACT
5801 GCATTTAAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATACACTATATCTTAAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATG
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6101 ATAGGGGGTGTAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATGGGAG

Bst1107I (6235)

6201 GTAAGCCAATGGGTTTTTCCATTACTGCAIGTATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATAGGGGTGAATCA
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6501 AACGGGTGCCTAGAGAAGGTGGCGGGGTAAGTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGT

Psp1406I (6617)

6601 GCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACACAGTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCC
6701 CTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTAAAGCT
6801 CAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTC

AseI (6973)

6901 TTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACAAACAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATA

SfiI (7024)

7001 GTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACC CGCGCGACGCTCGCCGGAGCGGTGCGAGTTCTGG
7101 ACCGACCGGCTCGGGTCTCCCGGACTTCGTGGAGGACGACTTCGCTGGTGTGGTCCGGGACGACGTGACCCTGTTATCAGCGCGGTCCAGGACCAGG
24▶ T D R L G F S R D F V E D D F A G V V R D D V T L F I S A V Q D Q
7201 TGGTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGCCCTGGACGAGCTGTACGCCGAGTGGTGGAGGTCGTGTCCACGAACTCCGGGACGCCTC
57▶ V V P D N T L A W V W V R G L D E L Y A E W S E V V S T N F R D A S
7301 CGGCGCGCCATGACCGAGATCGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGCGCCGCAACTGCGTGCACCTTTGTGCAGAGGAGCAG
90▶ G P A M T E I G E Q P W G R E F A L R D P A G N C V H F V A E E Q

XbaI (7408)

7401 GACTAAATCTAGAATTATCCCTAATACCTGCCACCCACTCTTAATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTGGCCATTTAAGTTT
124▶ D •
7501 AGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTCAGAAGGAAAGGAGAATGTTTTGTGGACCACCTTTGGTTTTCTTTTTGCGTGTGG

ScaI (7631)

7601 CAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGGAAACAACCTTGACCAAAAATTTGTCACAGAATTTTGGAGCCATTAAAAAGTTAAAT
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