pTRIOZ-mIgG2aL2

Plasmid for high yield production of recombinant murine IgG2a lambda 2 mAbs

Catalog code: ptrioz-migg2al2

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

For research use only

Version 22B16-MM

PRODUCT INFORMATION

Contents

- 20 µg of **pTRIOZ-mIgG2aL2** plasmid provided as lyophilized DNA
- 1 ml of Zeocin® (100 mg/ml)

Storage and Stability

- pTRIOZ-mIgG2aL2 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 required[1]. 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 subunits[2]. 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-mIgG2aL2 expresses the constant region of the heavy (CH) chain from murine IgG2a, and the constant region of the murine lambda 2 light chain (CL). pTRIOZ-mIgG2aL2 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, NcoI, Acc65I, and AvrII -3'*.
- **Murine λ2 light chain:** The constant region of the murine lambda 2 light chain (IGLC2).
- **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-mIgG2aL2

- **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 H_2O.
- 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 commerically 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-mIgG2aL2, the constant region of the murine IgG2a heavy chain is preceded by a MCS containing five unique 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.

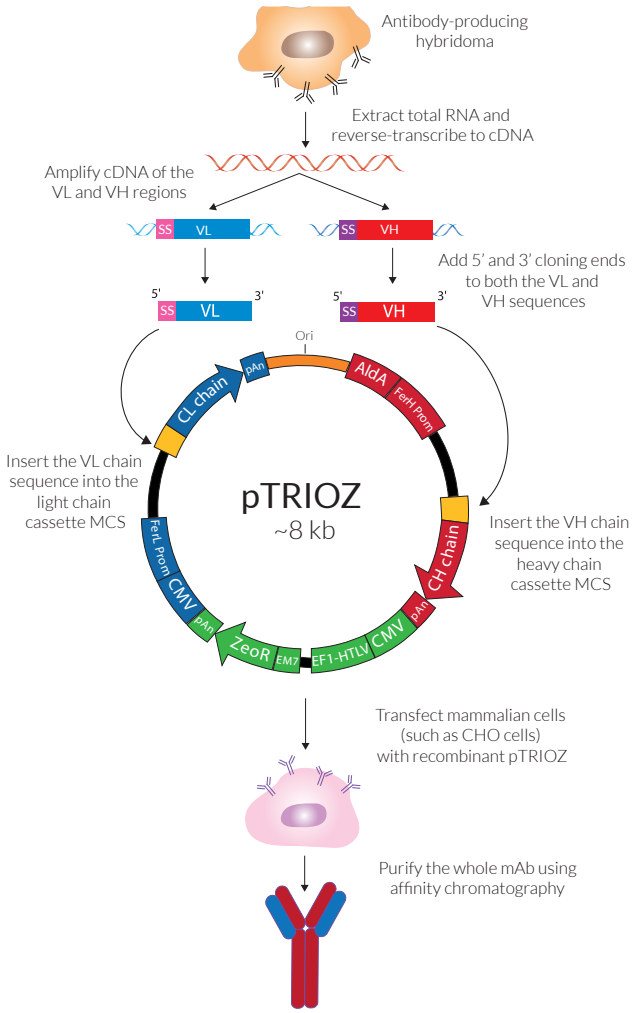
- Variable Light (VL) chain

In pTRIOZ-mIgG2a, the constant region of the murine lambda 2 light chain is preceded by a MCS containing six unique restriction sites: *SgrAI*, *AscI*, *PmeI*, *NcoI*, *Acc65I*, and *AvrII*. 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, *AvrII* 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 *AvrII* site at the 3' end of the VL chain, in frame with the constant region of the murine lambda 2 light chain. This ensures that no additional amino acids are introduced into the mAb sequence.

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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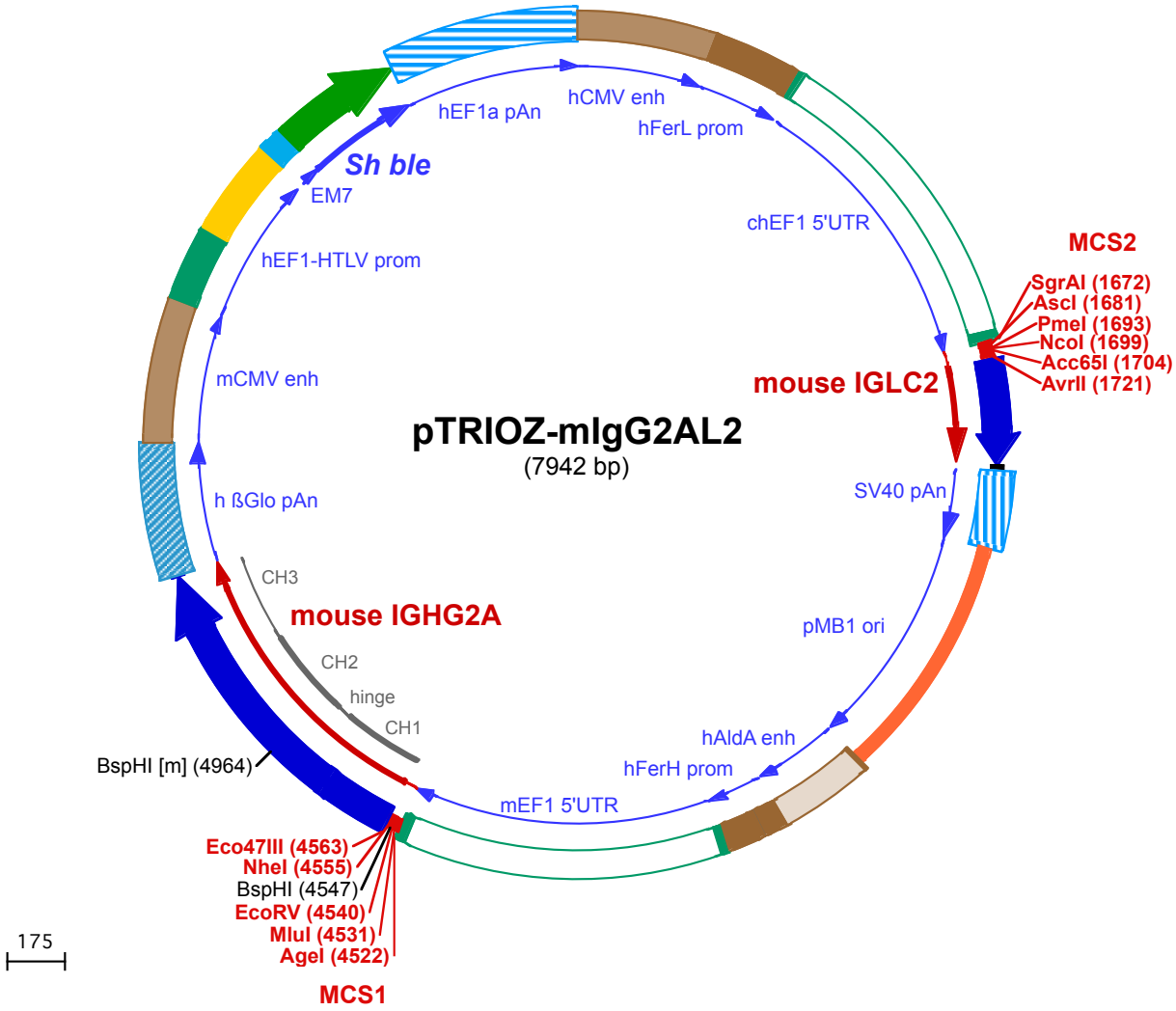
pTRIOZ-mIgG2AL2
(7942 bp)
hEF1a pAn
hCMV enh
hFerL prom
chEF1 5'UTR
MCS2
SgrAI (1672)
AscI (1681)
PmeI (1693)
NcoI (1699)
Acc65I (1704)
AvrII (1721)
mouse IGLC2
SV40 pAn
pMB1 ori
hAldA enh
hFerH prom
mEF1 5'UTR
Eco47III (4563)
NheI (4555)
BspHI (4547)
EcoRV (4540)
MluI (4531)
AgeI (4522)
MCS1
BspHI [m] (4964)
mouse IGHG2A
CH3
CH2
hinge
CH1
h ßGlo pAn
mCMV enh
hEF1-HTLV prom
EM7
Sh ble
175


AscI (1681)
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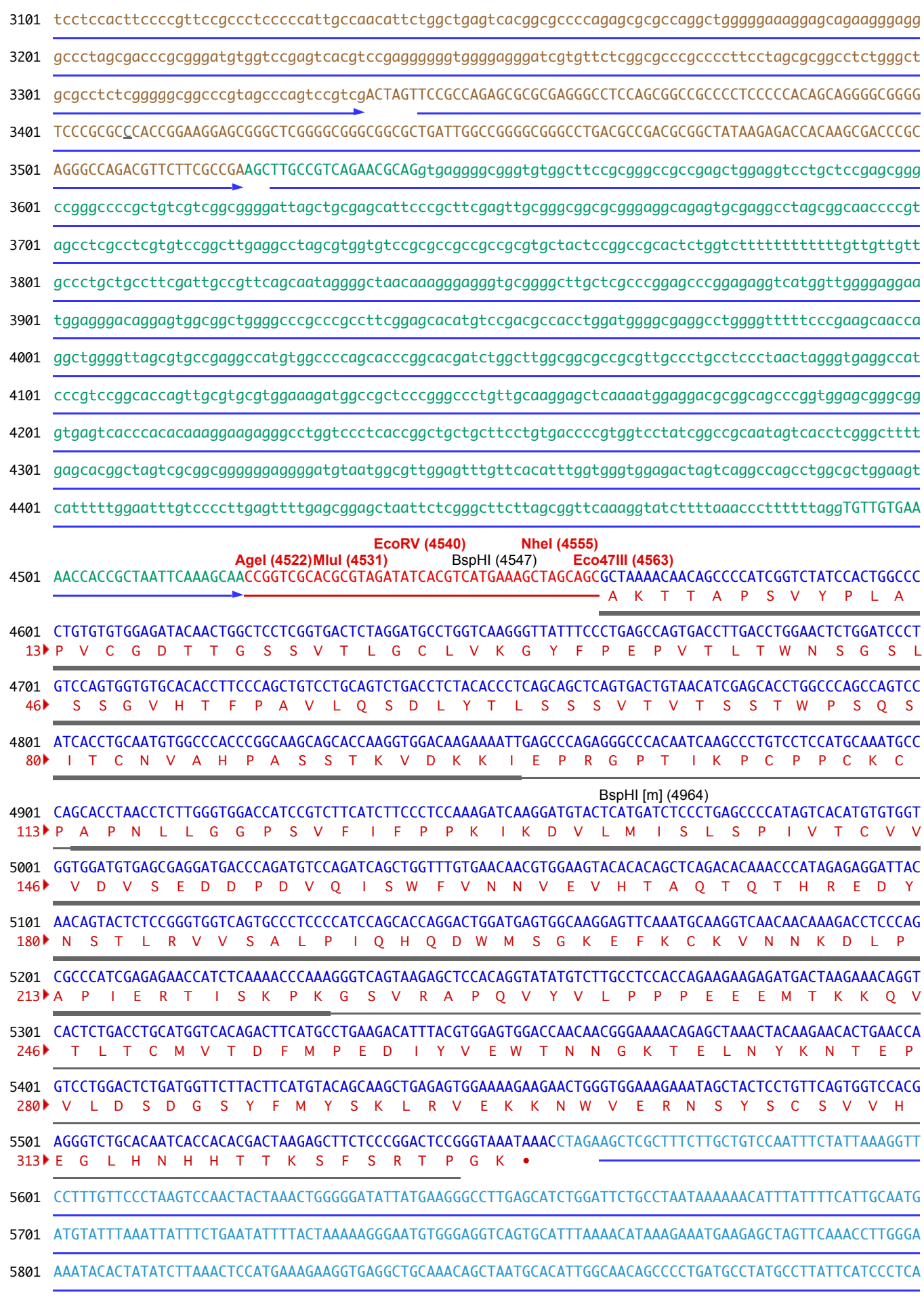
SgrAI (1672)
PmeI (1693)
Acc65I (1704)
AvrII (1721)


EcoRV (4540)
NheI (4555)
AgeI (4522)
MluI (4531)
BspHI (4547)
Eco47III (4563)
BspHI [m] (4964)


5901 GAAAAGGATTCAAGTAGAGGCTTGATTTGGAGGTTAAAGTTTTGCTATGCTGTATTTTACAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCA
6001 AGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCCAGTACATAAGGTCAATAGGGGGTGAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACAT
6101 TGAGTCAATAGGGACTTTCCAATGGGTTTTGCCCAGTACATAAGGTCAATGGGAGGTAAGCCAATGGGTTTTTCCCATTACTGACATGTATACTGAGTCA
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6301 TTTCCATTGGGTTTTGCCCAGTACAAAAGGTCAATAGGGGGTGAGTCAATGGGTTTTTCCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGT
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6901 CCGGCGCCTACAAACAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGAC
1 M A K L T
7001 CAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGGTCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTC
5 S 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
7101 GCTGGTGTGGTCCGGGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGG
39 A 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
7201 ACGAGCTGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCGAGATCGGCGAGCAGCCGTGGGGGCGGGA
72 D E L Y A E W S E V V S T N F R D A S G P A M T E I G E Q P W G R E
7301 GTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACTTTGTGGCAGAGGAGCAGGACTAAATCTAGAATTATCCCTAATACCTGCCACCCCACTCTTAA
105 F A L R D P A G N C V H F V A E E Q D •
7401 TCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTT
7501 CAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAA
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