pFUSEss-CHIg-mM*01

Plasmid featuring the constant region of the mouse IgM (allele 1) heavy chain, and the IL2 signal sequence

Catalog code: pfusess-mchm

For research use only

Version 24J28-MM-v37

PRODUCT INFORMATION

Content:
- 20 μg of **pFUSEss-CHIg-mM*01** plasmid provided as lyophilized DNA.
- 1 ml of Zeocin® (100 mg/ml)

Storage and Stability:
- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C and is stable 3 months.
- Resuspended DNA should be stored at -20°C and is stable up to 1 year.
- 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.

Materials required for antibody generation & isotype switching

- **pFUSE2ss-CLIg** plasmid that features the constant region of the kappa or lambda light chains. pFUSE2ss-CLIg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- **pFUSEss-CHIg** plasmid for the constant region of the heavy chain. This plasmid is selectable with Zeocin®.

GENERAL PRODUCT USE

pFUSEss-CHIg and pFUSE2ss-CLIg plasmids are designed to change a monoclonal antibody from one isotype to another, therefore, enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire antibodies from Fab or scFv fragments.

pFUSEss-CHIg and pFUSE2ss-CLIg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSEss-CHIg and pFUSE2ss-CLIg pair allows to generate an antibody that can be purified from the supernatant using the appropriate affinity chromatography.

Features of pFUSEss-CHIg and pFUSE2ss-CLIg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] 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[2]. 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.
- **MCS:** The multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh / hFerL prom:** This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin®-resistance gene in mammalian cells.
- **IL2 ss:** The human IL2 signal sequence contains 20 amino acids (MYRMQLLSCIALSLALVTNS) and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the immunoglobulin chain.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM 2KC is located within an intron and is spliced out in mammalian cells.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

pFUSEss-CHIg-mM*01 specific features

- **Mouse IGHM*01 (IgM allele1 heavy chain constant region):** When cloning your heavy chain variable region of choice in the MCS, care must be taken to insert the gene in-frame and to preserve the integrity of the heavy chain constant region.
- **Zeo:** Resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.

References:

1. Kim DW. *et al*. 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23. **2. Takebe Y. *et al*. 1988.** SR 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. 8(1):466-72. **3. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **4. Yu J. & Russell JE. 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

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PROTOCOL

Obtaining VH and VL sequences

The antibody sequence can be obtained by phage display or from an antibody producing hybridoma. To obtain the cDNA sequence of the VH and 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. Alternatively 5' RACE can be used . The resulting amplicons must be sequenced.

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.

Cloning into pFUSEss-CHIg and pFUSE2ss-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSEss-CHIg-mM*01, the constant region of the mouse IgM heavy chain is preceded by a multiple cloning site containing four unique restriction sites: EcoRI, EcoRV, XhoI, and NheI. Using EcoRI as the 5' cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids. Use NheI as the 3' cloning site in order to preserve the exact IgM amino acid sequence. We recommend introducing by PCR the EcoRI and NheI sites at the VH boundaries. Care must be taken to preserve the correct reading frame when placing the cloning sites on the variable sequence.

When generating the insert for VL, a BstAPI (pFUSE2ss-CLIg-mk; mouse kappa), or AvrII (pFUSE2ss-CLIg-ml1 / pFUSE2ss-CLIg-ml2; mouse lambda) site must be introduced at the 3' end.
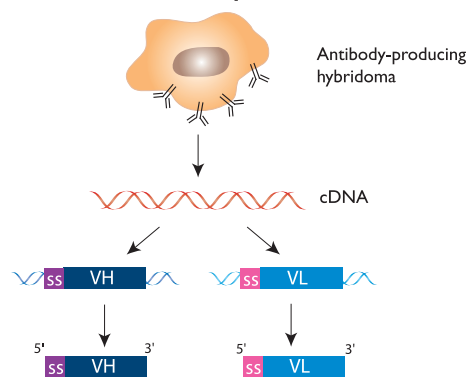
Choice of strategies for the transfection

Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.
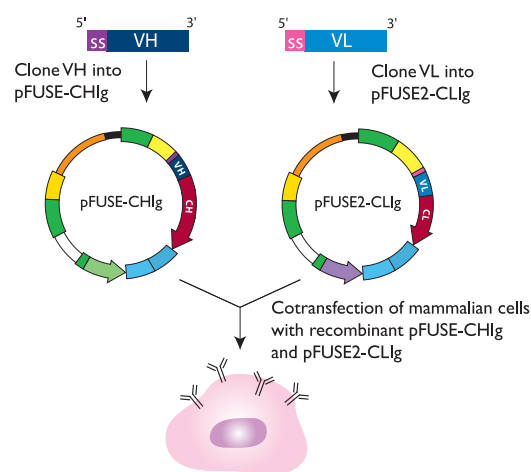
OR

A cotransfection can be performed with the plasmid coding for the light chain and the plasmid coding for the heavy chain. Since the pFUSE2ss-CLIg and pFUSEss-CHIg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of pFUSE2ss-CLIg and pFUSEss-CHIg plasmids. We recommend using a ratio of 3:2 of pFUSE2ss-CLIg:pFUSEss-CHIg plasmids. pFUSE2ss-CLIg plasmids feature the constant region of a kappa or lambda light chain. pFUSE2ss-CLIg plasmids are selectable with blasticidin. pFUSEss-CHIg plasmids are selectable with Zeocin®.

Antibody generation using pFUSE-CHIg & pFUSE-CLIg

1- Obtention of VH and VL sequences

Antibody-producing hybridoma

cDNA

2- Cloning into pFUSE-CHIg and pFUSE-CLIg

Clone VH into pFUSE-CHIg

Clone VL into pFUSE2-CLIg

pFUSE-CHIg

pFUSE2-CLIg

Cotransfection of mammalian cells with recombinant pFUSE-CHIg and pFUSE2-CLIg

To check for production of your antibody after transfection, you may take an aliquot of growth medium and perform SDS-PAGE, protein-specific ELISA, or the bioactivity assay of choice to determine that your cells are producing your antibody of interest.

The resulting IgM antibody can be purified from the supernatant using the appropriate affinity chromatography.

RELATED PRODUCTS

Product	Catalog Code
pFUSE2ss-CLIg-mk	pfuse2ss-mclk
pFUSE2ss-CLIg-ml1	pfuse2ss-mcll1
pFUSE2ss-CLIg-ml2	pfuse2ss-mcll2
LyoVec™	lyec-12
Zeocin®	ant-zn-1

NotI (4713)

SgrAI (553)
EcoRI (626)
EcoRV (634)
XhoI (637)
NheI (644)

hEF1-HTLV prom

ori

IL2ss

CH1

mIgM heavy chain (allele 1)

hCMV enh

CH2

pFUSEss-CHIg-mM*01
(4876 bp)

hFerL prom

CH3

CH4

EM2KC

Sh ble

SV40 pAn

SgrAI (2929)

ßGlo pAn

100

InvivoGen

24J04v37
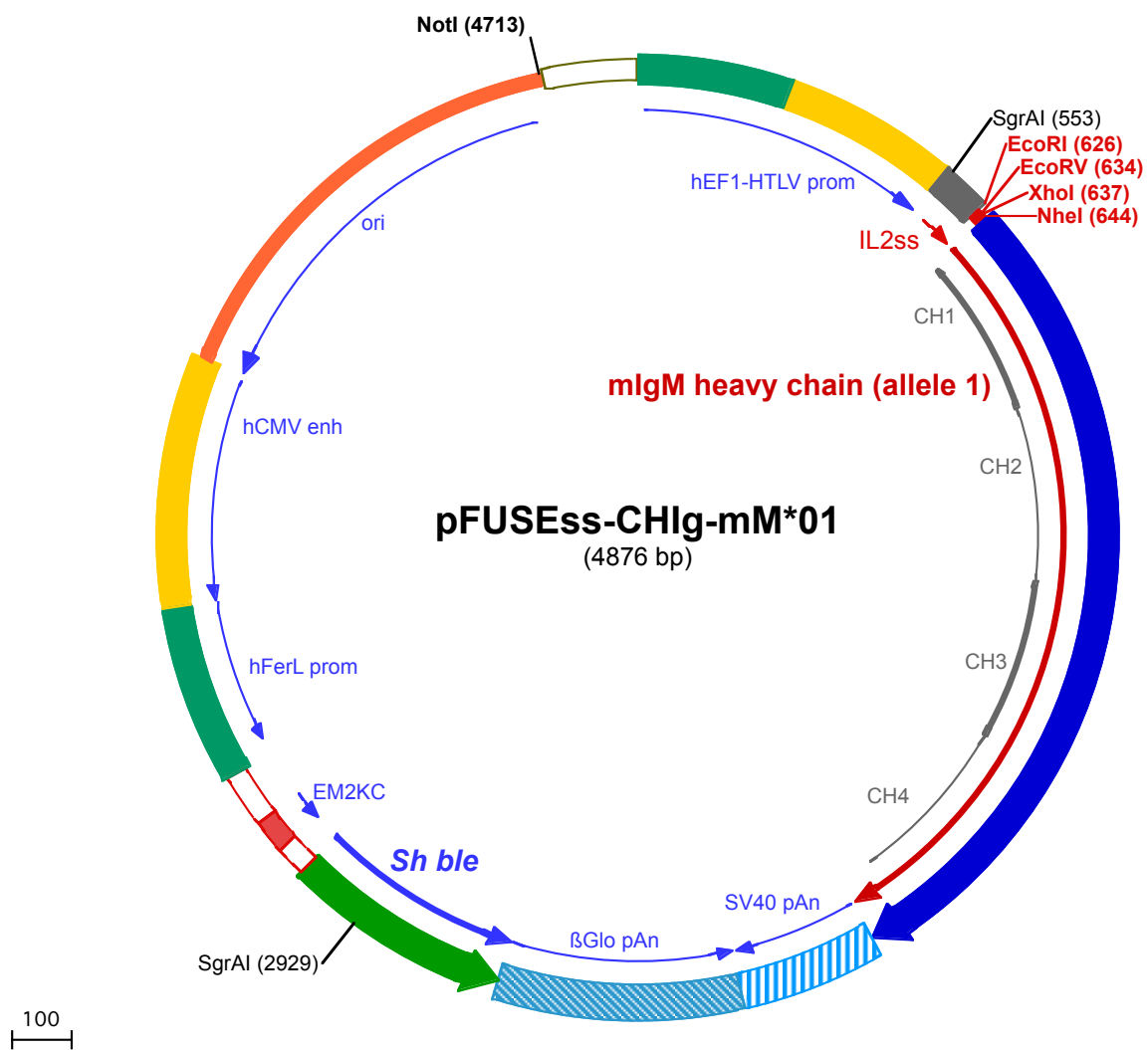
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101  GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

201  GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301  GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401  GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

                                                                         SgrAI (553)
501  TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
                                                                      1▶ M  Y  R  M  Q  L  L  S  C

                          EcoRV (634)     NheI (644)
                     EcoRI (626)    XhoI (637)
601  TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCTCGAGTGCTAGCTCAGAGAGTCAGTCCTTCCCAAATGTCTTCCCCCTCGTCTCCTGCGAGAGC
 10▶ I  A  L  S  L  A  L  V  T  N  S                1▶ S  S  E  S  Q  S  F  P  N  V  F  P  L  V  S  C  E  S

701  CCCCTGTCTGATAAGAATCTGGTGGCCATGGGCTGCCTGGCCCGGGACTTCCTGCCCAGCACCATTTCCTTCACCTGGAACTACCAGAACAACACTGAAG
 19▶ P  L  S  D  K  N  L  V  A  M  G  C  L  A  R  D  F  L  P  S  T  I  S  F  T  W  N  Y  Q  N  N  T  E

801  TCATCCAGGGTATCAGAACCTTCCCAACACTGAGGACAGGGGGCAAGTACCTAGCCACCTCGCAGGTGTTGCTGTCTCCCAAGAGCATCCTTGAAGGTTC
 52▶ V  I  Q  G  I  R  T  F  P  T  L  R  T  G  G  K  Y  L  A  T  S  Q  V  L  L  S  P  K  S  I  L  E  G  S

901  AGATGAATACCTGGTATGCAAAATCCACTACGGAGGCAAAAACAGAGATCTGCATGTGCCCATTCCAGCTGTCGCAGAGATGAACCCCAATGTAAATGTG
 85▶ D  E  Y  L  V  C  K  I  H  Y  G  G  K  N  R  D  L  H  V  P  I  P  A  V  A  E  M  N  P  N  V  N  V

1001 TTCGTCCCACCACGGGATGGCTTCTCTGGCCCTGCACCACGCAAGTCTAAACTCATCTGCGAGGCCACGAACTTCACTCCAAAACCGATCACAGTATCCT
119▶ F  V  P  P  R  D  G  F  S  G  P  A  P  R  K  S  L  I  C  E  A  T  N  F  T  P  K  P  I  T  V  S

1101 GGCTAAAGGATGGGAAGCTCGTGGAATCTGGCTTCACCACAGATCCGGTGACCATCGAGAACAAAGGATCCACACCCCAAACCTACAAGGTCATAAGCAC
152▶ W  L  K  D  G  K  L  V  E  S  G  F  T  T  D  P  V  T  I  E  N  K  G  S  T  P  Q  T  Y  K  V  I  S  T

1201 ACTTACCATCTCTGAAATCGACTGGCTGAACCTGAATGTGTACACCTGCCGTGTGGATCACAGGGGTCTCACCTTCTTGAAGAACGTGTCCTCCACATGT
185▶ L  T  I  S  E  I  D  W  L  N  L  N  V  Y  T  C  R  V  D  H  R  G  L  T  F  L  K  N  V  S  S  T  C

1301 GCTGCCAGTCCCTCCACAGACATCCTAACCTTCACCATCCCCCCCCTCCTTTGCCGACATCTTCCTCAGCAAGTCCGCTAACCTGACCTGTCTGGTCTCAA
219▶ A  A  S  P  S  T  D  I  L  T  F  T  I  P  P  S  F  A  D  I  F  L  S  K  S  A  N  L  T  C  L  V  S

1401 ACCTGGCAACCTATGAAACCCTGAATATCTCCTGGGCTTCTCAAAGTGGTGAACCACTGGAAACCAAAATTAAAATCATGGAAAGCCATCCCAATGGCAC
252▶ N  L  A  T  Y  E  T  L  N  I  S  W  A  S  Q  S  G  E  P  L  E  T  K  I  K  I  M  E  S  H  P  N  G  T

1501 CTTCAGTGCTAAGGGTGTGGCTAGTGTTTGTGTGGAAGACTGGAATAACAGGAAGGAATTTGTGTGTACTGTGACTCACAGGGATCTGCCTTCACCACAG
285▶ F  S  A  K  G  V  A  S  V  C  V  E  D  W  N  N  R  K  E  F  V  C  T  V  T  H  R  D  L  P  S  P  Q

1601 AAGAAATTCATCTCAAAACCCAATGAGGTGCACAAACATCCACCTGCTGTGTACCTGCTGCCACCAGCTCGTGAGCAACTGAACCTGAGGGAGTCAGCCA
319▶ K  K  F  I  S  K  P  N  E  V  H  K  H  P  P  A  V  Y  L  L  P  P  A  R  E  Q  L  N  L  R  E  S  A

1701 CAGTCACCTGCCTGGTGAAGGGCTTCTCTCCTGCAGACATCAGTGTGCAGTGGCTTCAGAGAGGGCAACTCTTGCCCCAAGAGAAGTATGTGACCAGTGC
352▶ T  V  T  C  L  V  K  G  F  S  P  A  D  I  S  V  Q  W  L  Q  R  G  Q  L  L  P  Q  E  K  Y  V  T  S  A

1801 CCCGATGCCAGAGCCTGGGGCCCCAGGCTTCTACTTTACCCACAGCATCCTGACTGTGACAGAGGAGGAATGGAACTCCGGAGAGACCTATACCTGTGTT
385▶ P  M  P  E  P  G  A  P  G  F  Y  F  T  H  S  I  L  T  V  T  E  E  E  W  N  S  G  E  T  Y  T  C  V

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419▶ V  G  H  E  A  L  P  H  L  V  T  E  R  T  V  D  K  S  T  G  K  P  T  L  Y  N  V  S  L  I  M  S  D

2001 CAGGCGGCACCTGCTATTGACTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTA
452▶ T  G  G  T  C  Y  •
2101 TTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCA

2201 GGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAAT

2301 CAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCA

2401 TGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTTAGTAAAA

2501 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTA
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2601 GTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTG
                                                                          125◄ • D Q E E A V F H

2701 CACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTG
116◄ V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F N T

2801 GACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGA
82◄ S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A S I

                                                        SgrAI (2929)
2901 TGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGC
49◄ F L T V D D R V V G A F D D E V F D R S F G L R D T W F E V A G A

3001 GACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagagggaaagagaagaaggttagtacaattgCTA
16◄ V D R A T L V P V A S T L K A M   ◄

3101 TAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgccacttttcctgcactgccccat

3201 ctcctgcccacccctttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGAAC
                                                  ◄

3301 TGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCGGTGGCAGGAGG

3401 CGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTT

3501 GTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACC
                                          ◄

3601 GCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGT

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3801 GTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGT

3901 TGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGT
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4201 AAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC

4301 GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG

4401 CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGT

4501 TACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAA

4601 AAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAA
                                                                                      ◄

              NotI (4713)
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