pFUSE-CHIg-hG4e1

Plasmid featuring a mutated constant region of the human IgG4 heavy chain

Catalog # pfuse-hchg4e1

For research use only

Version # 16J03v40-JC

PRODUCT INFORMATION

Content:

- 20 μg of **pFUSE-CHIg-hG4e1** plasmid provided as lyophilized DNA.
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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 *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

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

- pFUSE2-CLIg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLIg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).

- pFUSE-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™.

GENERAL PRODUCT USE

pFUSE-CLIg and pFUSE-CHIg 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 IgG antibodies from Fab or scFv fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

pFUSE-CHIg and pFUSE2-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 pFUSE-CHIg and pFUSE2-CLIg pair allows to generate an IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

Features of pFUSE-CHIg and pFUSE2-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.

• **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC 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].

pFUSE-CHIg-hG4e1 specific features

• **Human IgHG4e1 (Engineered IgG4 heavy chain constant region):** IgHG4e1 contains the S228P mutation that reduces Fab-arm exchange[5]. 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. **5. Labrijn AF. et al., 2009.** Therapeutic IgG4 antibodies engage in Fab- arm exchange with endogenous human IgG4 in vivo. Nat Biotechnol. 27(8):767-71.

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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 H_2O. Store resuspended plasmid at -20°C.

Cloning into pFUSE-CHIg and pFUSE2-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-CHIg-hG4e1, the constant region of the human IgG4 heavy chain is preceded by a multiple cloning site containing four restriction sites: EcoRI, EcoRV, XhoI and NheI. The first three restriction sites can be used for insertion of the 5'end of the variable region including the native signal sequence. If the immunoglobulin signal sequence is unknown, pFUSEss plasmids containing a signal sequence should be used. In pFUSE-CHIg-hG4e1, NheI must be used for insertion of the 3'end of the variable region. NheI must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an NheI site at the 3' end of the variable region in frame with the constant region.

When generating the insert for VL, a BsiWI (pFUSE2-CLIg-hK; human kappa), or AvrII (pFUSE2-CLIg-hL2; human lambda 2) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

Note: The 5'end of the variable region should encompass the native ATG initiation codon and the region immediately after which corresponds to the signal sequence. For proper initiation of translation, make sure that your insert contains a Kozak translation initiation sequence upstream of the ATG initiation codon such as (G/A)NNATGG.

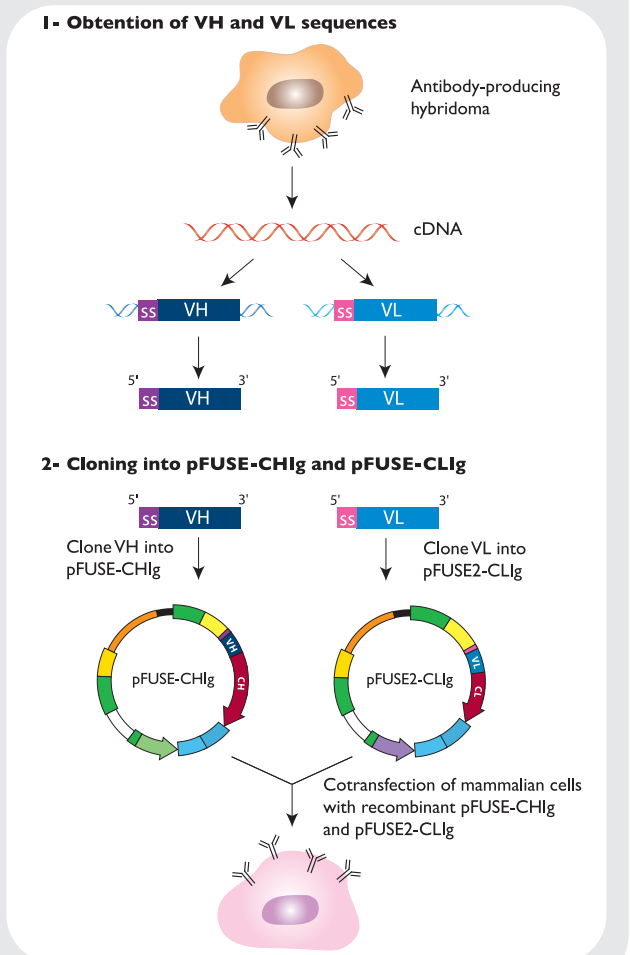
Choice of strategies for the transfection

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 pFUSE2-CLIg and pFUSE-CHIg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of pFUSE2-CLIg and pFUSE-CHIg plasmids. We recommend using a ratio of 3:2 of pFUSE2-CLIg:pFUSE-CHIg plasmids. pFUSE2-CLIg plasmids feature the constant region of the human lambda 2 light chain, or the human kappa light chain. pFUSE2-CLIg plasmids are selectable with blasticidin. pFUSE-CHIg plasmids are selectable with Zeocin™.

Antibody generation using pFUSE-CHIg & pFUSE-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 IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

RELATED PRODUCTS

Product	Catalog Code
pFUSE2-CLIg-hK	pfuse2-hclk
pFUSE2-CLIg-hL2	pfuse2-hcll2
pFUSE-CHIg-hG1	pfuse-hchg1
pFUSE-CHIg-hG2	pfuse-hchg2
pFUSE-CHIg-hG3	pfuse-hchg3
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar	fas-zn-s

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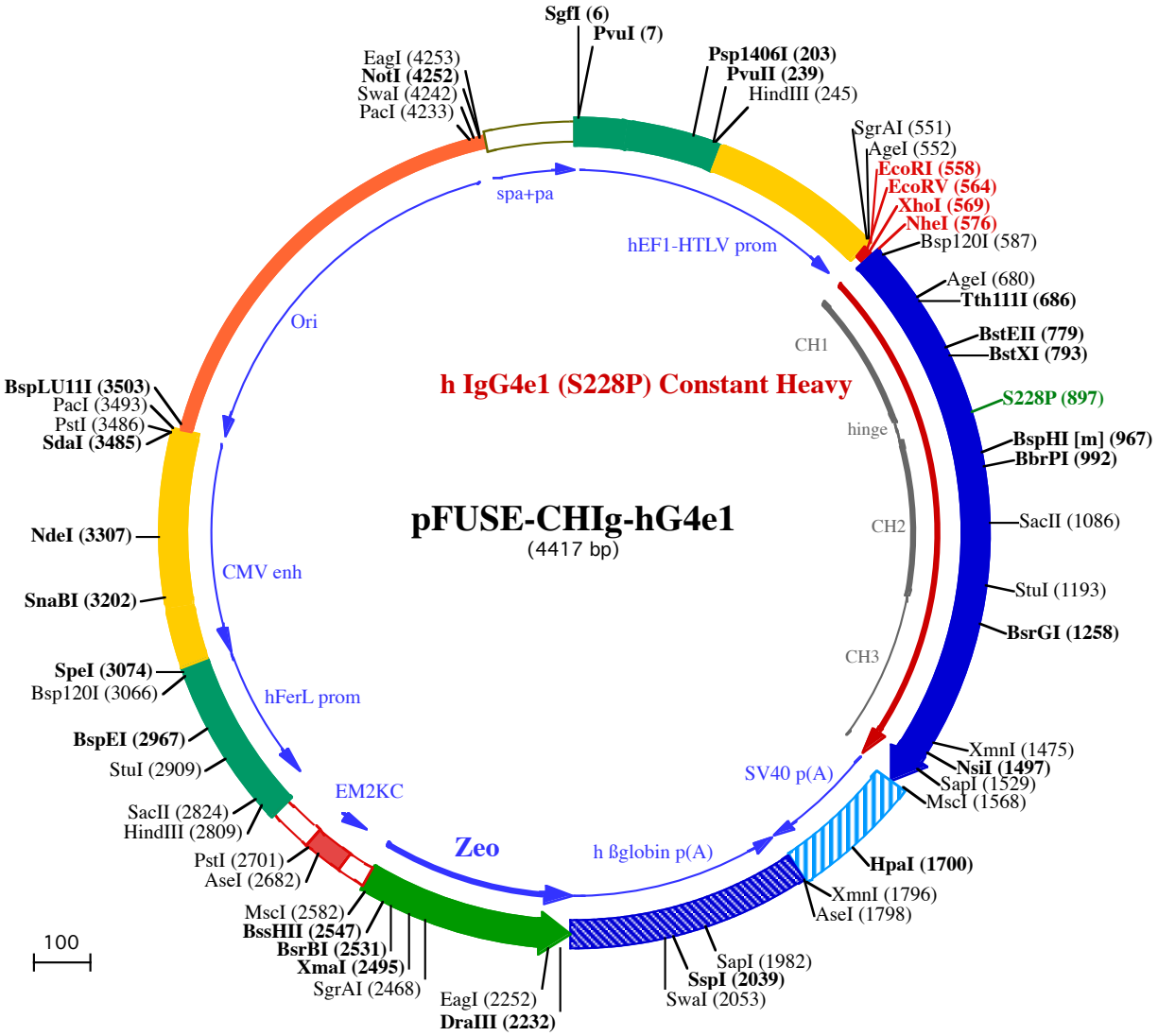
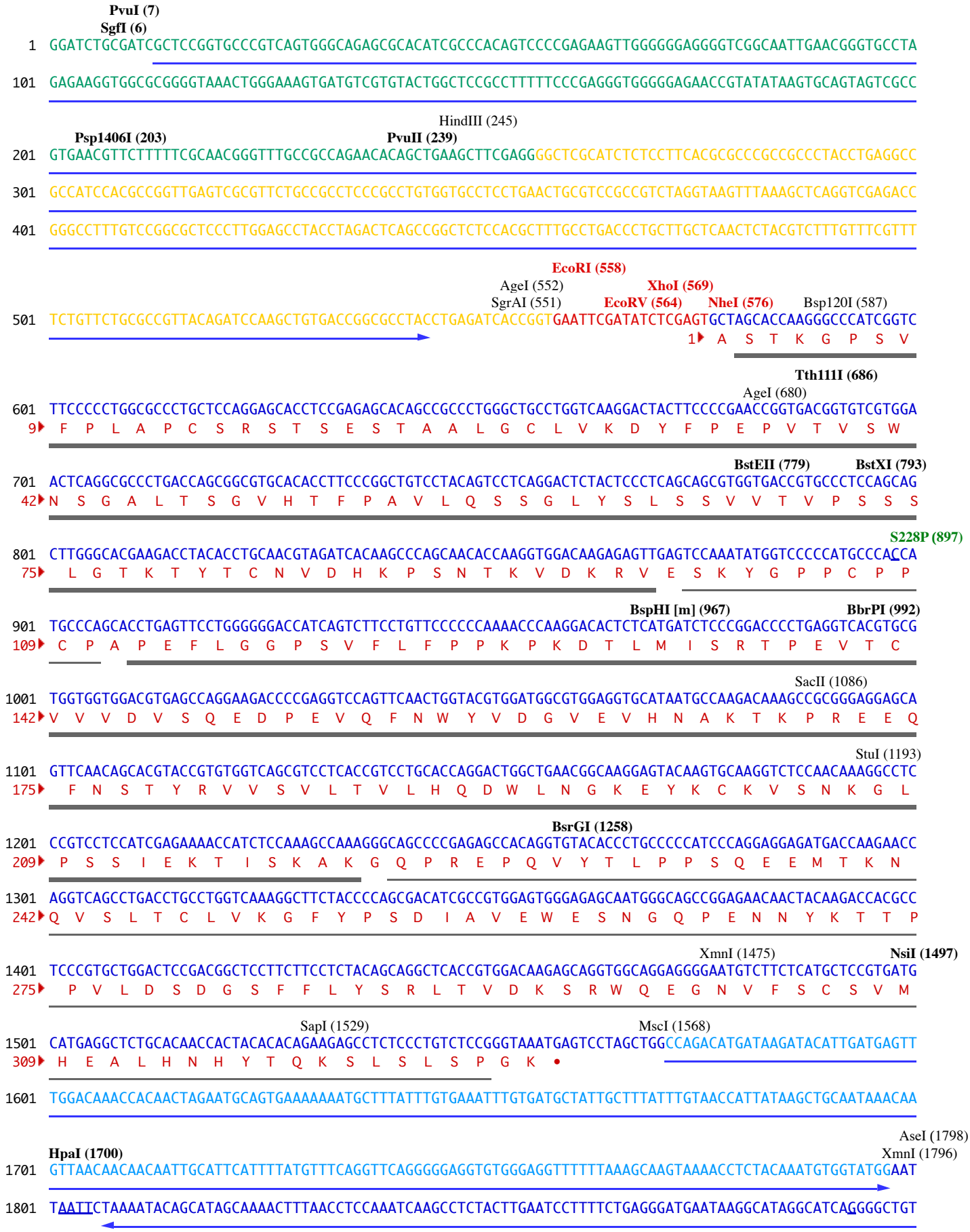
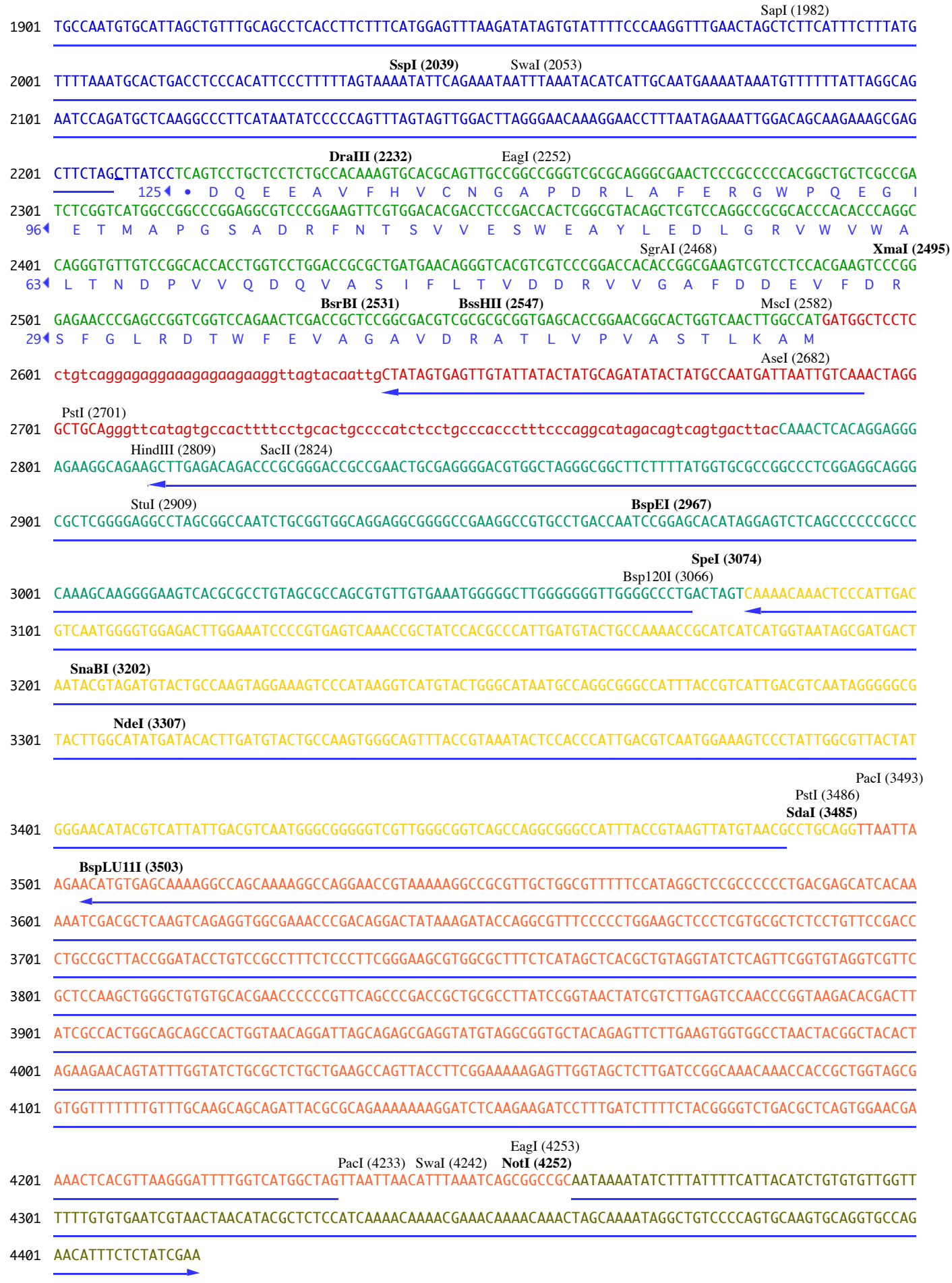

pFUSE-CHIg-hG4e1
(4417 bp)
h IgG4e1 (S228P) Constant Heavy
SgfI (6)
PvuI (7)
Psp1406I (203)
PvuII (239)
HindIII (245)
SgrAI (551)
AgeI (552)
EcoRI (558)
EcoRV (564)
XhoI (569)
NheI (576)
Bsp120I (587)
AgeI (680)
Tth111I (686)
BstEII (779)
BstXI (793)
S228P (897)
BspHI [m] (967)
BbrPI (992)
SacII (1086)
StuI (1193)
BsrGI (1258)
XmnI (1475)
NsiI (1497)
SapI (1529)
MscI (1568)
HpaI (1700)
XmnI (1796)
AseI (1798)
SapI (1982)
SspI (2039)
SwaI (2053)
EagI (2252)
DraIII (2232)
SgrAI (2468)
XmaI (2495)
BsrBI (2531)
BssHII (2547)
MscI (2582)
AseI (2682)
PstI (2701)
HindIII (2809)
SacII (2824)
StuI (2909)
BspEI (2967)
Bsp120I (3066)
SpeI (3074)
SnaBI (3202)
NdeI (3307)
SdaI (3485)
PstI (3486)
PacI (3493)
BspLU11I (3503)
PacI (4233)
SwaI (4242)
NotI (4252)
EagI (4253)
spa+pa
hEF1-HTLV prom
CH1
hinge
CH2
CH3
SV40 p(A)
h ßglobin p(A)
Zeo
EM2KC
hFerL prom
CMV enh
Ori
100


PvuI (7)
SgfI (6)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Psp1406I (203)
PvuII (239)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
EcoRI (558)
AgeI (552)
XhoI (569)
SgrAI (551)
EcoRV (564)
NheI (576)
Bsp120I (587)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGTGCTAGCACCAAGGGCCCATCGGTC
1 A S T K G P S V
Tth111I (686)
AgeI (680)
601 TTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
9 F P L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W
BstEII (779)
BstXI (793)
701 ACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
42 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S
S228P (897)
801 CTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCA
75 L G T K T Y T C N V D H K P S N T K V D K R V E S K Y G P P C P P
BspHI [m] (967)
BbrPI (992)
901 TGCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCG
109 C P A P E F L G G P S V F L F P P K P K D T L M I S R T P E V T C
SacII (1086)
1001 TGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCA
142 V V V D V S Q E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q
StuI (1193)
1101 GTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTC
175 F N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K G L
BsrGI (1258)
1201 CCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACC
209 P S S I E K T I S K A K G Q P R E P Q V Y T L P P S Q E E M T K N
1301 AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCC
242 Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P
XmnI (1475)
NsiI (1497)
1401 TCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATG
275 P V L D S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V M
SapI (1529)
MscI (1568)
1501 CATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTCCTAGCTGGCCAGACATGATAAGATACATTGATGAGTT
309 H E A L H N H Y T Q K S L S L S P G K •
1601 TGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAA
AseI (1798)
HpaI (1700)
XmnI (1796)
1701 GTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAT
1801 TAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGT


SapI (1982)
1901 TGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATG
SspI (2039)
SwaI (2053)
2001 TTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAG
2101 AATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAG
DraIII (2232)
EagI (2252)
2201 CTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGA
125 • D Q E E A V F H V C N G A P D R L A F E R G W P Q E G I
2301 TCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGC
96 E T M A P G S A D R F N T S V V E S W E A Y L E D L G R V W V W A
SgrAI (2468)
XmaI (2495)
2401 CAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGG
63 L T N D P V V Q D Q V A S I F L T V D D R V V G A F D D E V F D R
BsrBI (2531)
BssHII (2547)
MscI (2582)
2501 GAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTC
29 S F G L R D T W F E V A G A V D R A T L V P V A S T L K A M
AseI (2682)
2601 ctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGG
PstI (2701)
2701 GCTGCAgggttcatagtgccacttttcctgcactgccccatctcctgcccacccttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGG
HindIII (2809)
SacII (2824)
2801 AGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGG
StuI (2909)
BspEI (2967)
2901 CGCTCGGGGAGGCCTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCC
SpeI (3074)
Bsp120I (3066)
3001 CAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGAC
3101 GTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACT
SnaBI (3202)
3201 AATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCG
NdeI (3307)
3301 TACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTAT
PacI (3493)
PstI (3486)
SdaI (3485)
3401 GGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTA
BspLU11I (3503)
3501 AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAA
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4001 AGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCG
4101 GTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGA
EagI (4253)
PacI (4233)
SwaI (4242)
NotI (4252)
4201 AAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTT
4301 TTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG
4401 AACATTTCTCTATCGAA