pFUSEss-CHIg-hG4e1

Plasmid featuring a mutated constant region of the human IgG4 heavy chain and the IL2 signal sequence

Catalog # pfusess-hchg4e1

For research use only

Version # 16J03v40-JC

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSEss-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

- **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

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 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*. 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].

pFUSEss-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. Mol Cell Biol. 8(1):466-72. **3. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the SV40 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 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-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. Using EcoRI as the 5' cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids. In pFUSEss-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 (pFUSE2ss-CLIg-hK; human kappa), or AvrII (pFUSE2ss-CLIg-hL2; human lambda 2) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

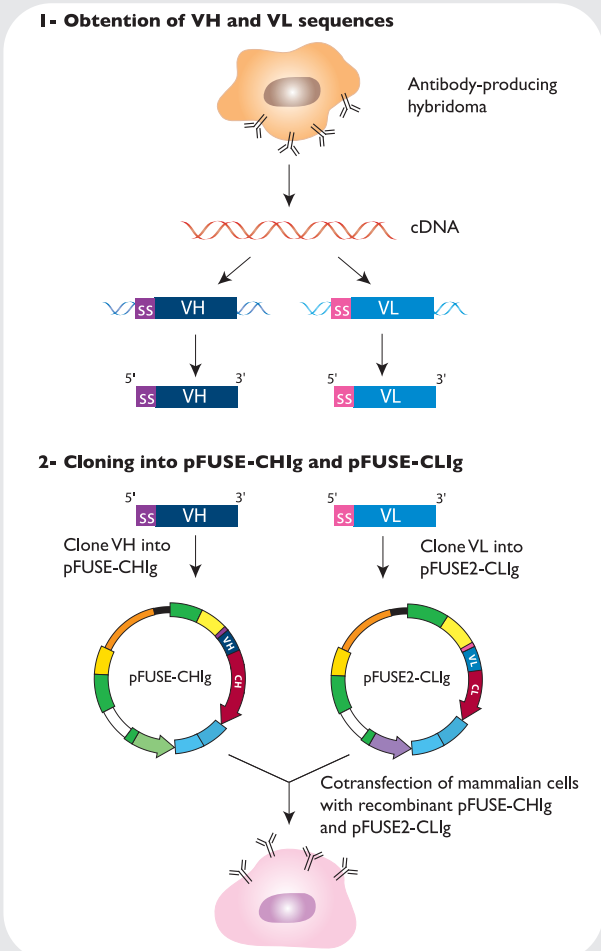
Choice of strategies for the transfection

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 the human lambda 2 light chain, or the human kappa light chain. pFUSE2ss-CLIg plasmids are selectable with blasticidin. pFUSEss-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
pFUSE2ss-CLIg-hK	pfuse2ss-hclk
pFUSE2ss-CLIg-hL2	pfuse2ss-hcll2
pFUSEss-CHIg-hG1	pfusess-hchg1
pFUSEss-CHIg-hG2	pfusess-hchg2
pFUSEss-CHIg-hG3	pfusess-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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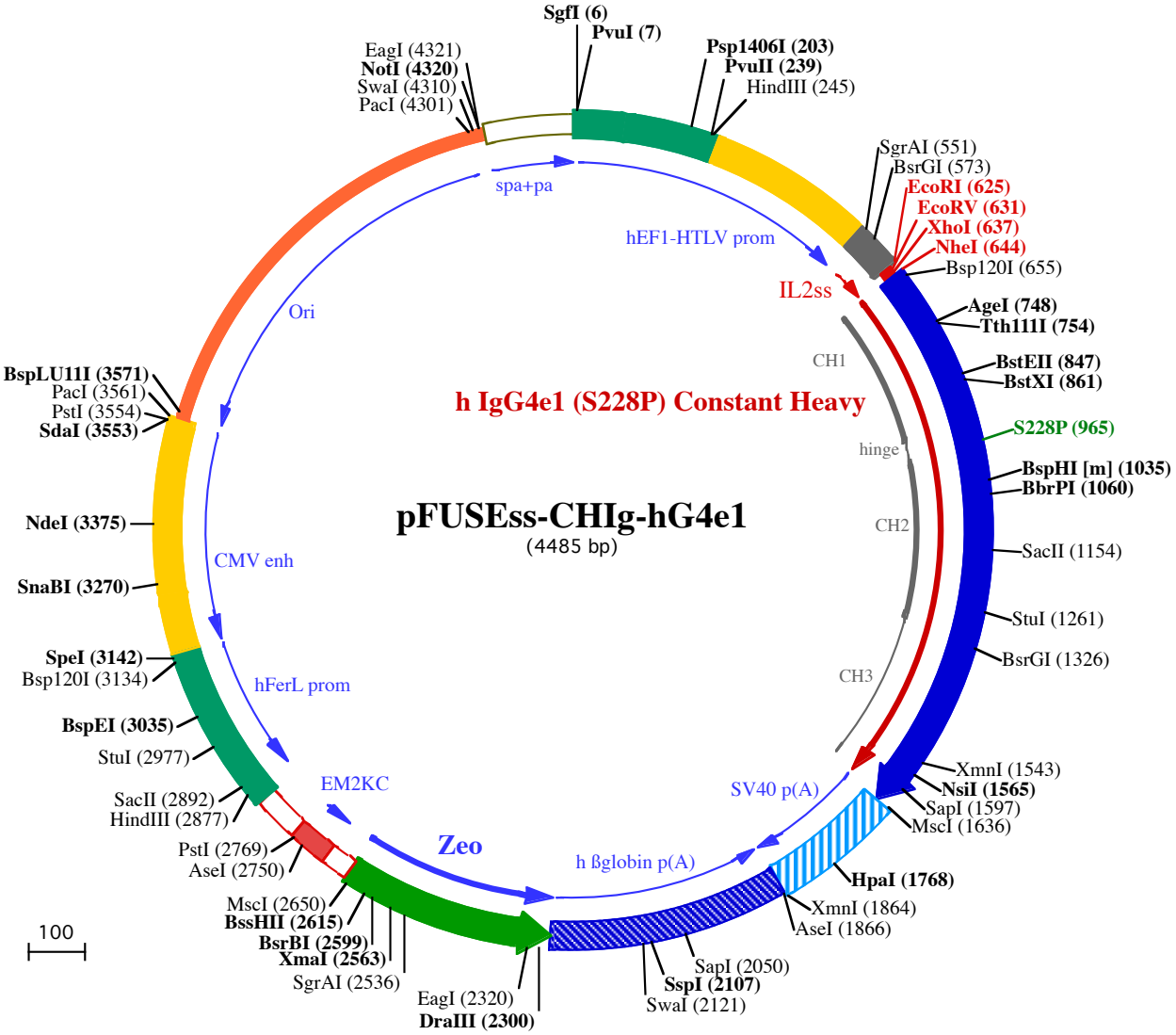
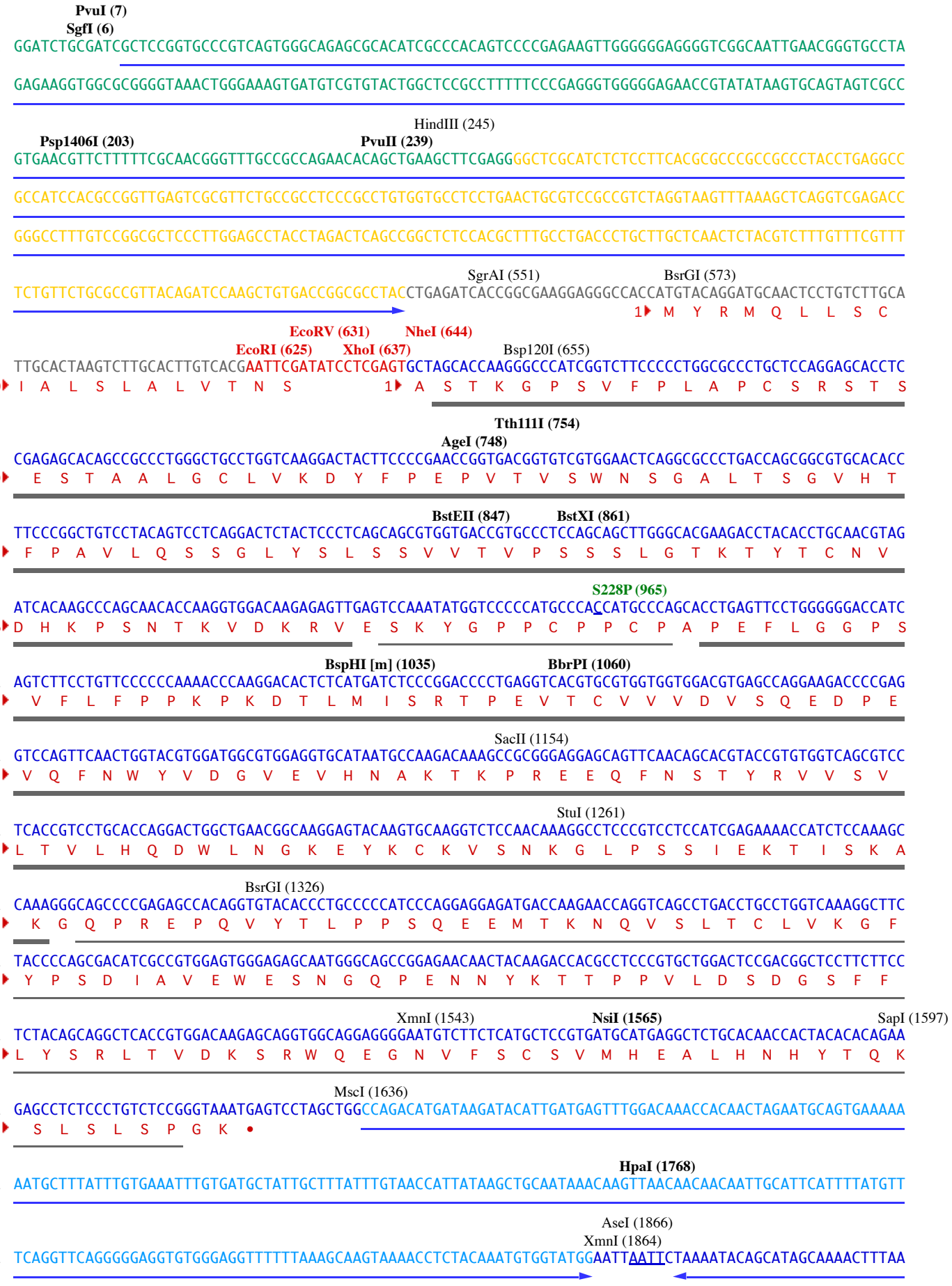

pFUSEss-CHIg-hG4e1
(4485 bp)
h IgG4e1 (S228P) Constant Heavy
SgfI (6)
PvuI (7)
Psp1406I (203)
PvuII (239)
HindIII (245)
SgrAI (551)
BsrGI (573)
EcoRI (625)
EcoRV (631)
XhoI (637)
NheI (644)
Bsp120I (655)
AgeI (748)
Tth111I (754)
BstEII (847)
BstXI (861)
S228P (965)
BspHI [m] (1035)
BbrPI (1060)
SacII (1154)
StuI (1261)
BsrGI (1326)
XmnI (1543)
NsiI (1565)
SapI (1597)
MscI (1636)
HpaI (1768)
XmnI (1864)
AseI (1866)
SapI (2050)
SspI (2107)
SwaI (2121)
EagI (2320)
DraIII (2300)
SgrAI (2536)
XmaI (2563)
BsrBI (2599)
BssHII (2615)
MscI (2650)
PstI (2769)
AseI (2750)
SacII (2892)
HindIII (2877)
StuI (2977)
BspEI (3035)
SpeI (3142)
Bsp120I (3134)
SnaBI (3270)
NdeI (3375)
BspLU11I (3571)
PacI (3561)
PstI (3554)
SdaI (3553)
EagI (4321)
NotI (4320)
SwaI (4310)
PacI (4301)
spa+pa
hEF1-HTLV prom
IL2ss
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
SgrAI (551)
BsrGI (573)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
1 M Y R M Q L L S C
EcoRV (631)
NheI (644)
EcoRI (625)
XhoI (637)
Bsp120I (655)
601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCCTCGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTC
10 I A L S L A L V T N S 1 A S T K G P S V F P L A P C S R S T S
Tth111I (754)
AgeI (748)
701 CGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
19 E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T
BstEII (847)
BstXI (861)
801 TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAG
53 F P A V L Q S S G L Y S L S S V V T V P S S S L G T K T Y T C N V
S228P (965)
901 ATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCATGCCCAGCACCTGAGTTCCTGGGGGGACCATC
86 D H K P S N T K V D K R V E S K Y G P P C P P C P A P E F L G G P S
BspHI [m] (1035)
BbrPI (1060)
1001 AGTCTTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAG
119 V F L F P P K P K D T L M I S R T P E V T C V V V D V S Q E D P E
SacII (1154)
1101 GTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCC
153 V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V
StuI (1261)
1201 TCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGC
186 L T V L H Q D W L N G K E Y K C K V S N K G L P S S I E K T I S K A
BsrGI (1326)
1301 CAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
219 K G Q P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F
1401 TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
253 Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F
XmnI (1543)
NsiI (1565)
SapI (1597)
1501 TCTACAGCAGGCTCACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAA
286 L Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K
MscI (1636)
1601 GAGCCTCTCCCTGTCTCCGGGTAAATGAGTCCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAA
319 S L S L S P G K •
HpaI (1768)
1701 AATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTT
AseI (1866)
XmnI (1864)
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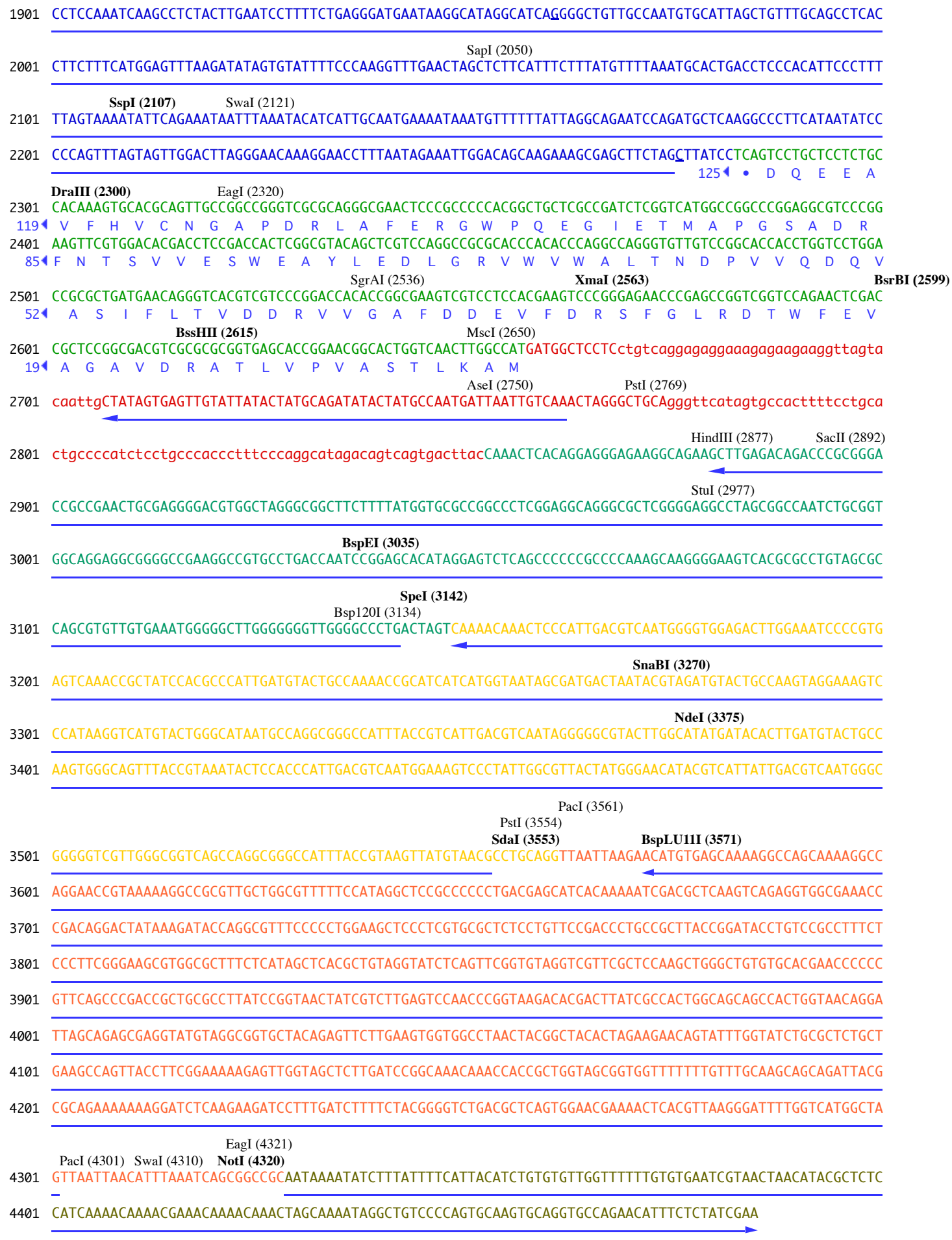
1901 CCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCAC
SapI (2050)
2001 CTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTT
SspI (2107)
SwaI (2121)
2101 TTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCC
2201 CCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGC
125 • D Q E E A
DraIII (2300)
EagI (2320)
2301 CACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGG
119 V F H 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
2401 AAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGA
85 F N T 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
SgrAI (2536)
XmaI (2563)
BsrBI (2599)
2501 CCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGAC
52 A S I 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
BssHII (2615)
MscI (2650)
2601 CGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagta
19 A G A V D R A T L V P V A S T L K A M
AseI (2750)
PstI (2769)
2701 caattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgccacttttcctgca
HindIII (2877)
SacII (2892)
2801 ctgccccatctcctgcccacccctttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGA
StuI (2977)
2901 CCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCGGT
BspEI (3035)
3001 GGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGC
SpeI (3142)
Bsp120I (3134)
3101 CAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTG
SnaBI (3270)
3201 AGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTC
NdeI (3375)
3301 CCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCC
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PacI (3561)
PstI (3554)
SdaI (3553)
BspLU11I (3571)
3501 GGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCC
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EagI (4321)
PacI (4301)
SwaI (4310)
NotI (4320)
4301 GTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTC
4401 CATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA