pFUSEss-CHIg-rG*03

Plasmid featuring the constant region of the rabbit Ig (allele 3/2) heavy chain and the IL2 signal sequence

Catalog code: pfusess-rchg

For research use only

Version 22G13-MMv31

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSEss-CHIg-rG*03** 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

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-rG specific features

• **Rabbit IgHG (IgG 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.

TECHNICAL SUPPORT
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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-rG, the constant region of the rabbit IgG heavy chain is preceded by a multiple cloning site containing three restriction sites: EcoRI, Eco RV, and XhoI. 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-rG, use XhoI as the 3' cloning site for the VH in order to preserve the IgG constant amino acid sequence. We recommend to introduce by PCR an XhoI site at the 3' end of the VH in frame with the constant region.

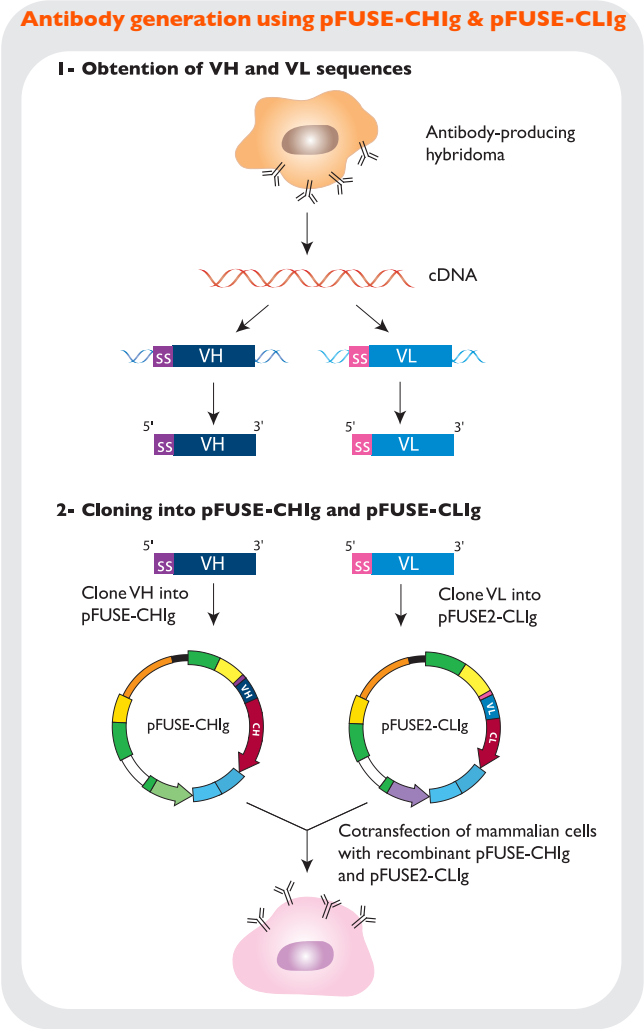
When generating the insert for VL, a BamHI (rabbit kappa; pFUSE2-CLIg-rk1 or pFUSE2-CLIg-rk2) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

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 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 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 rabbit lambda 1 or lambda 2 light chain. pFUSE2-CLIg plasmids are selectable with blasticidin. pFUSE-CHIg plasmids are selectable with Zeocin™.



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-rk1	pfuse2ss-rclk1
pFUSE2ss-CLIg-rk2	pfuse2ss-rcll2
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1

TECHNICAL SUPPORT
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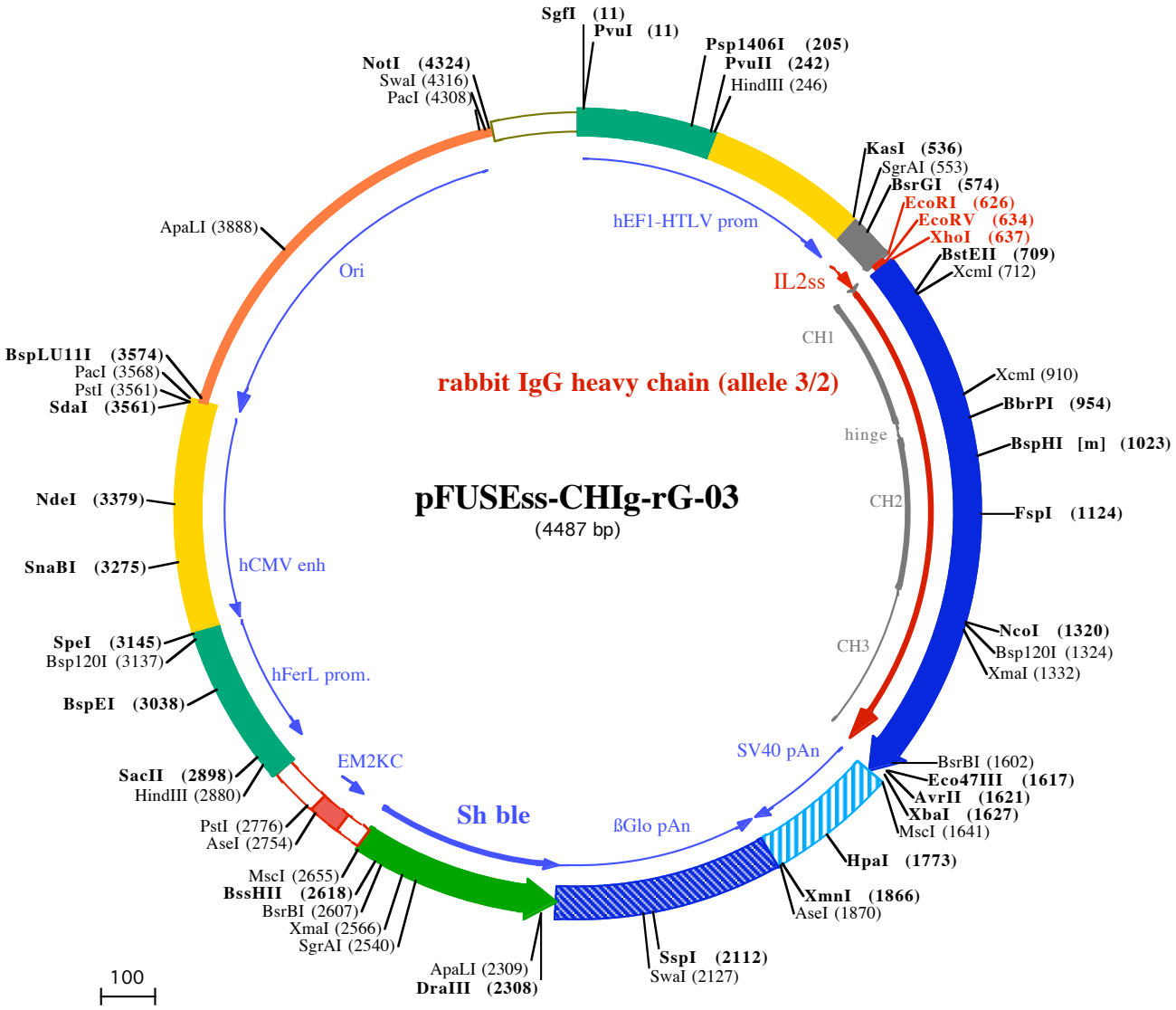
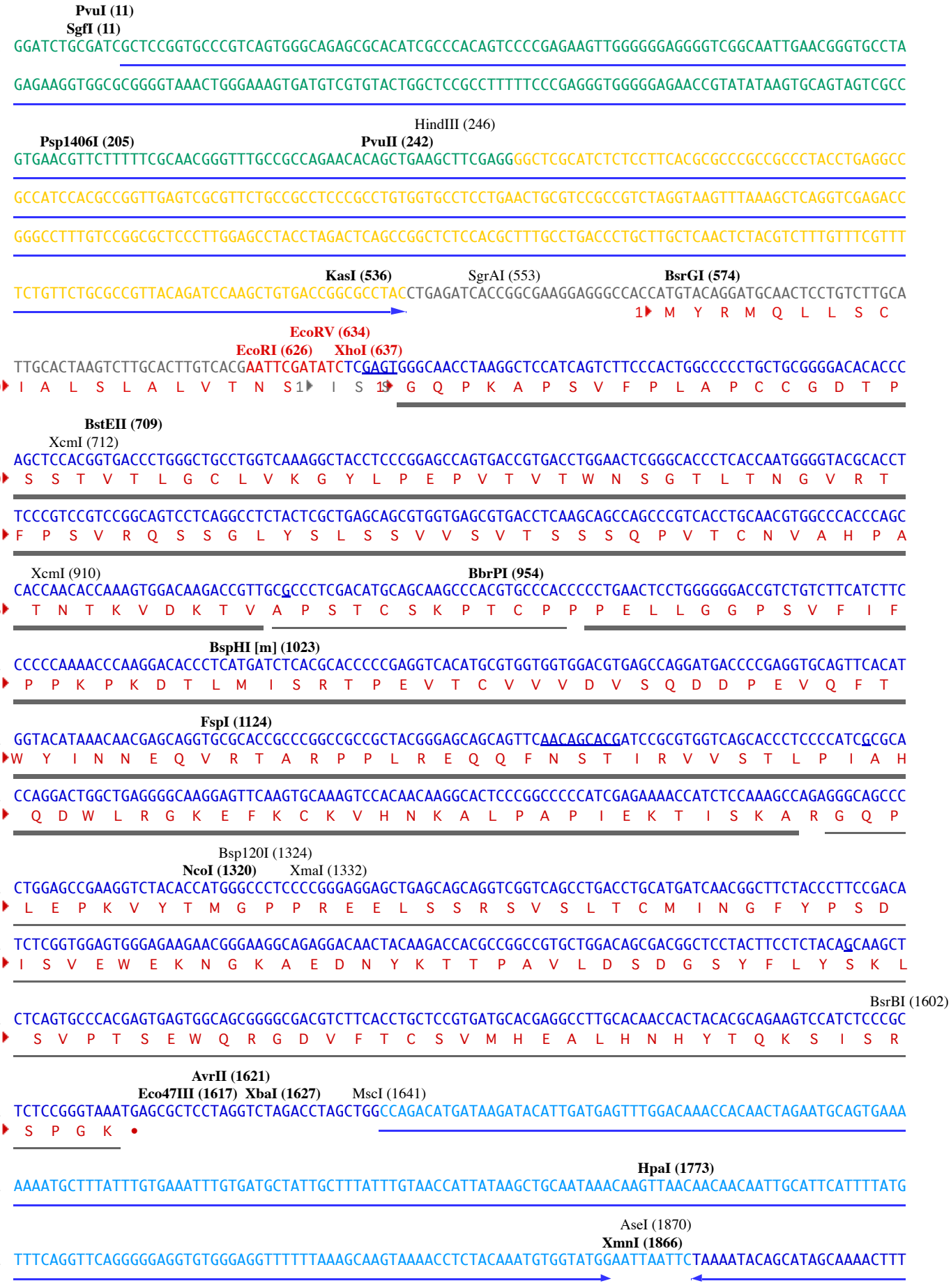

pFUSEss-CHIg-rG-03
(4487 bp)
rabbit IgG heavy chain (allele 3/2)
hEF1-HTLV prom
IL2ss
CH1
hinge
CH2
CH3
SV40 pAn
ßGlo pAn
Sh ble
EM2KC
hFerL prom.
hCMV enh
Ori
SgfI (11)
PvuI (11)
Psp1406I (205)
PvuII (242)
HindIII (246)
KasI (536)
SgrAI (553)
BsrGI (574)
EcoRI (626)
EcoRV (634)
XhoI (637)
BstEII (709)
XcmI (712)
XcmI (910)
BbrPI (954)
BspHI [m] (1023)
FspI (1124)
NcoI (1320)
Bsp120I (1324)
XmaI (1332)
BsrBI (1602)
Eco47III (1617)
AvrII (1621)
XbaI (1627)
MscI (1641)
HpaI (1773)
XmnI (1866)
AseI (1870)
SspI (2112)
SwaI (2127)
ApaLI (2309)
DraIII (2308)
MscI (2655)
BssHII (2618)
BsrBI (2607)
XmaI (2566)
SgrAI (2540)
PstI (2776)
AseI (2754)
SacII (2898)
HindIII (2880)
BspEI (3038)
SpeI (3145)
Bsp120I (3137)
SnaBI (3275)
NdeI (3379)
BspLU11I (3574)
PacI (3568)
PstI (3561)
SdaI (3561)
ApaLI (3888)
NotI (4324)
SwaI (4316)
PacI (4308)
100


PvuI (11)
SgfI (11)
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101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (246)
Psp1406I (205)
PvuII (242)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
KasI (536)
SgrAI (553)
BsrGI (574)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
1 M Y R M Q L L S C
EcoRV (634)
EcoRI (626)
XhoI (637)
601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCTCGAGTGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCC
10 I A L S L A L V T N S 1 I S 19 G Q P K A P S V F P L A P C C G D T P
BstEII (709)
XcmI (712)
701 AGCTCCACGGTGACCCTGGGCTGCCTGGTCAAAGGCTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCACCT
20 S S T V T L G C L V K G Y L P E P V T V T W N S G T L T N G V R T
801 TCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCAGC
53 F P S V R Q S S G L Y S L S S V V S V T S S S Q P V T C N V A H P A
XcmI (910)
BbrPI (954)
901 CACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCTGTCTTCATCTTC
86 T N T K V D K T V A P S T C S K P T C P P P E L L G G P S V F I F
BspHI [m] (1023)
1001 CCCCCAAAACCCAAGGACACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACAT
120 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 D D P E V Q F T
FspI (1124)
1101 GGTACATAAACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCA
153 W Y I N N E Q V R T A R P P L R E Q Q F N S T I R V V S T L P I A H
1201 CCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCC
186 Q D W L R G K E F K C K V H N K A L P A P I E K T I S K A R G Q P
Bsp120I (1324)
NcoI (1320)
XmaI (1332)
1301 CTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACA
220 L E P K V Y T M G P P R E E L S S R S V S L T C M I N G F Y P S D
1401 TCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAGCAAGCT
253 I S V E W E K N G K A E D N Y K T T P A V L D S D G S Y F L Y S K L
BsrBI (1602)
1501 CTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGC
286 S V P T S E W Q R G D V F T C S V M H E A L H N H Y T Q K S I S R
AvrII (1621)
Eco47III (1617)
XbaI (1627)
MscI (1641)
1601 TCTCCGGGTAAATGAGCGCTCCTAGGTCTAGACCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAA
320 S P G K •
HpaI (1773)
1701 AAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATG
AseI (1870)
XmnI (1866)
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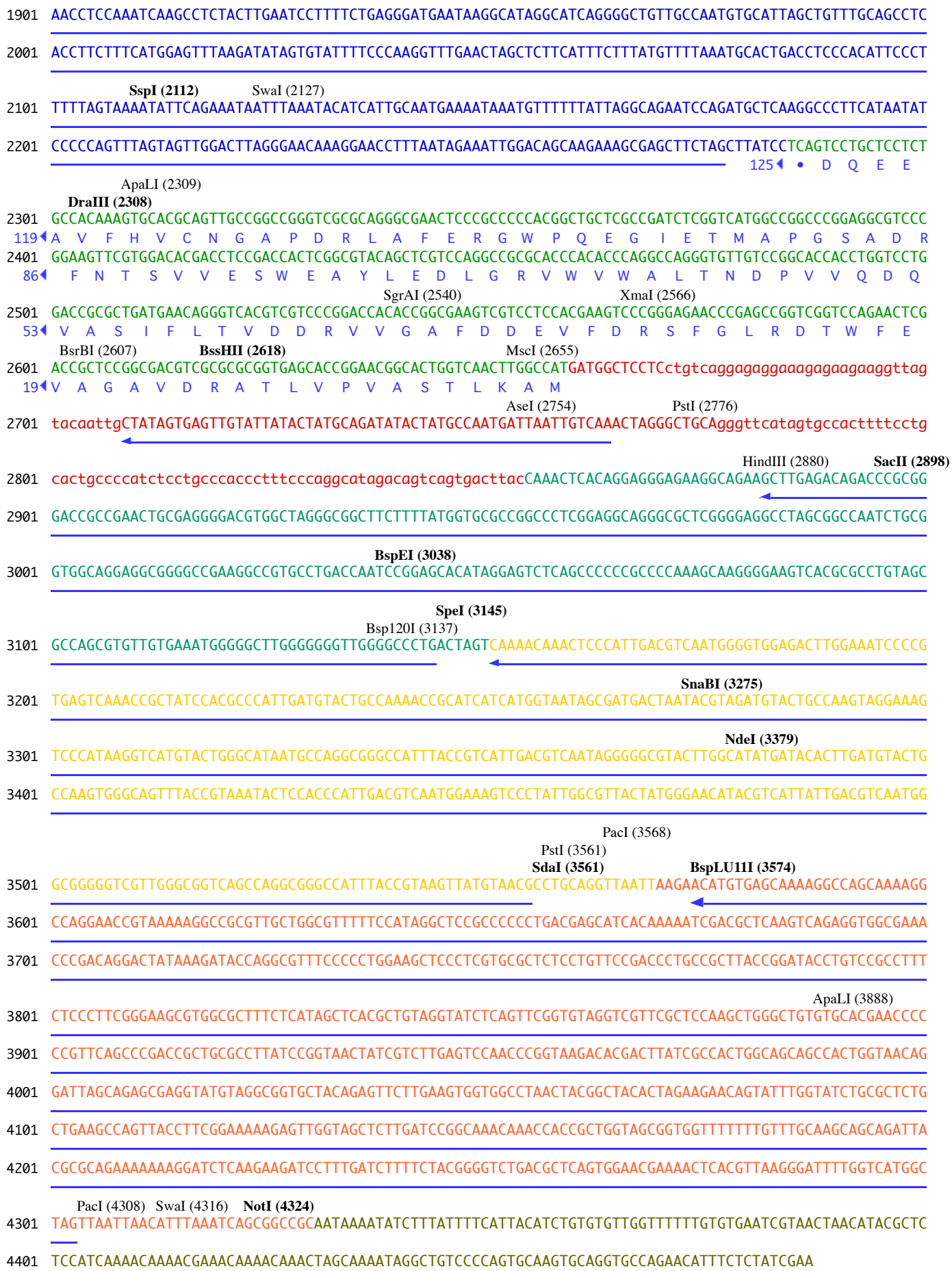
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SspI (2112)
SwaI (2127)
2101 TTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATAT
2201 CCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCT
125 • D Q E E
ApaLI (2309)
DraIII (2308)
2301 GCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCC
119 A 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 GGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTG
86 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
SgrAI (2540)
XmaI (2566)
2501 GACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCG
53 V 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
BsrBI (2607)
BssHII (2618)
MscI (2655)
2601 ACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttag
19 V A G A V D R A T L V P V A S T L K A M
AseI (2754)
PstI (2776)
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HindIII (2880)
SacII (2898)
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2901 GACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCAATCTGCG
BspEI (3038)
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SpeI (3145)
Bsp120I (3137)
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SnaBI (3275)
3201 TGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAG
NdeI (3379)
3301 TCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTG
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PacI (3568)
PstI (3561)
SdaI (3561)
BspLU11I (3574)
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ApaLI (3888)
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PacI (4308)
SwaI (4316)
NotI (4324)
4301 TAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTC
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