pFUSEN-hG2Fc

Plasmid designed for the fusion of an Fc domain to the N-terminus of a protein of interest

Catalog # pfcn-hg2

For research use only

Version 20K09-MM-v36

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSEN-hG2Fc** 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 and lyophilized.

GENERAL PRODUCT USE

pFUSEN-Fc is a family of plasmids developed to facilitate the construction of Fc-fusion proteins where the immunoglobulin G (IgG) Fc-domain is fused to the N-terminus of the protein of interest.

pFUSEN-Fc plasmids yield high levels of Fc-fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, CHO cells, monkey COS cells and human embryonic kidney (HEK) 293 cells, cells that are commonly used in protein purification systems.

A choice of cloning sites is provided to allow flexibility in the design of the fusion linker: either use pFUSEN linker, or bring forth your own linker with the protein of interest.

pFUSEN-Fc plasmids allow the secretion of Fc-Fusion proteins. They contain the human IL2 signal sequence (IL2ss). As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pFUSEN-Fc-transfected cells by SDS-PAGE. Furthermore, functional domains can be identified by immunoblotting and ligand blotting.

Fc-Fusion proteins can be easily purified by protein A or protein G affinity chromatography.

InvivoGen provides pFUSEN-Fc vectors featuring Fc regions from different species and isotypes. In humans, three options are available: IgG1, IgG1e2, or IgG2. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). IgG isoforms exert different levels of effector functions. The engineered IgG1e2 contains mutations in the FcRn binding sites leading to higher FcRn binding affinity and reduced pH dependence.

PLASMID FEATURES

• **Human IgG2-Fc :** The Fc region comprises the CH2 and CH3 domains of the IgG2 heavy chain, with the hinge region. The first and second cysteines of the hinge have been replaced by serines to prevent detrimental disulfite bridges. The last amino acid (lysine) of the Fc region has been replaced by an alanine for better fusion result.
Human IgG2 dispays low ADCC and CDC.

• **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.

• **IL2 ss:** The IL2 signal sequence contains 20 amino acids 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 fusion protein.

• **Cloning sites & fusion linker:** The protein of interest can be cloned either as a BamHI—NheI fragment, or as an EcoRV—NheI fragment, or as a BsiWI—NheI fragment. With BamHI or EcoRV cloning, the protein of interest will be separated from the Fc-domain by a flexible linker (Gly_4Ser dimer). With BsiWI cloning, the flexible linker will not be retained, allowing for a different fusion design.
The provided cloning sites 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.

• **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*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

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

TECHNICAL SUPPORT
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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.

METHODS

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.

Plasmid amplification and cloning

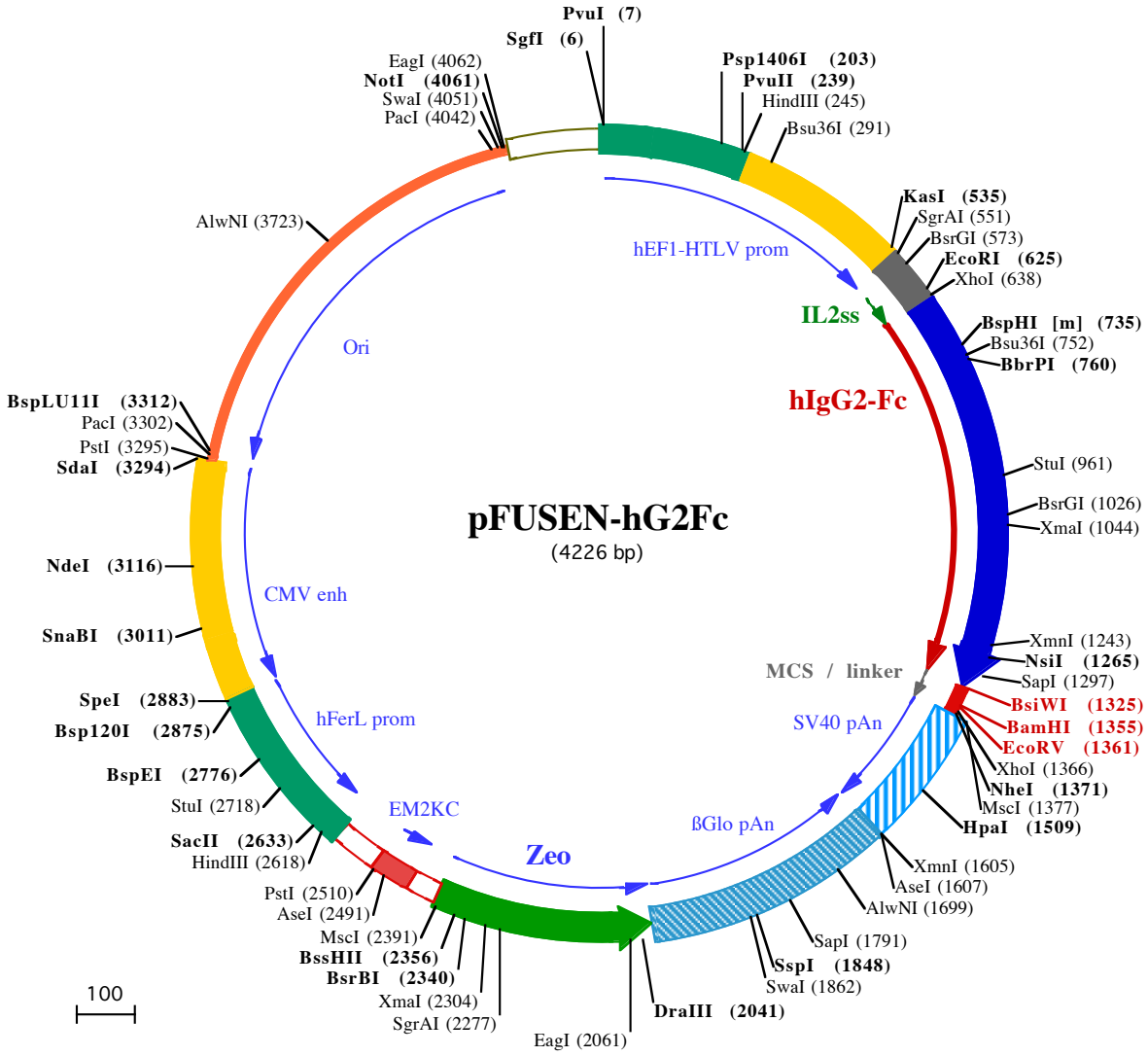
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

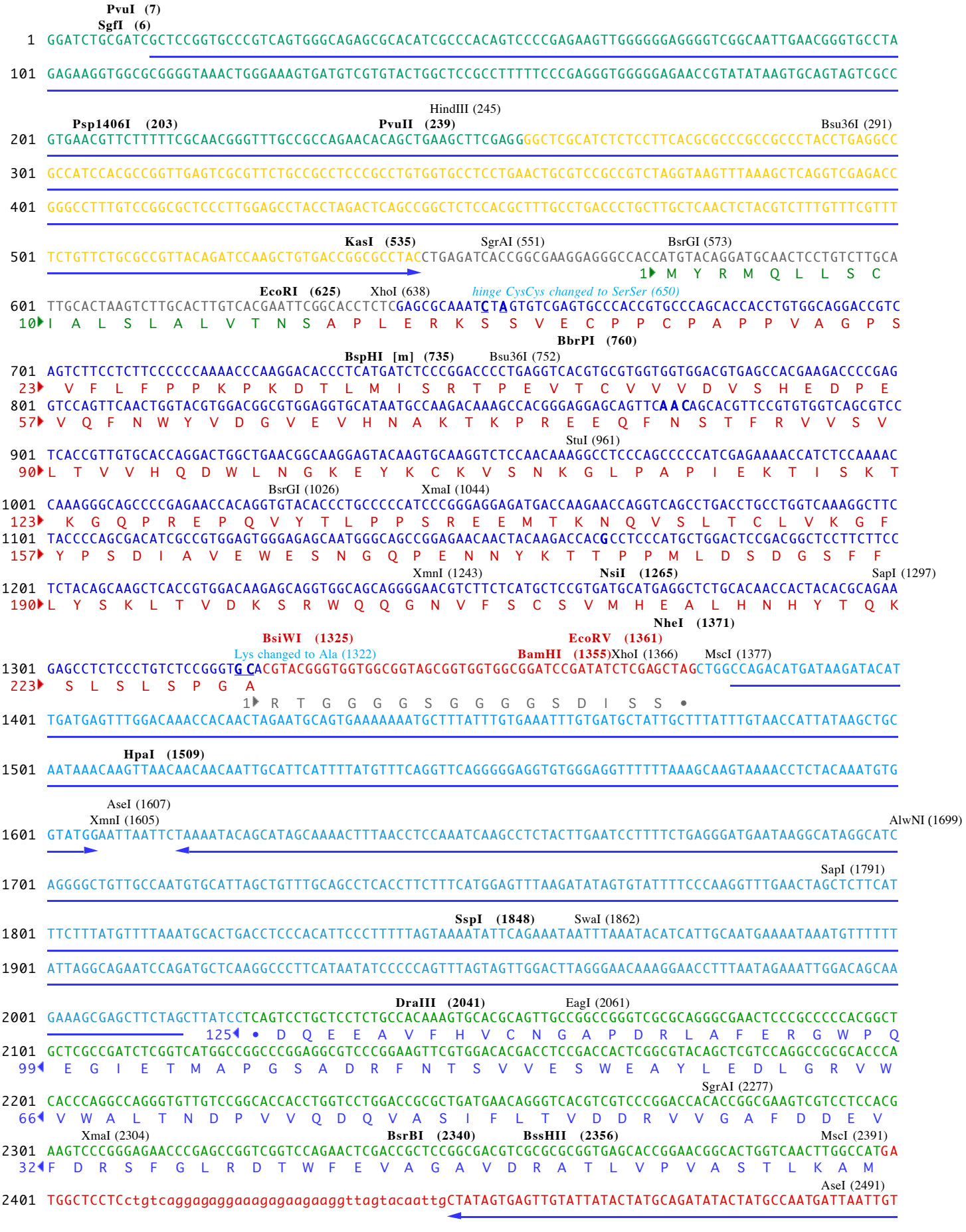
Zeocin™ usage

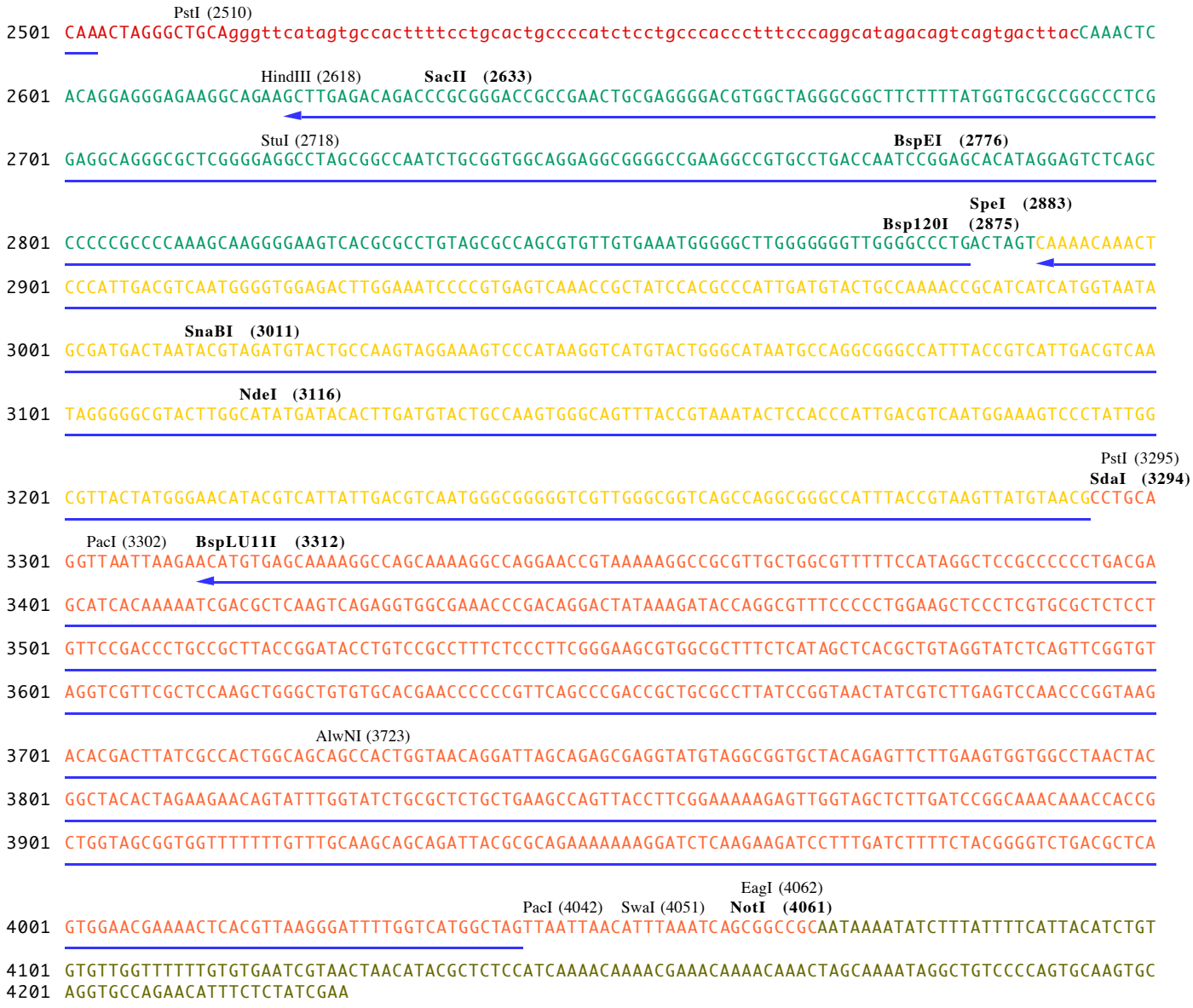
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1


pFUSEN-hG2Fc
(4226 bp)
PvuI (7)
SgfI (6)
Psp1406I (203)
PvuII (239)
HindIII (245)
Bsu36I (291)
KasI (535)
SgrAI (551)
BsrGI (573)
EcoRI (625)
XhoI (638)
BspHI [m] (735)
Bsu36I (752)
BbrPI (760)
StuI (961)
BsrGI (1026)
XmaI (1044)
XmnI (1243)
NsiI (1265)
SapI (1297)
BsiWI (1325)
BamHI (1355)
EcoRV (1361)
XhoI (1366)
NheI (1371)
MscI (1377)
HpaI (1509)
XmnI (1605)
AseI (1607)
AlwNI (1699)
SapI (1791)
SspI (1848)
SwaI (1862)
DraIII (2041)
EagI (2061)
XmaI (2304)
SgrAI (2277)
BssHII (2356)
BsrBI (2340)
MscI (2391)
AseI (2491)
PstI (2510)
HindIII (2618)
SacII (2633)
StuI (2718)
BspEI (2776)
Bsp120I (2875)
SpeI (2883)
SnaBI (3011)
NdeI (3116)
SdaI (3294)
PstI (3295)
PacI (3302)
BspLU11I (3312)
AlwNI (3723)
EagI (4062)
NotI (4061)
SwaI (4051)
PacI (4042)
hEF1-HTLV prom
IL2ss
hIgG2-Fc
MCS / linker
SV40 pAn
ßGlo pAn
Zeo
EM2KC
hFerL prom
CMV enh
Ori
100


PvuI (7)
SgfI (6)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Psp1406I (203)
PvuII (239)
Bsu36I (291)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
KasI (535)
SgrAI (551)
BsrGI (573)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
1 M Y R M Q L L S C
EcoRI (625)
XhoI (638)
hinge CysCys changed to SerSer (650)
601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGGCACCTCTCGAGCGCAAATCTAGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTC
10 I A L S L A L V T N S A P L E R K S S V E C P P C P A P P V A G P S
BbrPI (760)
BspHI [m] (735)
Bsu36I (752)
701 AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAG
23 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 H E D P E
801 GTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCC
57 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 F R V V S V
StuI (961)
901 TCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAAC
90 L T V V H Q D W L N G K E Y K C K V S N K G L P A P I E K T I S K T
BsrGI (1026)
XmaI (1044)
1001 CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
123 K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F
1101 TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCATGCTGGACTCCGACGGCTCCTTCTTCC
157 Y P S D I A V E W E S N G Q P E N N Y K T T P P M L D S D G S F F
XmnI (1243)
NsiI (1265)
SapI (1297)
1201 TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
190 L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K
NheI (1371)
BsiWI (1325)
EcoRV (1361)
Lys changed to Ala (1322)
BamHI (1355)
XhoI (1366)
MscI (1377)
1301 GAGCCTCTCCCTGTCTCCGGGTGCACGTACGGGTGGTGGCGGTAGCGGTGGTGGCGGATCCGATATCTCGAGCTAGCTGGCCAGACATGATAAGATACAT
223 S L S L S P G A
1 R T G G G G S G G G G S D I S S •
1401 TGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGC
HpaI (1509)
1501 AATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTG
AseI (1607)
XmnI (1605)
AlwNI (1699)
1601 GTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATC
SapI (1791)
1701 AGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCAT
SspI (1848)
SwaI (1862)
1801 TTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTT
1901 ATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAA
DraIII (2041)
EagI (2061)
2001 GAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCT
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
2101 GCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCA
99 E G I 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
SgrAI (2277)
2201 CACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACG
66 V W A 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
XmaI (2304)
BsrBI (2340)
BssHII (2356)
MscI (2391)
2301 AAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGA
32 F D R 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 (2491)
2401 TGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGT


PstI (2510)
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