CAUTION

Before using this product, please read the Limited Use License statement below:

Important Limited Use License information for pFUSEN-Lucia-hG1e2Fc

The purchase of the pFUSEN-Lucia-hG1e2Fc vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact InvivoGen, 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

pFUSEN-Lucia-hG1e2Fc

Plasmid designed for Lucia::Fc fusion to the N-terminus of a protein of interest

Catalog # pfcn-lchg1e2

For research use only

Version 20K09-MM-v36

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSEN-Lucia-hG1e2Fc** 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 / pFUSEN-Lucia-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 / pFUSEN-Lucia-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.

pFUSEN-Lucia-hG1e2Fc plasmid allows the production of Lucia-Fc fusion proteins. This plasmid can be used to make recombinant Lucia-Fc fusion proteins or can be used as a transfection control in experiments with other pFUSEN-Fc constructs. Quantification of Lucia-Fc expression can be determined utilizing InvivoGen's QUANTI-Luc™ (rep-qlc1 or rep-qlc2).

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.

InvivoGen provides pFUSEN-Lucia-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

- **Lucia luciferase** is a secreted coelenterazine-utilizing luciferase reporter protein with advantageous characteristics when associated with Fc-fusion proteins. It possesses superior carrier ability for excellent secretion of the chimeric protein. It provides a simple means to screen for recombinant clones and it minimally affects the activity of the protein of interest.
- **Human IgG1e2-Fc :** The Fc region comprises the CH2 and CH3 domains of the IgG1 heavy chain, with the hinge region. The first cysteine of the hinge has been replaced by a serine to prevent detrimental disulfite bridges. The last amino acid (lysine) of the Fc region has been replaced by an alanine for better fusion result. The Fc region binds to neonatal FcR (FcRn), a receptor expressed on the surface of endothelial cells. This interaction, which is pH-dependent, protects the IgG from lysosomal degradation thus mediating the serum persistence of IgG antibodies. The human "IgG1e2" Fc domain was engineered to introduce mutations in the FcRn binding sites leading to higher FcRn binding affinity and reduced pH dependence[5]. hIgG1e2 Fc contains the following mutations: M252Y/S254T/T256E and H433K/N434F.
- **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.
- **Cloning sites & fusion linker:** The protein of interest can be cloned either as a BamHI—NheI fragment, or as a BsiWI—NheI fragment. With BamHI cloning, the protein of interest will be separated from the Fc-domain by a flexible linker (Gly4Ser 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.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

• **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 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.Vaccaro C. et al. 2005. Engineering the Fc rregion of immunoglobulin G to modulate in vivo antibody levels. Nat Biotechnol. 23(10):1283-8.

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

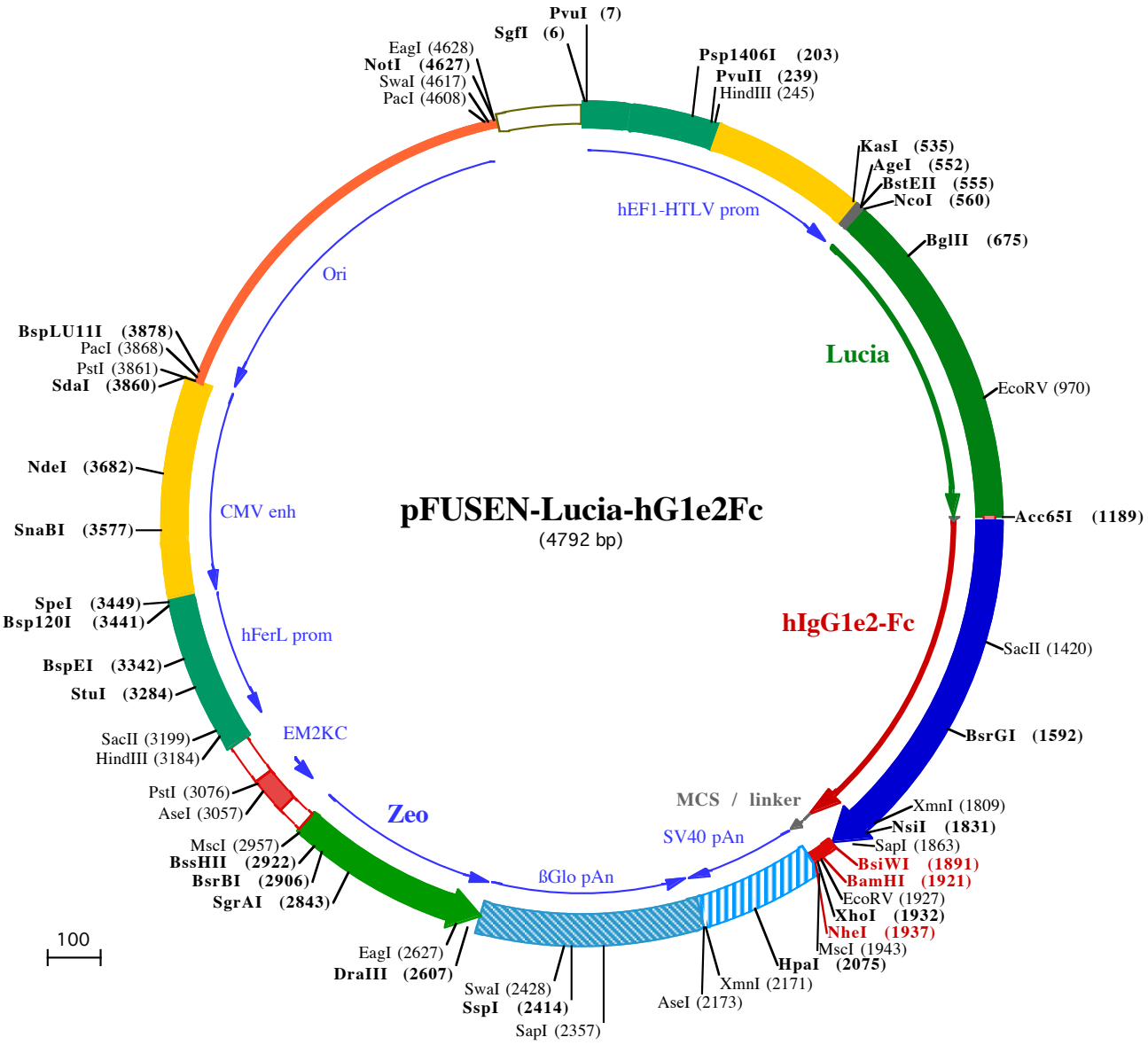
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

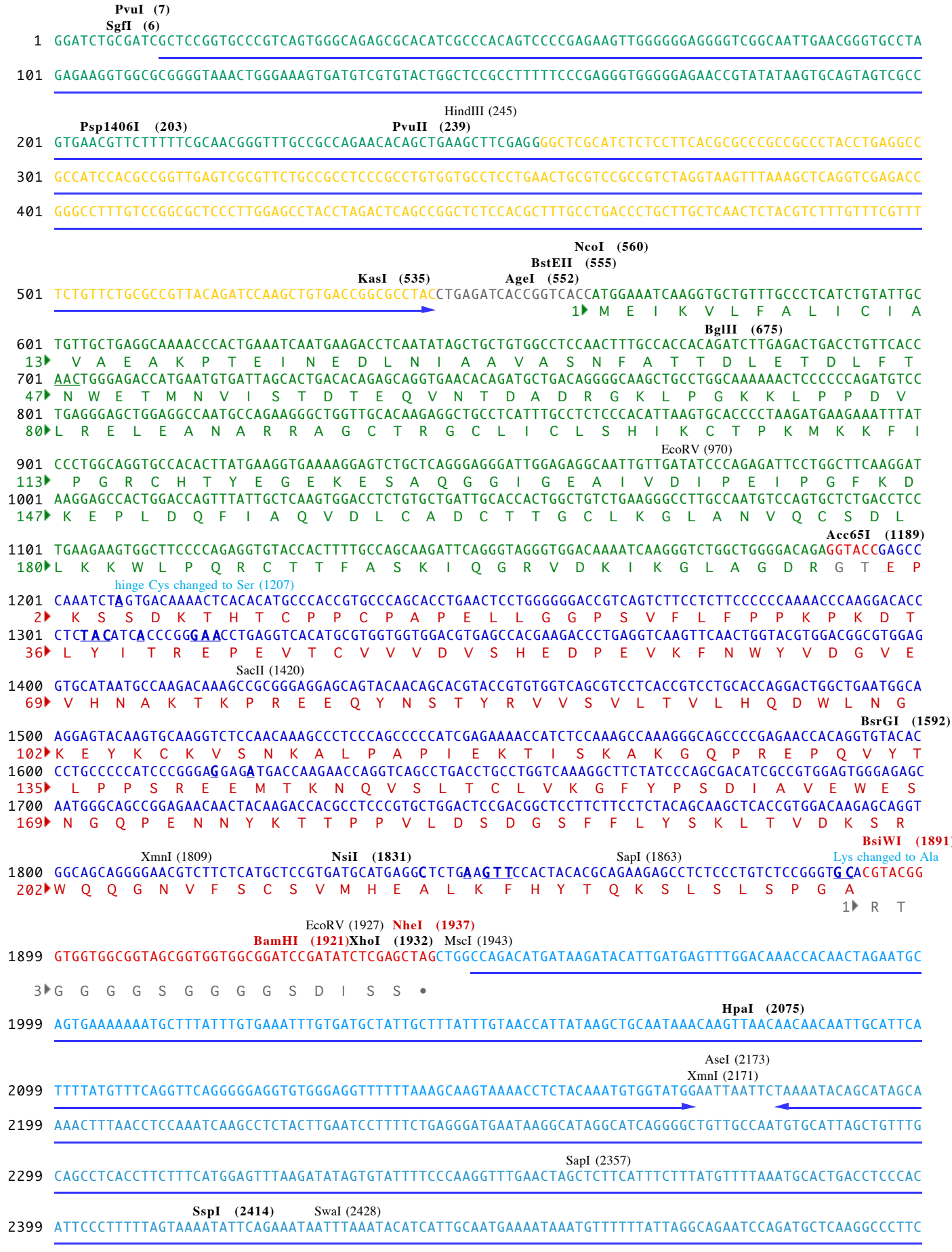
This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

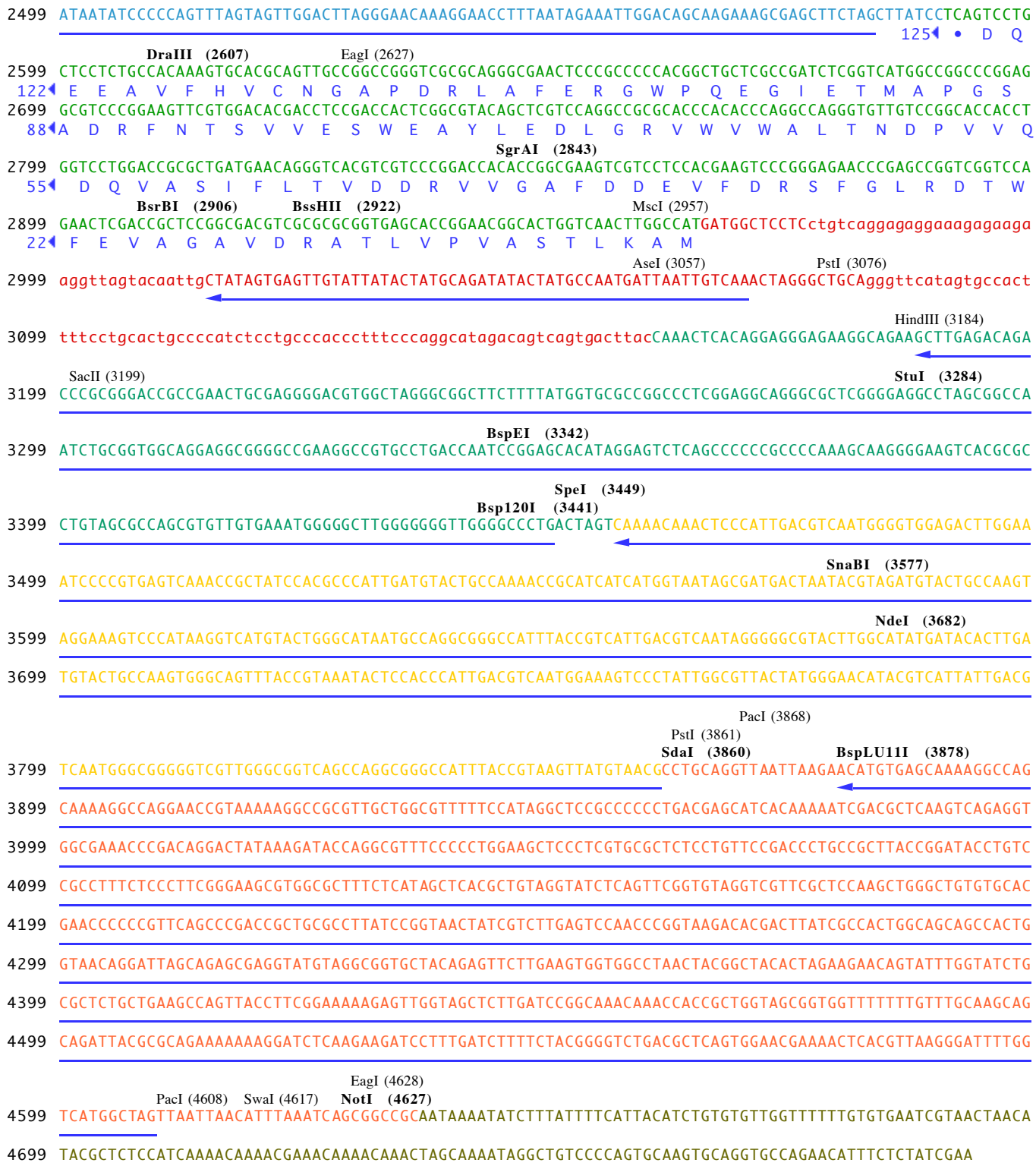
RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1
QUANTI-Luc™	rep-qlc1


pFUSEN-Lucia-hG1e2Fc
(4792 bp)
PvuI (7)
SgfI (6)
EagI (4628)
NotI (4627)
SwaI (4617)
PacI (4608)
Psp1406I (203)
PvuII (239)
HindIII (245)
KasI (535)
AgeI (552)
BstEII (555)
NcoI (560)
BglII (675)
hEF1-HTLV prom
Ori
Lucia
EcoRV (970)
Acc65I (1189)
SacII (1420)
BsrGI (1592)
hIgG1e2-Fc
MCS / linker
XmnI (1809)
NsiI (1831)
SapI (1863)
BsiWI (1891)
BamHI (1921)
EcoRV (1927)
XhoI (1932)
NheI (1937)
MscI (1943)
HpaI (2075)
XmnI (2171)
AseI (2173)
SwaI (2428)
SspI (2414)
SapI (2357)
EagI (2627)
DraIII (2607)
ßGlo pAn
SV40 pAn
Zeo
EM2KC
hFerL prom
CMV enh
BspLU11I (3878)
PacI (3868)
PstI (3861)
SdaI (3860)
NdeI (3682)
SnaBI (3577)
SpeI (3449)
Bsp120I (3441)
BspEI (3342)
StuI (3284)
SacII (3199)
HindIII (3184)
PstI (3076)
AseI (3057)
MscI (2957)
BssHII (2922)
BsrBI (2906)
SgrAI (2843)
100

InvivoGen


PvuI (7)
SgfI (6)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
HindIII (245)
Psp1406I (203)
PvuII (239)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
NcoI (560)
BstEII (555)
KasI (535)
AgeI (552)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGC
1 M E I K V L F A L I C I A
BglII (675)
601 TGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACC
13 V A E A K P T E I N E D L N I A A V A S N F A T T D L E T D L F T
701 AACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCC
47 N W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P P D V
801 TGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTAT
80 L R E L E A N A R R A G C T R G C L I C L S H I K C T P K M K K F I
EcoRV (970)
901 CCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGAT
113 P G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G F K D
1001 AAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCC
147 K E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D L
Acc65I (1189)
1101 TGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAGGTACCGAGCC
180 L K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R G T E P
hinge Cys changed to Ser (1207)
1201 CAAATCTAGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACC
2 K S S D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T
1301 CTCTTACATCACCCGGGAACCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
36 L Y I T R E P E V T C V V V D V S H E D P E V K F N W Y V D G V E
SacII (1420)
1400 GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
69 V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G
BsrGI (1592)
1500 AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACAC
102 K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T
1600 CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
135 L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S
1700 AATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGT
169 N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R
BsiWI (1891)
XmnI (1809)
NsiI (1831)
SapI (1863)
Lys changed to Ala
1800 GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGAAGTTCCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTGCACGTACGG
202 W Q Q G N V F S C S V M H E A L K F H Y T Q K S L S L S P G A
1 R T
EcoRV (1927)
NheI (1937)
BamHI (1921)
XhoI (1932)
MscI (1943)
1899 GTGGTGGCGGTAGCGGTGGTGGCGGATCCGATATCTCGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGC
3 G G G G S G G G G S D I S S •
HpaI (2075)
1999 AGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCA
AseI (2173)
XmnI (2171)
2099 TTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCA
2199 AAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTG
SapI (2357)
2299 CAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCAC
SspI (2414)
SwaI (2428)
2399 ATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTC


2499 ATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTG
125 • D Q
DraIII (2607)
EagI (2627)
2599 CTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTGGTCATGGCCGGCCCGGAG
122 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 E T M A P G S
2699 GCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCT
88 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 L T N D P V V Q
SgrAI (2843)
2799 GGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCA
55 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 S F G L R D T W
BsrBI (2906)
BssHII (2922)
MscI (2957)
2899 GAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaaga
22 F E V A G A V D R A T L V P V A S T L K A M
AseI (3057)
PstI (3076)
2999 aggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtgccact
HindIII (3184)
3099 tttcctgcactgccccatctcctgcccacccttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAGACAGA
SacII (3199)
StuI (3284)
3199 CCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGCGGCCA
BspEI (3342)
3299 ATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCACGCGC
SpeI (3449)
Bsp120I (3441)
3399 CTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAA
SnaBI (3577)
3499 ATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT
NdeI (3682)
3599 AGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGA
3699 TGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACG
PacI (3868)
PstI (3861)
SdaI (3860)
BspLU11I (3878)
3799 TCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAG
3899 CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGT
3999 GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC
4099 CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCAC
4199 GAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
4299 GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTG
4399 CGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAG
4499 CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGG
EagI (4628)
PacI (4608)
SwaI (4617)
NotI (4627)
4599 TCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACA
4699 TACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA