STOP

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

Important Limited Use License information for pFUSE-Lucia-mG2a-Fc

The purchase of the pFUSE-Lucia-mG2a-Fc 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.

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InvivoGen
www.invivogen.com

pFUSE-Lucia-mG2a-Fc

Plasmid designed for the expression of a Lucia-Fc fusion protein

Catalog # pfuse-mg2alc

For research use only

Version 22H31-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-Lucia-mG2a-Fc** 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.
- Resuspended DNA should be stored at -20 °C and is stable for 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

pFUSE-Fc is a family of plasmids developed to facilitate the construction of Fc-fusion proteins by fusing the effector region of a protein to the Fc region of an immunoglobulin G (IgG).

pFUSE-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.

pFUSE-Lucia-mG2a-Fc plasmids allow 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 pFUSE-hFc constructs. Quantification of Lucia-Fc expression can be determined utilizing InvivoGen's QUANTI-Luc™ (rep-qlc1 or rep-qlc2).

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.

- **mIgG2a Fc (mouse):** The Fc region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-fusion protein, allowing each part of the molecule to function independently. Mouse IgG2a dispays high 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.

- **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.

- **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.

TECHNICAL SUPPORT
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InvivoGen Asia: +852 3-622-34-80
E-mail: info@invivogen.com

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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 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

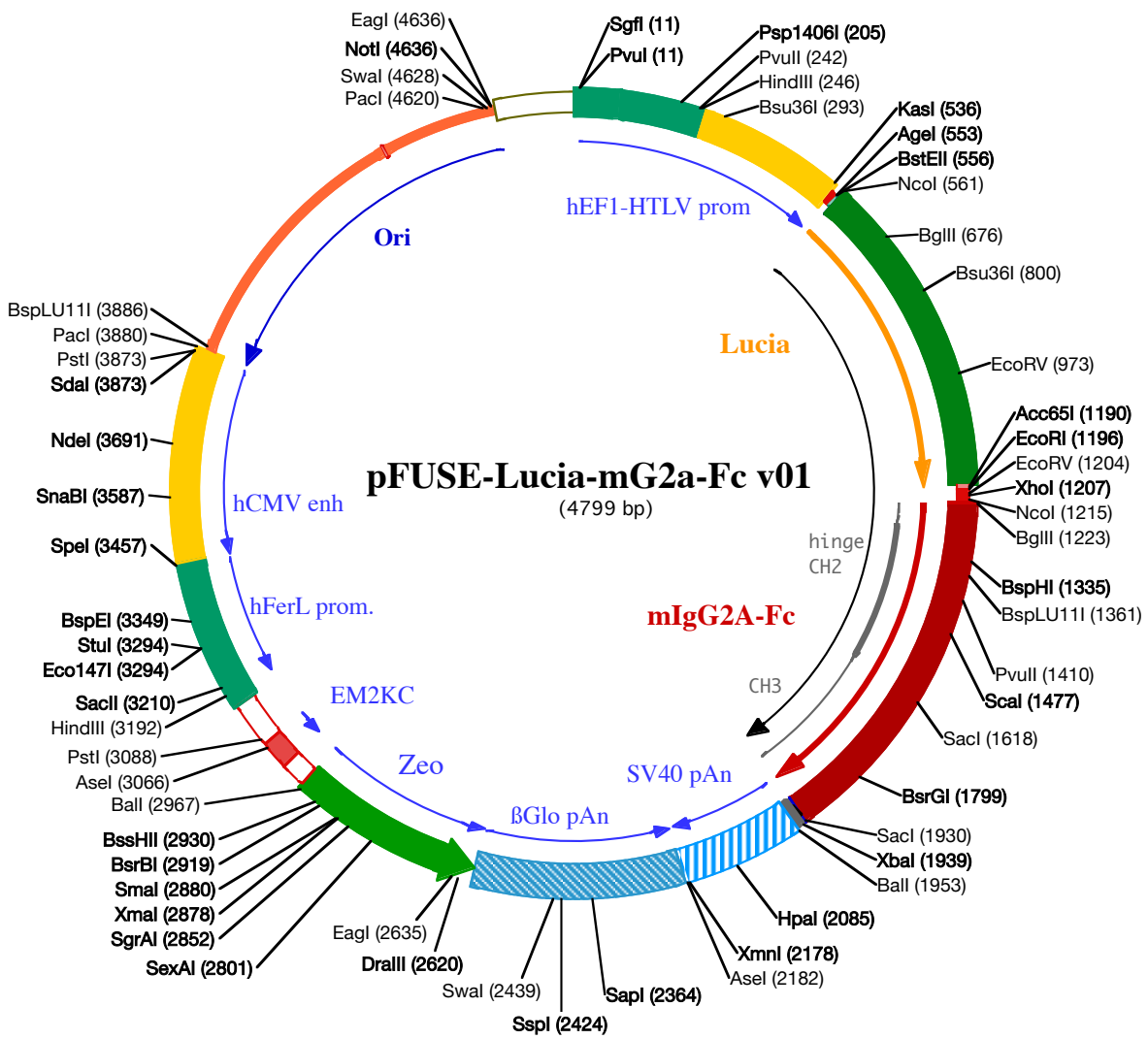| Product	Catalog Code
ChemiComp GT116	gt116-11
QUANTI-Luc™	rep-qlc1
Zeocin®	ant-zn-1

TECHNICAL SUPPORT
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InvivoGen Asia: +852 3-622-34-80
E-mail: info@invivogen.com

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pFUSE-Lucia-mG2a-Fc v01
(4799 bp)

EagI (4636)
NotI (4636)
SwaI (4628)
PacI (4620)

SgfI (11)
PvuI (11)

Psp1406I (205)
PvuII (242)
HindIII (246)
Bsu36I (293)

KasI (536)
AgeI (553)
BstEII (556)
NcoI (561)

BglII (676)

Bsu36I (800)

EcoRV (973)

Acc65I (1190)
EcoRI (1196)
EcoRV (1204)
XhoI (1207)
NcoI (1215)
BglII (1223)

BspHI (1335)
BspLU11I (1361)

PvuII (1410)
ScaI (1477)

SacI (1618)

BsrGI (1799)
SacI (1930)
XbaI (1939)
BalI (1953)

HpaI (2085)
XmnI (2178)
AseI (2182)

SapI (2364)

SspI (2424)

SwaI (2439)

DraIII (2620)

EagI (2635)

SexAI (2801)
SgrAI (2852)
XmaI (2878)
SmaI (2880)
BsrBI (2919)
BssHII (2930)

BalI (2967)
AseI (3066)
PstI (3088)
HindIII (3192)
SacII (3210)
Eco147I (3294)
StuI (3294)
BspEI (3349)

SpeI (3457)

SnaBI (3587)

NdeI (3691)

SdaI (3873)
PstI (3873)
PacI (3880)
BspLU11I (3886)

Ori

hEF1-HTLV prom

Lucia

hinge
CH2

mIgG2A-Fc

CH3

SV40 pAn

ßGlo pAn

Zeo

EM2KC

hFerL prom.

hCMV enh

115

Pvul (11)
Sgfl (11)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)
Psp1406I (205)   PvuII (242)   Bsu36I (293)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

NcoI (561)
BstEII (556)
KasI (536)   AgeI (553)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTcaCCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGC

1▶ M E I K V L F A L I C I A
BglII (676)
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
Bsu36I (800)
701 AACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGAGTGCC

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 (973)
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
EcoRI (1196)
Acc65I (1190)
1101 TGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAGGTACCGAATT

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 F
XhoI (1207)   BglII (1223)
EcoRV (1204)   NcoI (1215)
1201 CGATATCTCGAGCACCATGGTTAGATCTCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTCTTGGGTGGACCATCC
                               1▶ P R G P T I K P C P P C K C P A P N L L G G P S

213▶ D I S S T M V R S P R G P T I K P C P P C K C P A P N L L G G P S
BspHI (1335)   BspLU11I (1361)
1301 GTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATG
25▶ V F I F P P K I K D V L M I S L S P I V T C V V V D V S E D D P D

247▶ V F I F P P K I K D V L M I S L S P I V T C V V V D V S E D D P D
PvuII (1410)   ScaI (1477)
1401 TCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCT
58▶ V Q I S W F V N N V E V H T A Q T Q T H R E D Y N S T L R V V S A L

280▶ V Q I S W F V N N V E V H T A Q T Q T H R E D Y N S T L R V V S A L
1501 CCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCC
91▶ P I Q H Q D W M S G K E F K C K V N N K D L P A P I E R T I S K P

313▶ P I Q H Q D W M S G K E F K C K V N N K D L P A P I E R T I S K P
SacI (1618)
1601 AAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCA
125▶ K G S V R A P Q V Y V L P P P E E E M T K K Q V T L T C M V T D F

347▶ K G S V R A P Q V Y V L P P P E E E M T K K Q V T L T C M V T D F

BsrGI (1799)

1701 TGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCAT
158▶ M P E D I Y V E W T N N G K T E L N Y K N T E P V L D S D G S Y F M

380▶ M P E D I Y V E W T N N G K T E L N Y K N T E P V L D S D G S Y F M
1801 GTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAG
191▶ Y S K L R V E K K N W V E R N S Y S C S V V H E G L H N H H T T K

413▶ Y S K L R V E K K N W V E R N S Y S C S V V H E G L H N H H T T K

SacI (1930)   XbaI (1939)   BalI (1953)
1901 AGCTTCTCCCGGACTCCGGGTAAATGAGCTCAGCTAGGTCTAGACCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAG
225▶ S F S R T P G K •

447▶ S F S R T P G K •

HpaI (2085)
2001 AATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGC

AseI (2182)
Xmnl (2178)
2101 ATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCA

2201 TAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCT

SapI (2364)
2301 GTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCT

SspI (2424)   SwaI (2439)
2401 CCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGC

2501 CCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAG
125◀ •

DraIII (2620)   EagI (2635)
2601 TCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCC
123◀ 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 E T M A P G
2701 CGGAGGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCAC
90◀ 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 L T N D P V

XmaI (2878)
SexAI (2801)   SgrAI (2852)   SmaI (2880)
2801 CACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCG
57◀ 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 S F G L R D

BsrBI (2919)   BssHII (2930)   BalI (2967)
2901 GTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaaga
23◀ 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 (3066)   PstI (3088)
3001 gaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggttcatagtg

HindIII (3192)
3101 ccacttttcctgcactgccccatctcctgcccacccttttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGAAGCTTGAG

Stul (3294)
SacII (3210)   Eco147I (3294)
3201 ACAGACCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCCTAGC

BspEI (3349)
3301 GGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGGGGAAGTCA

SpeI (3457)
3401 CGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACT

SnaBI (3587)
3501 TGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGC

NdeI (3691)
3601 CAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACA

3701 CTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTAT

PacI (3880)
PstI (3873)
Sdal (3873)   BspLU11I (3886)
3801 TGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAG

3901 GCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA

4001 GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC

4101 CTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG

4201 TGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC

```
4301  CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGT

4401  ATCTGCGCTtgattgcAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCA

4501  AGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGAT
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                              SwaI (4628)   EagI (4636)
                   PacI (4620)   NotI (4636)
```
4601  TTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAAC

4701  TAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
```