

# pINFUSE-hIgG4-Fc1

Plasmid designed for the construction of Fc-Fusion proteins

Catalog # pfc1-hgin4

For research use only

Version # 08C12-SV

## PRODUCT INFORMATION

### Content:

- 20  $\mu$ g of pINFUSE-hIgG4-Fc1 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 and lyophilized.

## GENERAL PRODUCT USE

pINFUSE-Fc is a family of plasmid 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).

pINFUSE-Fc plasmids yield high levels of Fc-fusion proteins. The level of expression is usually in the  $\mu$ g/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.

pINFUSE-Fc plasmids allow the secretion of Fc-Fusion proteins. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pINFUSE-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 single-step protein A or protein G affinity chromatography.

InvivoGen provides pINFUSE-Fc vectors featuring Fc regions containing introns from different species and isotypes. In humans, there are four isotypes: IgG1, IgG2, IgG3 and IgG4. 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 increasing in the order of IgG4<IgG2<IgG1≤IgG3.

## PLASMID FEATURES

- **human genomic IgG2-Fc (with introns):** 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. A short intron is present between each region (one intron between the hinge and CH2 and one intron between CH2 and CH3). The presence of introns is known to enhance the level of gene expression as splicing is known to promote rapid and efficient mRNA export<sup>1</sup>. Human IgG4 displays low ADCC and CDC.
- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 $\alpha$  (EF-1 $\alpha$ ) core promoter<sup>2</sup> 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<sup>3</sup>. The EF-1 $\alpha$  promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 $\alpha$  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<sup>4</sup>.
- **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*.
- **$\beta$ G10 pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>5</sup>.

### TECHNICAL SUPPORT

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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  $\mu\text{g}/\mu\text{l}$ , resuspend the DNA in 20  $\mu\text{l}$  of sterile  $\text{H}_2\text{O}$ . Store resuspended plasmid at  $-20^\circ\text{C}$ .

### **Selection of bacteria with *E. coli* Fast-Media®**

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Zeo is available separately: #fas-zn-l (liquid), #fas-zn-s (agar).

- 1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to  $45^\circ\text{C}$  before pouring plates. Let liquid media cool to  $37^\circ\text{C}$  before seeding bacteria.

*Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

### **References:**

1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. RNA. 9(5):607-17.
2. 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.
3. 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.
4. 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.
5. 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.

## RELATED PRODUCTS

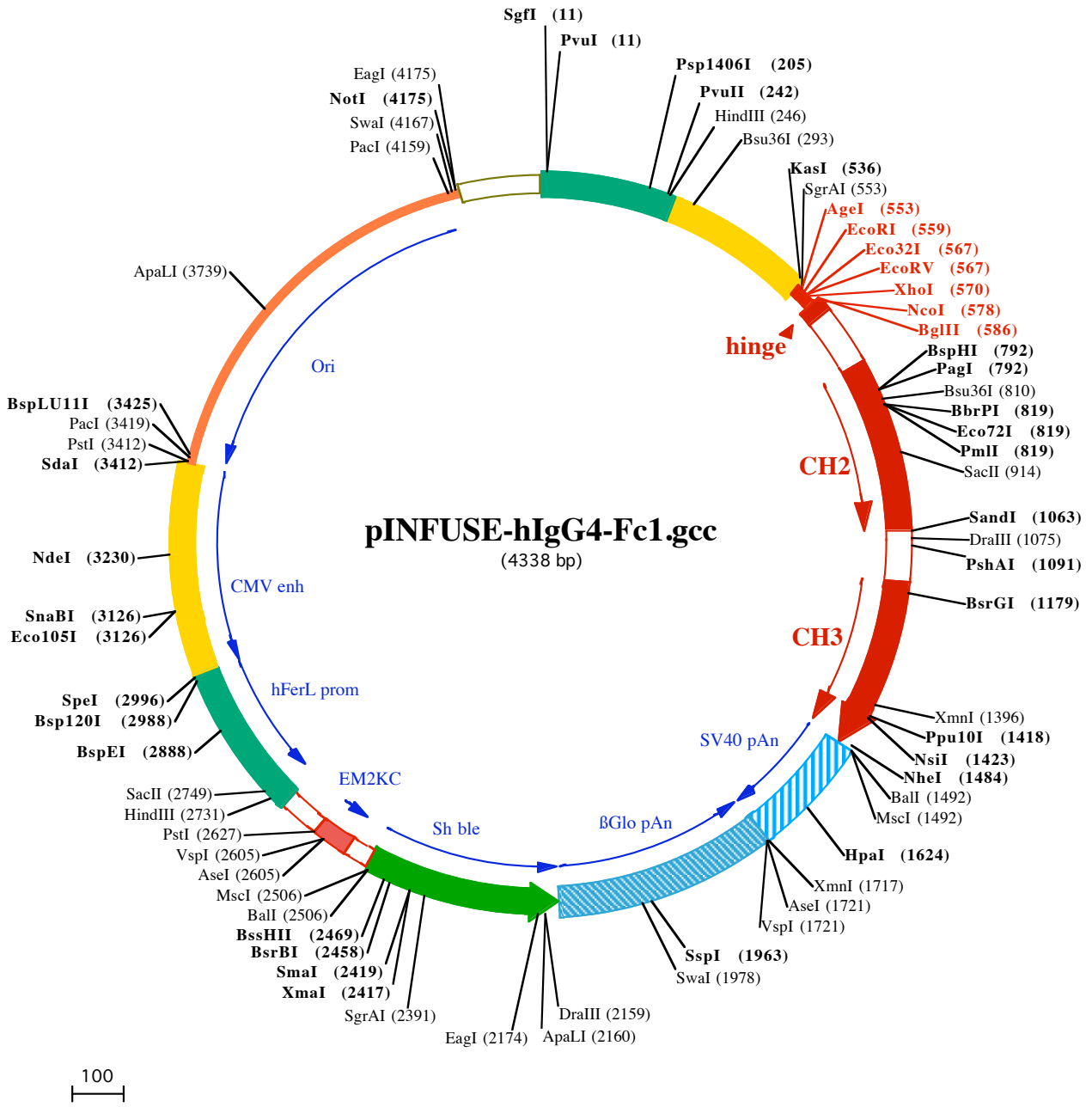
Product	Catalog Code
Zeocin™	ant-zn-l
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar	fas-zn-s

### [TECHNICAL SUPPORT](#)

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**PvuI (11)**  
**SgfI (11)**  
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGAGAGCGCACATGCCACAGTCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA  
101 GAGAAGTGGCGGGGTAACGGGAAAGTGATGTCGTGACTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

**HindIII (246)**  
**Psp1406I (205)** **PvuII (242)** **Bsu36I (293)**  
201 GTGAACGTTCTTTTTTCGCAACGGGTTTCCCGCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGGC  
301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACCTGCGTCCGCGCTTAGGTAAGTTAAAGTCTAGGTCGAGACC  
401 GGGCTTTGTCCGGCGCTCCCTTGAGCGCTACCTAGACTACAGCGGCTCTCCACGCTTTCGCTCAACTCTACGCTCTTTGTTTCGTTT

**EcoRI (559)** **XhoI (570)**  
**AgeI (553)** **EcoRV (567)** **BglIII (586)**  
**KasI (536)** **SgrAI (553)** **Eco32I (567)** **NeoI (578)**  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTCCCCATGC  
1▶ProProCys  
601 CCATCATGCCAGgtaagccaaccaggcctcgccctccagctcaaggcgggacaggtgcctagagtagcctgcatccagggacagggcccagccgggt  
4▶ProSerCysPro

**PagI (792)**  
**BspHI (792)**  
701 gctgagcgcacccctccatctcttctcagCACCTGAGTTCCTGGGGGACCATCAGTCTTCTGTTCCCCCAAACCAAGGACACTCTCATGATCT  
1▶ProGl uPheLeuGl yGl yProSer Val PheLeuPheP roP roLysP roLysAspThr LeuMe t l eS

**PmlI (819)**  
**Eco72I (819)**  
**BbrPI (819)**  
**Bsu36I (810)**  
801 CCCGGACCCCTGAGGTCAGTGCCTGGTGGTGGACGTGAGCCAGGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGTGCATAATGC  
23▶erArgThrProGl uVal ThrCysVal Val ValAspVal Ser Gl nGl uAspProGl uVal Gl nPheAsnTrpTyrValAspGl yVal Gl uVal Hi sAsnAl  
901 CAAGACAAAGCCGCGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAAGCGTCTCACCGTCTGCACCGGACTGGCTGAACGGCAAGGAGTACAAG  
56▶aLysThrLysP roArgGl uGl uGl nPheAsnSer Thr TyrArgVal Val Ser Val LeuThr Val LeuHi sGl nAspTrpLeuAsnGl yLysGl uTyrLys

**DraIII (1075)**  
**SandI (1063)** **PshAI (1091)**  
1001 TGCAAGGTCTCCAACAAGGCCTCCGTCCTCCATCGAGAAAACCATCTCCAAAGCAAAGgtgggaccacggggtgcgagggccacatggacagagggt  
90▶CysLysVal SerAsnLysGl yLeuProSer Ser l l eGl uLysThr l l eSer LysAl aLys

**BsrGI (1179)**  
1101 cagctcggccaccctctgccctgggagtgaccgctgtgccacctctgtccctacagGGCAGCCCGAGAGCCACAGGTGTACACCCTGCCCCATCCC  
1▶Gl nProArgGl uP roGl nVal TyrThr LeuP roP roSer G  
1201 AGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTACCCAGCGACATCGCGTGGAGTGGAGAGCAATGGGCAGCCGGA  
14▶l nGl uGl uMetThr LysAsnGl nVal Ser LeuThr CysLeuVal LysGl yPheTyrP roSerAsp l l eAl aVal Gl uTrpGl uSerAsnGl yGl nP roGl

**XmnI (1396)**  
1301 GAACAACATAAGACCAGCCTCCGCTGGACTCCGACGGCTCTTCTTCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAAT  
47▶uAsnAsnTyrLysThr Thr ProP roVal LeuAspSerAspGl ySer PhePheLeuTyrSer ArgLeuThr Val AspLysSer ArgT rpGl nGl uGl yAsn

**MscI (1492)**  
**BalI (1492)**  
**NheI (1484)**  
1401 GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGTAGCTGGCCAGACAT  
81▶Val PheSer CysSer ValMetHi sGl uAl aLeuHi sAsnHi sTyrThr Gl nLysSer LeuSer LeuSer Pr oGl yLys●●●  
1501 GATAAGATACATTGATGAGTTTGACAAACCACTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACC

**HpaI (1624)**  
1601 ATTATAAGTGCATAAACAAGTTAAACAACAACATTGCATTATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACC

**VspI (1721)**  
**AseI (1721)**  
**XmnI (1717)**  
1701 TCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAA  
1801 GGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTTGA

**SspI (1963)** **Swal (1978)**  
1901 CTAGCTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATGTAATAATTCAGAAATAATTTAAATACATCATTGCAATGAAAA  
2001 TAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAA

**ApaLI (2160)** **EagI (2174)**  
**DraIII (2159)**  
2101 ATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTCTGCCACAAAGTGACGCGAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCC  
125▶●●●AspGl nGl uGl uAl aVal PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uAr  
2201 GCGCCACGGCTGCTCGCGATCTCGTTCATGGCCGGCCGAGGCGTCCCGAAGTTCGTGGACACGACTCCGACACTCGCGTACAGCTCGTCCAG  
103▶gl yTrpP roGl nGl uGl y l eGl uThr MetAl aP roGl ySerAl aAspArgPheAsnThr Ser Val Val Gl uSer TrpGl uAl aTyrLeuGl uAspLeu  
2301 GCGCGCACCCACCCAGGCCAGGTTGTGTCGGCACCACTGGTCTGGACCGGCTGATGAACAGGGTACGCTGCTCCGGACCAACCGGCGAAG  
70▶Gl yArgVal TrpVal TrpAl aLeuThrAsnAspP roVal Val Gl nAspGl nVal l Al aSer l l ePheLeuThr Val AspAspArgVal Val Gl yAl aPheA

**XmaI (2417)** **SmaI (2419)** **BsrBI (2458)** **BssHIII (2469)**  
2401 TCGTCTCCACGAAGTCCCGGAGAACCAGCCGCTCGGTCCAGAAGTCCGACCGTCCGGCGAGCTCGCGCGGTGAGCACCAGGACCGGCACTGGTCA  
36▶spAspGl uVal PheAspArgSer PheGl yLeuArgAspThr TrpPheGl uVal Al aGl yAl aVal AspArgAl aThr LeuVal P roVal Al aSer Thr Le  
2501 ACTTGGCCATGATGGCTCCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGGATTGTATTATACTATGCAGATATACTATGCCA  
3▶uLysAl aMet

VspI (2605)  
AseI (2605) PstI (2627)

2601 ATGATTAATTGTCAAAC TAGGGCTGCAgggttcatagtgccacttttctgcaactgccccatctcctgcccaccctttccaggcatagacagtcagtg

HindIII (2731) SacII (2749)

2701 cttaCCAAACACAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTG

BspEI (2888)

2801 CGCCGGCCCTCGGAGGCAGGGCGCTCGGGGAGCCTAGCGCCAATCTGCGGTGGCAGGAGCGGGGCCAAGGCCGTGCTGACCAATCCGGAGCACAT

SpeI (2996)  
Bsp120I (2988)

2901 AGGAGTCTCAGCCCCCGCCCAAAGCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTGGGGCCCTGACTAG

3001 TCAAACCAAACCTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGTATCCACGCCATTGATGTACTGCCAAACCGCATC

SnaBI (3126)  
Eco105I (3126)

3101 ATCATGGTAATAGCGATGACTAATACGTAGATGTAAGTACTGCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGT

NdeI (3230)

3201 CATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAA

3301 AGTCCCTATTGGCGTACTATGGGAACATACGTCAATTATTGACGTCAATGGCGGGGGTCTTGGCGGGTCAAGCCAGGCGGGCCATTTACCGTAAGTTAT

PacI (3419)  
PstI (3412)  
SdaI (3412) BspLU11I (3425)

3401 GTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCTCCG

3501 CCCCCGTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTC

3601 GTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATC

ApaLI (3739)

3701 TCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC

3801 CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG

3901 TGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCA

4001 AACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGC

EagI (4175)  
PacI (4159) SwaI (4167) NotI (4175)

4101 GTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGAATAAAATATCTTTATTTT

4201 CATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCC

4301 CAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA