

pFUSE-CHlg-hD

Plasmid featuring the constant region of the human IgD (allele 2) heavy chain

Catalog code: pfuse-hchd2

For research use only

Version 24J28-MM-v37

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE-CHlg-hD 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 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

- pFUSE2-CLlg plasmid that feature the constant region of the kappa or lambda light chains. pFUSE2-CLlg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- pFUSE-CHlg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin®.

GENERAL PRODUCT USE

pFUSE-CHlg and pFUSE2-CLlg 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 antibodies from Fab or scFv fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

pFUSE-CHlg and pFUSE2-CLlg 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-CHlg and pFUSE2-CLlg pair allows to generate an antibody that can be purified from the supernatant using the appropriate affinity chromatography.

Features of pFUSE-CHlg and pFUSE2-CLlg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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 mRNA³.
- **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.
- **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

pFUSE-CHlg-hD specific features

- **Human IGHD (IgD allele2 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

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 H₂O. Store resuspended plasmid at -20°C.

Cloning into pFUSE-CHIg and pFUSE2-CLiG

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-CHIg-hD, the constant region of the human IgD heavy chain is preceded by a multiple cloning site containing six restriction sites: AgeI, EcoRI, EcoRV, XhoI, NheI and Eco47III. The first four restriction sites can be used for insertion of the 5' end of the variable region including the native signal sequence. If the immunoglobulin signal sequence is unknown, pFUSEss plasmids containing a signal sequence should be used. In pFUSE-CHIg-hD, Eco47III must be used for insertion of the 3' end of the variable region. Eco47III must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an Eco47III site at the 3' end of the variable region in frame with the constant region.

Note:

- The 5' end of the variable region should encompass the native ATG initiation codon and the region immediately after which corresponds to the signal sequence. For proper initiation of translation, make sure that your insert contains a Kozak translation initiation sequence upstream of the ATG initiation codon such as (G/A)NNATG.

- When generating the insert for VL, a BsiWI (pFUSE2-CLiG-hk; human kappa), or AvrII (pFUSE2-CLiG-hl2; human lambda 2) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

Antibody production

Cotransfect mammalian cells, such as 293 and CHO cells, with the recombinant plasmids pFUSE2-CLiG encoding the light chain and pFUSE-CHIg encoding the heavy chain. Antibody production depends greatly on the ratio of heavy chain and light chain expression. Typically, pFUSE-CHIg to pFUSE2-CLiG ratio of 2:3 is used to cotransfect mammalian cells. Since both plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of plasmids.

OR

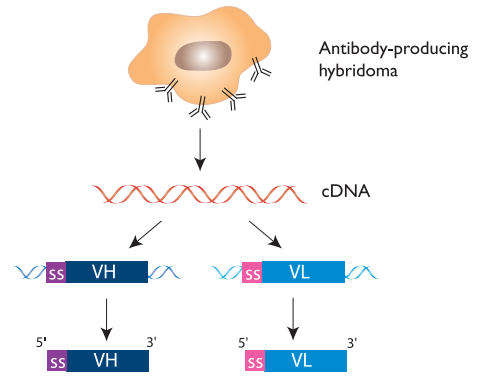
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

Use blasticidin and Zeocin® to select pFUSE2-CLiG and pFUSE-CHIg respectively.

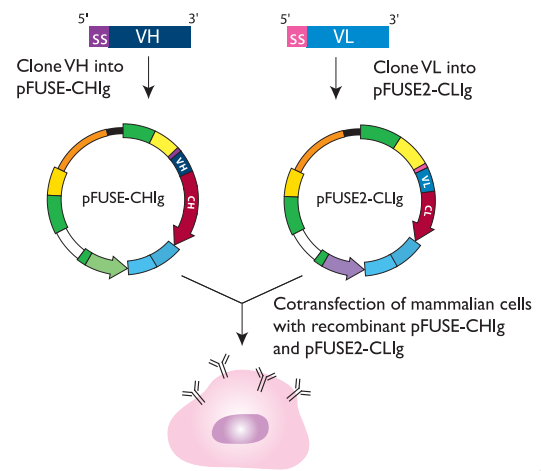
Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.

Antibody generation using pFUSE-CHIg & pFUSE2-CLiG

1- Obtention of VH and VL sequences



2- Cloning into pFUSE-CHIg and pFUSE2-CLiG



Antibody purification

The resulting IgG antibody can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

RELATED PRODUCTS

Product	Catalog Code
pFUSE2-CLiG-hk	pfuse2-hclk
pFUSE2-CLiG-hl2	pfuse2-hcll2
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Zeocin®	ant-zn-1

TECHNICAL SUPPORT

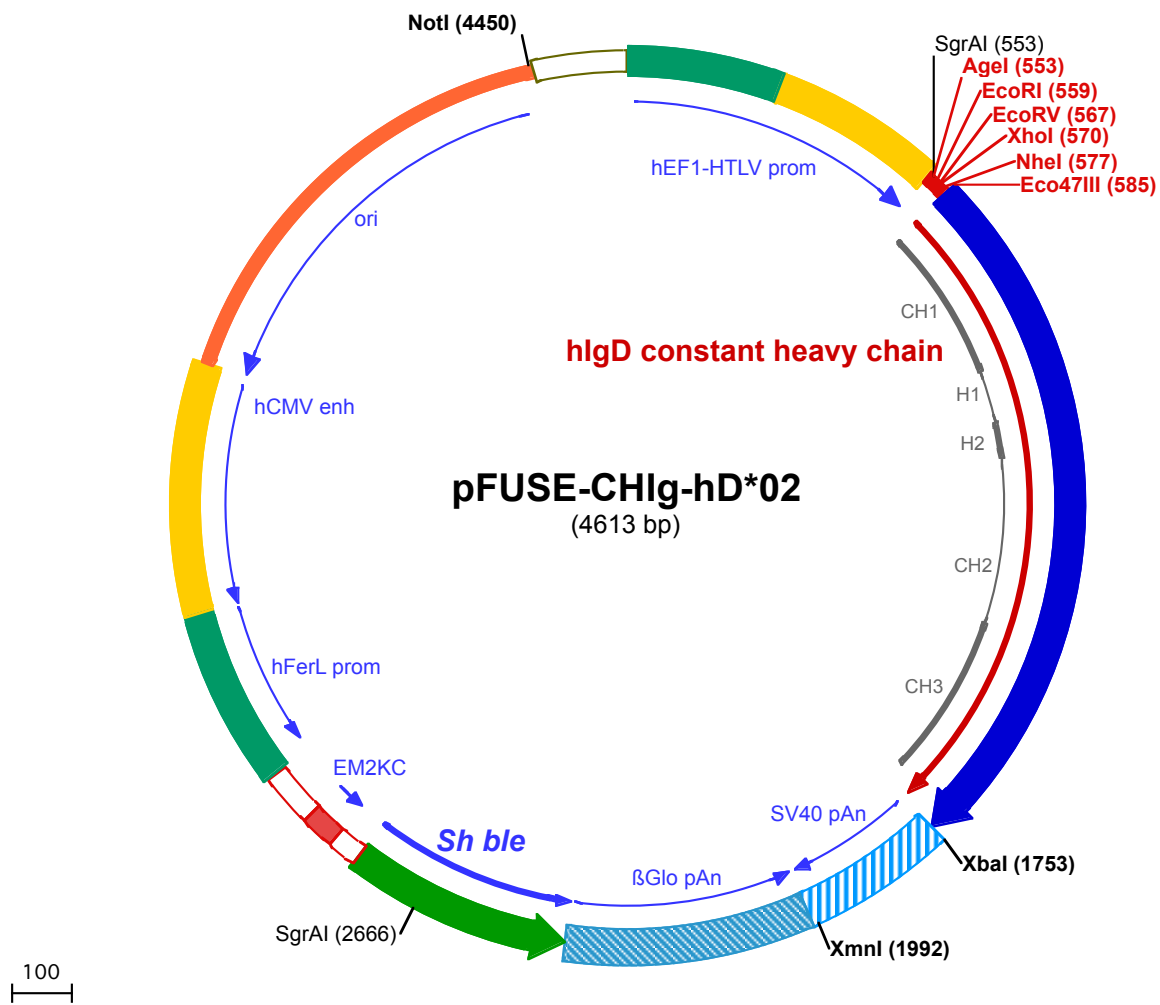
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1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCGAGCGCACATGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGTTGAGTCCGCTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGTGCCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTAACTCTACGCTTTTGTTCGTTT

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGTGAATTCGATATCTCGAGTGTAGCAGCGCTCCACCAAGGCT
Agel (553) EcoRI (559) XhoI (570) Eco47III (585)
SgrAI (553) EcoRV (567) NheI (577)
1▶ A P T K A

601 CCGATGTGTTCCCATCATATCAGGTCGAGACACCCAAAGGATAACAGCCCTGTGGTCTGGCATGCTTGATAACTGGGTACCACCAACCTCCGTTGA
6▶ P D V F P I I S G C R H P K D N S P V V L A C L I T G Y H P T S V

701 CTGTCACCTGGTACATGGGACACAGAGCCAGCCAGAGAACCTTCCTGAGATACAAGACGGGACAGCTACTACATGACAAGCAGCCAGCTCTCCAC
39▶ T V T W Y M G T Q S Q P Q R T F P E I Q R R D S Y Y M T S S Q L S T

801 CCCCTCCAGCAGTGGCGCAAGGCGAGTACAATGCGTGGTCCAGCACACCCAGCAAGAGTAAGAAGGAGATCTCCGCTGGCCAGAGTCTCAAAG
72▶ P L Q Q W R Q G E Y K C V V Q H T A S K S K K E I F R W P E S P K

901 GCACAGGCTCCTCAGTGCCTGCAACCCCAAGCAGAGGGCAGCCTCGCAAGCAACCACAGCCCAGCCACCACCGTAACACAGGAAGAGGAG
106▶ A Q A S S V P T A Q P Q A E G S L A K A T T A P A T T R N T G R G

1001 GAGAAGAGAAGAAGAAGGAGAAGGAGAAGGAACAAGAAGAGAGAGAGACAAGACACCAGAGTGTCCGAGCCACCCAGCCTTTGGCGTCTACCT
139▶ G E E K K K E K E E Q E E R E T K T P E C P S H T Q P L G V Y L

1101 GCTAACCCCTGCAGTGCAGGACCTGTGGCTCCGGGACAAGCCACCTTACCTGCTTCGTGGTGGGAGTGCCTGAAGGATGCTCACCTGACCTGGGAG
172▶ L T P A V Q D L W L R D K A T F T C F V V G S D L K D A H L T W E

1201 GTGGTGGGAAGTCCCCACAGGGGGCGTGGAGGAAGGGTGTGGAGCGGCACAGCAACGGTCCCAGAGCCAGCACAGCCGTGTGACCCTGCCAGGT
206▶ V A G K V P T G G V E E G L L E R H S N G S Q S Q H S R L T L P R

1301 CCTTGTGGAACGCGGGGACCTCCGTACCTGCACACTGAACCATCCAGCCTCCACCCAGAGGTTGATGGCGTGAGAGAACCCTGCGCAGGCACC
239▶ S L W N A G T S V T C T L N H P S L P P Q R L M A L R E P A A Q A P

1401 CGTCAAGCTTTCCTGAACCTGCTGGCCTCGTCTGACCTCCCGAGGCGGCTCGTGGCTCCTGTGTGAGGTGTCTGGCTTCTCGCCCCAACATCTC
272▶ V K L S L N L L A S S D P P E A A S W L L C E V S G F S P P N I L

1501 CTGATGTGGTGGAGGACCAGCGTGAGGTGAACACTTCTGGTTCGCCCCGACGCCCCCTCCACAGCCAGGAGCACCACGTTCTGGCCTGGAGTG
306▶ L M W L E D Q R E V N T S G F A P A R P P P Q P R S T T F W A W S

1601 TGCTGCGTGTCCAGCCCCGCCAGCCCTCAGCCAGCCACCTACACGTGTGTGGTCCAGCCAGGACTCCCGGACTGTCTCAACGCCAGCCGGAGCCT
339▶ V L R V P A P P S P Q P A T Y T C V V S H E D S R T L L N A S R S L

1701 AGAAGTCAGTATGTAACAGACCATGGCCCATGAAATGATCCCGACAGATCTAGACCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGA
372▶ E V S Y V T D H G P M K •

1801 CAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTA

1901 ACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAAT
2001 TCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCC

2101 AATGTGCATTAGCTGTTTGCAGCCTCACCTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTGCTCTTCAATTTCTTTATGTTTT
2201 AAATGCACTGACCTCCACATCCCTTTTATGTAATAATTCAAGAAATAATTAATAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATC

2301 CAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAAGGAACCTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTC
2401 TAGCTTATCCTCAGTCTGCTCCTGTCACAAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTC

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 E G I E

XmnI (1992)

2501 GGCATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCAGGCCAGG
95 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 V W A L
SgrAI (2666)

2601 GTGTTGTCGGCACCACCTGGTCTGGACCGCGTGATGAACAGGGTCACGTCGTCCCGACCACACCGGCGAAGTCGTCTCCACGAAGTCCCGGGAGA
61 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 F D R S F

2701 ACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCATGATGGCTCCTCctgt
28 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

2801 caggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTG
←

2901 CAgggttcatagtgccacttttcctgcactgccccatctcctgcccaccctttcccaggcatagacagtcagtgcacttacCAAACCTACAGGAGGGAGAA
3001 GGCAGAAGCTTGAGACAGACCCGCGGGACCGCGAAGTGCAGAGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCT
←

3101 CGGGGAGGCCTAGCGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAA
←

3201 GCAAGGGGAAGTCACGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAACAAACTCCATTGACGTCA
←

3301 ATGGGGTGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATA
←

3401 CGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACT
←

3501 TGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGA
←

3601 ACATACGTCAATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCTGCAGGTTAATTAAGAA
←

3701 CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAT
←

3801 CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTGC
←

3901 CGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGGTCGTTCCGCTC
←

4001 CAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG
←

4101 CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAA
←

4201 GAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGG
←

4301 TTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGGAAACGAAAAAC
←

4401 TCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTT
NotI (4450)

4501 GTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACA
4601 TTTCTCTATCGAA