pFUSE-mIgG3-Fc2

Plasmid containing a mouse IgG3 Fc region

Catalog # pfuse-mg3fc2

For research use only

Version 20K05-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-mIgG3-Fc2 (ssIL2)** 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.

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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 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).

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-Fc2 (IL2ss) plasmids allow the secretion of Fc-Fusion proteins. They contain the IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pFUSE-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 pFUSE-Fc vectors featuring Fc regions from different species and isotypes. Three murine isotypes are available: IgG1, IgG2a and IgG3. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (FcγRs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the targeted cells by triggering the complement cascade at the cell surfaceIgG isoforms exert different levels of effector functions increasing in the order of mIgG1<mIgG3<mIgG2a.

PLASMID FEATURES

• **mIgG3 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.
The Fc region of mouse IgG3 mediates high ADCC and low CDC[1].

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[2] 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[3]. 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 antigenic protein.

• **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[4].

• **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[5].

References:

1. Dangl JL. *et al.*, Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. EMBO J. 7(7):1989-94.
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.

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 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

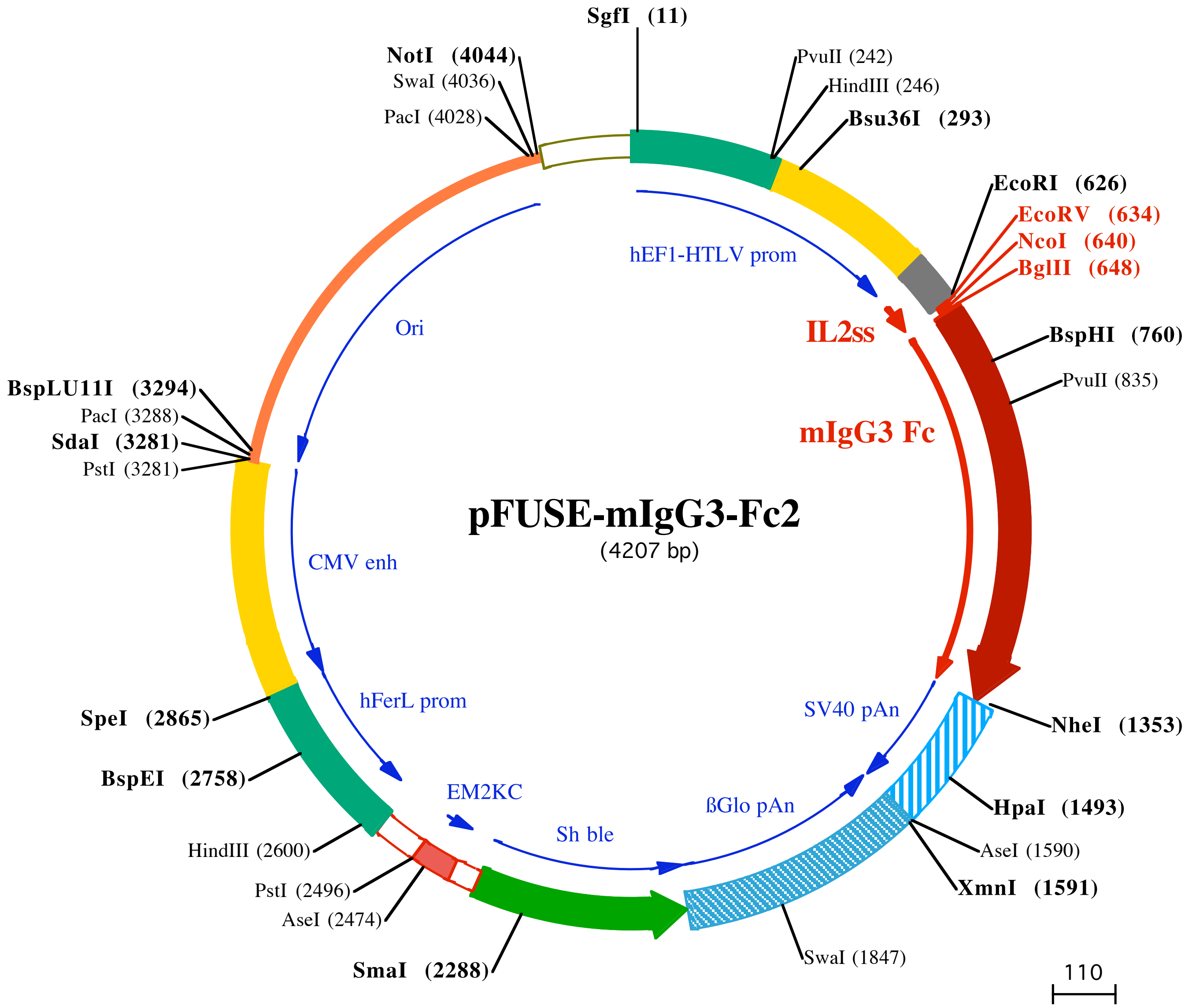
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

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


pFUSE-mIgG3-Fc2
(4207 bp)
SgfI (11)
PvuII (242)
HindIII (246)
Bsu36I (293)
EcoRI (626)
EcoRV (634)
NcoI (640)
BglII (648)
BspHI (760)
PvuII (835)
NheI (1353)
HpaI (1493)
AseI (1590)
XmnI (1591)
SwaI (1847)
SmaI (2288)
AseI (2474)
PstI (2496)
HindIII (2600)
BspEI (2758)
SpeI (2865)
PstI (3281)
SdaI (3281)
PacI (3288)
BspLU11I (3294)
PacI (4028)
SwaI (4036)
NotI (4044)
hEF1-HTLV prom
IL2ss
mIgG3 Fc
SV40 pAn
ßGlo pAn
Sh ble
EM2KC
hFerL prom
CMV enh
Ori
110

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               SgfI (11)
1    GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101  GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

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

301  GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401  GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

501  TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCAccggcGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA
                                                                                   1 MetTyrArgMetGlnLeuLeuSerCysI
                                       EcoRV (634)       BglII (648)
                                 EcoRI (626)      NcoI (640)
601  TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCGGCCATGGTTAGATCTCCTAGAATACCCAAGCCCAGTACCCCCCCAGGTTCTTCATGCCCACC
 10  leAlaLeuSerLeuAlaLeuValThrAsnSer                           1 ProArgIleProLysProSerThrProProGlySerSerCysProPr
                                                                       BspHI (760)
701  TGGTAACATCTTGGGTGGACCATCCGTCTTCATCTTCCCCCCAAAGCCCAAGGATGCACTCATGATCTCCCTAACCCCCAAGGTTACGTGTGTGGTGGTG
 16  oGlyAsnIleLeuGlyGlyProSerValPheIlePheProProLysProLysAspAlaLeuMetIleSerLeuThrProLysValThrCysValValVal
                                         PvuII (835)
801  GATGTGAGCGAGGATGACCCAGATGTCCATGTCAGCTGGTTTGTGGACAACAAAGAAGTACACACAGCCTGGACACAGCCCCGTGAAGCTCAGTACAACA
 50  AspValSerGluAspAspProAspValHisValSerTrpPheValAspAsnLysGluValHisThrAlaTrpThrGlnProArgGluAlaGlnTyrAsnS
901  GTACCTTCCGAGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGGGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGCCCTCCCAGCCCC
 83  erThrPheArgValValSerAlaLeuProIleGlnHisGlnAspTrpMetArgGlyLysGluPheLysCysLysValAsnAsnLysAlaLeuProAlaPr
1001 CATCGAGAGAACCATCTCAAAACCCAAAGGAAGAGCCCAGACACCTCAAGTATACACCATACCCCCACCTCGTGAACAAATGTCCAAGAAGAAGGTTAGT
116  oIleGluArgThrIleSerLysProLysGlyArgAlaGlnThrProGlnValTyrThrIleProProProArgGluGlnMetSerLysLysLysValSer
1101 CTGACCTGCCTGGTCACCAACTTCTTCTCTGAAGCCATCAGTGTGGAGTGGGAAAGGAACGGAGAACTGGAGCAGGATTACAAGAACACTCCACCCATCC
150  LeuThrCysLeuValThrAsnPhePheSerGluAlaIleSerValGluTrpGluArgAsnGlyGluLeuGluGlnAspTyrLysAsnThrProProIleL
1201 TGGACTCAGATGGGACCTACTTCCTCTACAGCAAGCTCACTGTGGATACAGACAGTTGGTTGCAAGGAGAAATTTTTACCTGCTCCGTGGTGCATGAGGC
183  euAspSerAspGlyThrTyrPheLeuTyrSerLysLeuThrValAspThrAspSerTrpLeuGlnGlyGluIlePheThrCysSerValValHisGluAl
                                                                  NheI (1353)
1301 TCTCCATAACCACCACACACAGAAGAACCTGTCTCGCTCCCCTGGTAAATGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACC
216  aLeuHisAsnHisHisThrGlnLysAsnLeuSerArgSerProGlyLys•••
                                                                                                HpaI (1493)
1401 ACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA

                                                                                              AseI (1590)
                                                                                          XmnI (1591)
1501 ACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAA

1601 ATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTG

1701 CATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGC

                                                    SwaI (1847)
1801 ACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATG

1901 CTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTT

2001 ATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCAT
125   •••AspGlnGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpProGlnGluGlyIleGluThrMet
2101 GGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTG
 93  AlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnA
                                                                                          SmaI (2288)
2201 TCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGA
 59  spProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLe
2301 GCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggag
 26  uArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet
                                                                            AseI (2474)      PstI (2496)
2401 aggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAgggt

                                                                                                  HindIII (2600)
2501 tcatagtgccacttttcctgcactgccccatctcctgcccaccctttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCAGA
2601 AGCTTGAGACAGACCCGCGGGACCGCCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGGCCCTCGGAGGCAGGGCGCTCGGGGA

                                                                 BspEI (2758)
2701 GGCCTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGG

                                                                         SpeI (2865)
2801 GGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGG

2901 TGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGA

3001 TGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCAT

3101 ATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATAC
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PacI (3288)
PstI (3281)
SdaI (3281) **BspLU11I (3294)**

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3201 GTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTG
3301 AGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGC
3401 TCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTA
3501 CCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT
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3701 GCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAG
3801 TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTT
3901 TGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGT
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PacI (4028) SwaI (4036) **NotI (4044)**

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4001 TAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGA
4101 ATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTC
4201 TATCGAA
```