# pUNO1-hSTING-MRP

Expression vector containing an isoform of human STING lacking exon 7

Catalog code: puno1-hsting-mrp

https://www.invivogen.com/hsting-mrp

For research use only

Version 19K10-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 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 at least for 1 year.
- Store blasticidin at 4°C or -20°C. *

*The expiry date is specified on the product label.

### Quality control

- Plasmid construct has been confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

## GENERAL PRODUCT USE

• **Subclone gene into another vector.** Two unique restriction sites flank the gene, allowing convenient excision. The 5' site is BspEI which is compatible with AgeI, XmaI, NgoMIV and SgrAI. The 3' site is NheI which is compatible with XbaI, SpeI, and AvrII.

• **Stable gene expression in mammalian cells.** pUNO1 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO1 plasmids contain the blasticidin-resistance gene (*bsr*) driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO1 allows high levels of expression and secretion of the gene product.

## 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 water. Store resuspended plasmid at -20°C.

### Plasmid amplification and cloning

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

### Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

## PLASMID FEATURES

• **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **CMV promoter & enhancer** drives the expression of the blasticidin resistance in mammalian cells.

• **Human STING-MRP**

ORF size: 852 bp Cloning fragment size: 961 bp

STING (stimulator of interferon genes; also known as TMEM173, MITA, MPYS, and ERIS) is essential for the IFN response to microbial or self-DNA, and acts as a direct sensor of cyclic dinucleotides (CDNs). CDNs are important messengers in bacteria, affecting numerous responses of the prokaryotic cell, but also in mammalian cells, acting as agonists of the innate immune response. hSTING-MRP (MITA-related protein), discovered and identified in HEK293T cells[1], is an alternatively spliced isoform of hSTING lacking exon 7 that acts as a dominant negative mutant of STING. It was recently reported to block STING-mediated IFN response while retaining the ability to active NF-κB[1].

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[2] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[3] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[4].

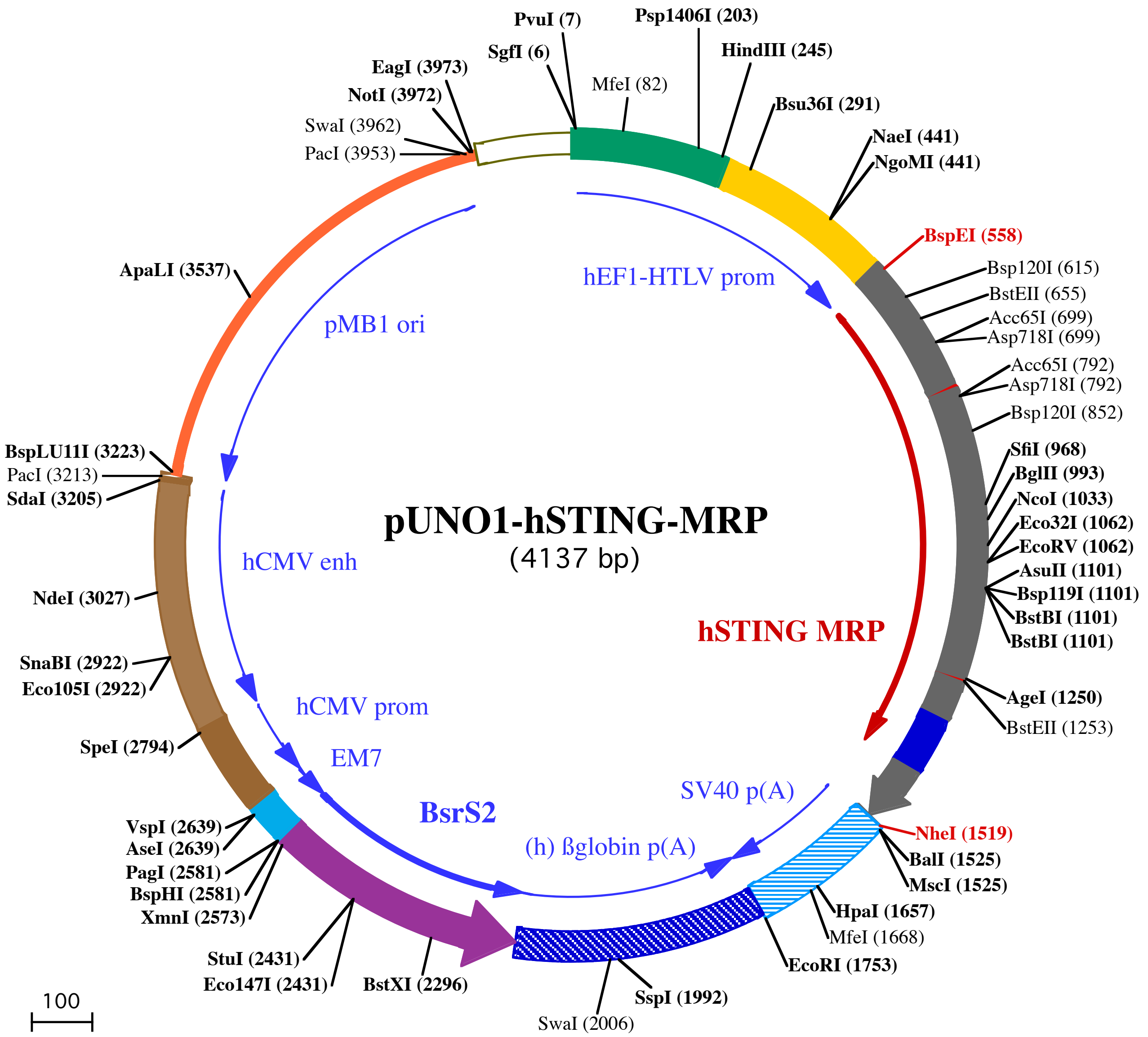
• **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference[5] and possible recombination events with the SV40 polyadenylation signal.


**1. Chen H. *et al.*, 2014.** An alternative splicing isoform of MITA antagonizes MITA-mediated induction of type I IFNs. J Immunol 192(3):1162-70. **2. Kim D. *et al.*, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 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 J., 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 J., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.


## RELATED PRODUCTS

| Product | Description | Cat. Code |
|---|---|---|
| Blasticidin | Selection antibiotic | ant-bl-1 |
| ChemiComp GT116 | Competent *E. coli* | gt116-11 |


pUNO1-hSTING-MRP
(4137 bp)
PvuI (7)
SgfI (6)
Psp1406I (203)
HindIII (245)
MfeI (82)
Bsu36I (291)
NaeI (441)
NgoMI (441)
BspEI (558)
Bsp120I (615)
BstEII (655)
Acc65I (699)
Asp718I (699)
Acc65I (792)
Asp718I (792)
Bsp120I (852)
SfiI (968)
BglII (993)
NcoI (1033)
Eco32I (1062)
EcoRV (1062)
AsuII (1101)
Bsp119I (1101)
BstBI (1101)
BstBI (1101)
AgeI (1250)
BstEII (1253)
NheI (1519)
BalI (1525)
MscI (1525)
HpaI (1657)
MfeI (1668)
EcoRI (1753)
SspI (1992)
SwaI (2006)
BstXI (2296)
StuI (2431)
Eco147I (2431)
XmnI (2573)
BspHI (2581)
PagI (2581)
AseI (2639)
VspI (2639)
SpeI (2794)
Eco105I (2922)
SnaBI (2922)
NdeI (3027)
SdaI (3205)
PacI (3213)
BspLU11I (3223)
ApaLI (3537)
PacI (3953)
SwaI (3962)
NotI (3972)
EagI (3973)
hEF1-HTLV prom
pMB1 ori
hCMV enh
hCMV prom
EM7
BsrS2
hSTING MRP
SV40 p(A)
(h) ßglobin p(A)
100

```
PvuI (7)
SgfI (6)                                                                 MfeI (82)
1    GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101  GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

     Psp1406I (203)                      HindIII (245)                              Bsu36I (291)
201  GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301  GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

                                  NgoMI (441)
                                  NaeI (441)
401  GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

                                                        BspEI (558)
501  TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCTCCGGAAGATGCCCACTCCAGCCTGCATCCATCCATCCCGT
                                                        1▸ M  P  H  S  S  L  H  P  S  I  P
                                                                                   Asp718I (699)
            Bsp120I (615)                      BstEII (655)                        Acc65I (699)
601  GTCCCAGGGGTCACGGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCACCAGAGCACACTCTCCG
12▸  C  P  R  G  H  G  A  Q  K  A  A  L  V  L  L  S  A  C  L  V  T  L  W  G  L  G  E  P  P  E  H  T  L  R
                                                                               Asp718I (792)
                                                                               Acc65I (792)
701  GTACCTGGTCCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACCGG
45▸   Y  L  V  L  H  L  A  S  L  Q  L  G  L  L  L  N  G  V  C  S  L  A  E  E  L  R  H  I  H  S  R  Y  R
                                              Bsp120I (852)
801  GGCAGCTACTGGAGGACTGTGCGGGCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCCCAAATG
79▸   G  S  Y  W  R  T  V  R  A  C  L  G  C  P  L  R  R  G  A  L  L  L  L  S  I  Y  F  Y  Y  S  L  P  N
                                                         SfiI (968)              BglII (993)
901  CGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCTCCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTC
112▸ A  V  G  P  P  F  T  W  M  L  A  L  L  G  L  S  Q  A  L  N  I  L  L  G  L  K  G  L  A  P  A  E  I  S
                                                EcoRV (1062)
                           NcoI (1033)          Eco32I (1062)
1001 TGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCCCATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGG
145▸  A  V  C  E  K  G  N  F  N  V  A  H  G  L  A  W  S  Y  Y  I  G  Y  L  R  L  I  L  P  E  L  Q  A  R
 BstBI (1101)
 BstBI (1101)
 Bsp119I (1101)
 AsuII (1101)
1101 ATTCGAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCCATTGGACTGTGGGGTGCCTGATAACC
179▸  I  R  T  Y  N  Q  H  Y  N  N  L  L  R  G  A  V  S  Q  R  L  Y  I  L  L  P  L  D  C  G  V  P  D  N
                                                BstEII (1253)
                                              AgeI (1250)
1201 TGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCCCAGCAGACCGGTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATCTA
212▸ L  S  M  A  D  P  N  I  R  F  L  D  K  L  P  Q  Q  T  G  D  R  A  G  I  K  D  R  V  Y  S  N  S  I  Y
1301 TGAGCTTCTGGAGAACGGGCAGCGGAACCTGCAGATGACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGG
245▸  E  L  L  E  N  G  Q  R  N  L  Q  M  T  A  A  S  R  C  P  R  R  F  S  G  T  C  G  R  R  K  R  K  R
1401 TTACTGTGGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGTGGAATGGAAAAGCCCCTCCCTCT
279▸  L  L  W  A  A  •
                    MscI (1525)
                    BalI (1525)
                NheI (1519)
1501 CCGCACGGATTTCTCTTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATT

                                                    HpaI (1657)   MfeI (1668)
1601 TGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGG

                                                  EcoRI (1753)
1701 GGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCC

1801 TCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGT

                                                                                      SspI (1992)
1901 TTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCA

     SwaI (2006)
2001 GAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTT

2101 GGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTTCCTCAA
                                                      141◂ •  N  R  T  Y  K  L  P  I  L  E  E  I
                                                                                    BstXI (2296)
2201 TGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCAC
128◂  T  T  K  V  L  K  G  N  M  E  I  L  V  F  C  D  P  A  Y  D  S  I  L  E  R  C  M  G  C  P  S  V  V
2301 CCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCAATGGCAATG
95◂  R  I  S  R  D  V  E  D  S  Y  P  H  R  V  A  V  I  T  D  F  D  K  Q  G  N  S  V  A  S  G  I  A  I
                          StuI (2431)
                          Eco147I (2431)
2401 GCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCCTGATGGCCGCCCCGACATGGTGCT
61◂  A  E  A  C  V  T  V  R  G  I  Y  A  E  I  H  V  A  S  I  I  E  G  T  K  T  R  I  A  A  G  V  H  H  K
```

```
                                                                                       PagI (2581)
                                                                                       BspHI (2581)
                                                                               XmnI (2573)
2501 TGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCTCCTATA
  28   N  D  E  Y  L  M  T   I  K  E  T  A  V  E  V  L  E  L  D  Q  Q  S   I  N  F  T  K  M
                                                  VspI (2639)
                                                  AseI (2639)
2601 GTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGC
                                                                                                  SpeI (2794)
2701 TCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGT
2801 CAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCA
                     SnaBI (2922)
                     Eco105I (2922)
2901 TCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTC
                          NdeI (3027)
3001 ATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAA
3101 GTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATG
             PacI (3213)
       SdaI (3205)      BspLU11I (3223)
3201 TAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGC
3301 CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG
3401 TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCT
                                   ApaLI (3537)
3501 CAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCC
3601 AACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGT
3701 GGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAA
3801 ACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGG
                                                                               EagI (3973)
                                                          PacI (3953) SwaI (3962) NotI (3972)
3901 TCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTC
4001 ATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCC
4101 AGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
```