

pUNO1Fc-SARS2-S2

Plasmid designed for the production of the SARS-CoV-2 Spike S2::Fc fusion protein

Catalog code: p1fc-cov2-s2

<https://invivogen.com/sars2-spike-s2-tag-production-vectors>

For research use only

Version 21C17-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pUNO1Fc-SARS2-S2 (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20°C.
- Resuspended DNA stored at -20°C 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

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

PLASMID FEATURES

SARS-CoV-2 Spike S2::hIgG1 Fc cassette

- **SV40 enhancer** is comprised of a 72-base-pair repeat and allows the enhancement of gene expression in a wide range of hosts. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids¹.
- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter² 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 Repeat³ 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.

- **Codon-optimized Spike S2 subunit ORF**

pUNO1Fc-SARS2-S2 contains the coding sequence for the S2 subunit of the Spike (S) protein from the original SARS-CoV-2 Wuhan-Hu-1 isolate. To improve expression of the S2 subunit, the gene is codon-optimized. Furthermore, to facilitate secretion of the protein the coding sequence is preceded by the native SARS-CoV-2 signal sequence.

The S protein mediates the binding and entry of SARS-CoV-2 to host cells. In particular, the S2 subunit contains various features such as a fusion peptide and heptad repeats (HR), and is thus responsible for viral-host membrane fusion⁴.

For more information visit: <https://www.invivogen.com/sars2-spike>

- **TEV sequence** (Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser)) is cleavable by the TEV (Tobacco Etch Virus) protease between the Gln and Gly/Ser residues.

- **Fc** is the the Fc region of the human IgG1 cloned at the C-terminus of the gene of interest and followed by a Stop codon. This tag 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.

- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA⁵.

Antibiotic selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **bsr (blasticidin resistance 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.
- **Human beta-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference and recombination events with the SV40 pAn signal⁶.

General features of pUNO1Fc-SARS2-S2

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

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 as a 10 mg/ml colorless solution in HEPES buffer.

APPLICATIONS

Detection and purification of the S2 subunit

pUNO1Fc-SARS2-S2 has been designed to generate the Spike S2 subunit in mammalian cells with a C-terminal human IgG1-Fc (Fc) tag. This facilitates the detection of the secreted protein with an anti-Fc antibody, and its purification using [protein G](#) or [protein L](#) affinity resins.

TECHNICAL SUPPORT

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E-mail: info@invivogen.com

- Subcloning the gene into another vector

For subcloning, two unique restriction sites flank the S2 subunit gene:

- **5' site: AgeI** (compatible with XmaI, BspEI, NgoMIV, and SgrAI)
- **3' site: MscI** (compatible with Ball, MIsI, MLuNI, Mox20I, and Msp20I)

REFERENCES

1. Dean DA. *et al.* 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22. 2. Kim D. *et al.*, 1990. Use of the human elongation factor 1 α 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. Huang, Y. *et al.* 2020. Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. *Acta Pharmacol Sin* 41, 1141-1149. 5. 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. 6. 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 <i>E. coli</i> | gt116-11 |
| Protein G / Agarose | Immunoglobulin binding protein | gel-agg-2 |
| Protein L / Agarose | Immunoglobulin binding protein | gel-protl-2 |

COVID-19 Product Range

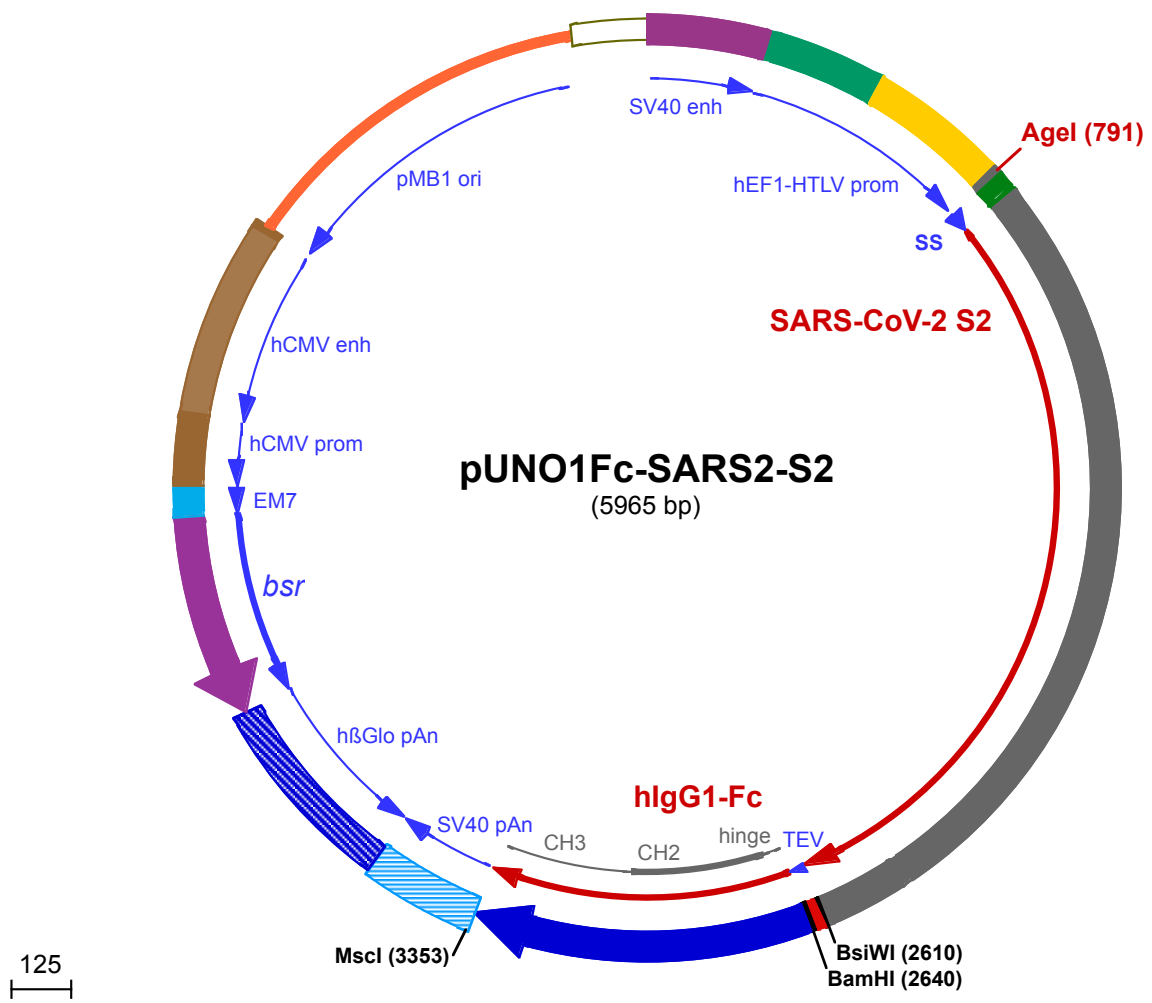
| | | |
|--------------------------|-------------------|-----------------|
| HEK-Blue™ hACE2 Cells | Cell line | hkb-hace2 |
| A549-hACE2-TMPRSS2 Cells | Cell Line | a549-hace2-tpsa |
| pUNO1-hACE2 | Expression vector | puno1-hace2 |
| pUNO1-hTMPRSS2a | Expression vector | puno1-htp2a |
| pUNO1-hTMPRSS2b | Expression vector | puno1-htp2b |

For a complete list of InvivoGen's COVID-19 related products visit:
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1 GGACCTGCAGGGCTGAATAACCTCTGAAAGAGGAAGCTGGTTAGGTACCTCTGAGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTG
101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGGAAAGTCCCAGGCTCCCAGCAGGCAG
201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCTCCGGTGGCCGTGAGTGGGAGAGCGCACATCGCCACAGTCCCCGA
301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCCC
401 GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGGCTC
501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGC
601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCCTCACGCTTTGC

Agel (791)

701 CTGACCCTGCTTGTCAACTCTACGCTTTTGTTCGTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGGTCAA
801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTGGAGCTCCCAATGCAGTGTAGCATCCAAAGTATCATTGCCTACACAATGAGCCTCGGTGCTGAGAAT
1 M F V F L V L L P L V S S Q 1 S V A S Q S I I A Y T M S L G A E N
901 TCTGTCGCTACAGCAACAATCCATTGCTATCCCTACTAACTCACAATCAGTGTGACAACCTGAAATTCGCCGATCTATGACCAAAACAAGCGTTG
19 S V A Y S N N S I A I P T N F T I S V T T E I L P V S M T K T S V
1001 ACTGCACCATGTACATCTGTGGCGATTCTACGAATGTAGCAATCTCCTCCTGCAATACGGATCATTCTGCACTCAGCTGAATCGTGCCTCACAGGTAT
52 D C T M Y I C G D S T E C S N L L L Q Y G S F C T Q L N R A L T G I
1101 TGCAGTTGAGCAGACAAGAATACGCAGGAAGTGTTCGCCAGGTGAAGCAATCTACAAAACCTCCACCCATAAAAGACTTTGGCGGATTCAATTTCTCA
85 A V E Q D K N T Q E V F A Q V K Q I Y K T P P I K D F G G F N F S
1201 CAGATCCTGCCGATCCCTCAAAACCTCCTCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAACCTCGCAGATGCCGTTTCATCAAGCAGT
119 Q I L P D P S K P S K R S F I E D L L F N K V T L A D A G F I K Q
1301 ATGGCGATTGTCTGGGAGACATCGCCGCTCGGACCTGATCTGTGCACAGAAGTTCAATGGACTGACCGTGTGCTCCCTTGTGACCGCAGGATGAT
152 Y G D C L G D I A A R D L I C A Q K F N G L T V L P P L L T D E M I
1401 AGCCCAATACACTAGCGCCTGCTGGCCGGCACCATCACTTCTGGGTGGACATTGGAGCTGGCGCTGCCCTCAGATTCTTTTGTATGCAGATGGCC
185 A Q Y T S A L L A G T I T S G W T F G A G A A L Q I P F A M Q M A
1501 TACCGCTTAAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACAGAACTCATCGCAACAGTTCAACAGTGTATCGGTAAGATACAGGATA
219 Y R F N G I G V T Q N V L Y E N Q K L I A N Q F N S A I G K I Q D
1601 GCCTGTCTCACTGCCAGCGCATTGGGAAAGTTGCAGGATGTAGTGAACCAGAATGCCAGGCACCTAACACCCTGGTGAACAGCTCTCTTCAAATTT
252 S L S S T A S A L G K L Q D V V N Q N A Q A L N T L V K Q L S S N F
1701 TGGTGCCATTTCTAGCGTGTGAATGACATACTGAGCCGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATAGGCTGATAACTGGGCGCCTCAGTCT
285 G A I S S V L N D I L S R L L D K V E A E V Q I D R L I T G R L Q S
1801 CTTCAGACCTATGTGACCAGCAGCTCATCCGCGTGTGAAATTCGCGCATCCGCTAACCTGGCAGCAACCAAAATGTCCGAGTGTGTGCTGGGTGAGT
319 L Q T Y V T Q Q L I R A A E I R A S A N L A A T K M S E C V L G Q
1901 CTAAGAGAGTGGACTTTTGGCGGAAGGGGTATCACCTGATGTCTTTTCTCAGTCTGCACCCCATGGTGTGGTCTTTCTGCAGCTGACTTATGTCACG
352 S K R A V D F C T G C K G Y H L M S F P Q S A P H G V V F L H V T Y V P A
2001 TCAGGAAAAGAACTTCACTACAGCCCAAGCATCTGCCACGATGGGAAAGCCCACTTTCCAGGGAAGGCGTATTCTGTCCAATGGTACTCATTGTGTTT
385 Q E K N F T T A P A I C H D G K A H F P R E G V F V S N G T H W F
2101 G T C A C T C A G A G A A T T T C A C G A G C C C A G A T T A T A A C C A C T G A C A A T A C A T T T G T A T C C G G C A A T T G T A T G T G T T A T C G G G A T T G A A T A A T A C T G
419 V T Q R N F Y E P Q I I T T D N T F V S G N C D V V I G I V N N T
2201 TTTACGATCCTTTGCAGCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAATATTTAAGAATCACACATCACCTGACGTGCACCTCGGAGATATTT
452 V Y D P L Q P E L D S F K E E L D K Y F K N H T S P D V D L G D I S
2301 AGGAATCAATGCTTCCGTGGTCAATATTGAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCTGCAGGAGTTG
485 G I N A S V V N I Q K E I D R L N E V A K N L N E S L I D L Q E L
2401 GGCAAGTACGAACAGTATATCAAATGGCCTTGGTACATTTGGCTTGGGTTTATTGCTGGGCTGATAGCTATCGTATGGTGAACATATGTTGTTGTTGCA
519 G K Y E Q Y I K W P W Y I W L G F I A G L I A I V M V T I M L C C
2501 T G A C A T C C T G T G T A G T T G T G T G A A G G G C T G C T G C A T G C G G C A G C T G T T G C A A G T T T G A C G A G G A C G A T T C C G A G C C T G T G C T G A A G G G C T C A A A C T
552 M T S C C S C L K G C C S C G S C C K F D E D D S E P V L K G V K L

BsiWI (2610)

BamHI (2640)

2601 GCATTATACGCGTACGGAGAACCTGTACTTCCAGGGCTCTGGATCCGAGCCAAATCTAGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAA
585 H Y T 1 E P K S S D K T H T C P P C P A P E
1 R T E N L Y F Q G S G S
2701 G C C A G G G G G G A C C G T C A G T C T T C T T C C C C C A A A A C C A A G G A C C A A C T G A T G A T C T C C C G A C C C C T G A G G T C A C A T G C G T G G T G G T G G A C G T G A
19 A E G G P S V F L F P P K P K D Q L M I S R T P E V T C V V V D V
2801 G C C A C G A A G A C C C T G A G G T C A A G T T C A A C T G G T A C G T G G A C G G C G T G G A G G T G C A T A A T G C C A A G A C A A A G C C G G G A G G A G C A G T A C A A C A G C A C G T A
52 S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y
2901 C C G T G T G G T C A G C G T C C T C A C C G T C C T G C A C C A G G A C T G G C T G A A T G G C A A G G A G T A C A A G T G C A A G G T C T C C A C A A A G C C C T C C C A G C C I C C A T C G A G
85 R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A S I E

3001 AAAACCATCTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCT
119▶ K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T

3101 GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCTCCCGTGTGGACTC
152▶ C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S

3201 CGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGCTGCATGAGGCTCTGCAC
185▶ D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V L H E A L H

MscI (3353)

3301 AACCACTACAGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAC
219▶ N H Y T Q K S L S L S P G K •

3401 TAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAAT

3501 TGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTCTAAAATACAGCAT

3601 AGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTG

3701 TTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTGCTTTCATTTCTTTATGTTTTAAATGCACTGACCTC

3801 CCACATTCCTTTTATAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCC

3901 CTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTG
141◀ • N R

4001 GTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCT
137▶ T Y K L P I L E E I T T K V L K G N M E I L V F C D P A Y D S I L E

4101 CTCTGCACATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCTTCTGCC
104▶ R C M G C P S V V R I S R D V E D S Y P H R V A V I T D F D K Q G

4201 GTTGCTCAGCAGACCAATGGCAATGGCTTCAAGCAGACAGTACCTGCAATGTAGGCTCAATGTGGACAGCAGAGATGATCTCCCAAGTCTTG
71▶ N S V A S G I A I A E A C V T V R G I Y A E I H V A S I I E G T K

4301 GTCCTGATGGCCGCCGACATGGTGTCTGTCCTCATAGAGCATGGTGTCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATG
37▶ T R I A A G V H H K N D E Y L M T I K E T A V E V L E L D Q Q S I N

4401 TGAAGTCTTCATGGTGGCCCTCTATAGTGTGATTATACTATGCGCATATACTATGCGCATGATTAATTGTCAAACAGCGTGGATGGCGTCTCC
4▶ F T K M

4501 AGTTATCTGACGTTCACTAAACGAGCTCTGTTATATAGACCTCCACCGTACACGCTACCGCCATTTCGTCATGGGGCGGAGTTGTACGACA

4601 TTTTGAAAGTCCCGTTGATTTACTAGTCAAAACAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGC

4701 CCATTGATGACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAGTGCATGACTGGG

4801 CATAATGCCAGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGGCTACTTGGCATATGATACACTTGATGACTGCCAAGTGGGCGAGTTACCGTAA

4901 ATACTCCACCCATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTATTATTGACGTCAATGGGCGGGGCTGTTGGGCGGTACG

5001 CCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGC

5101 GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC

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