# pUNO1-SpikeV6 

# Expression vector encoding the SARS-CoV-2 New York variant (B.1.526 lineage) Spike gene <br> Catalog code: p1-spike-v6 <br> https://www.invivogen.com/ny-b1526-spike-expression-vectors 

For research use only
Version 21E11-ED

## PRODUCT INFORMATION

## Contents

- $20 \mu \mathrm{~g}$ of lyophilized pUNO1-SpikeV6 (plasmid DNA)
- $2 \times 1 \mathrm{ml}$ of Blasticidin ( $10 \mathrm{mg} / \mathrm{ml}$ )


## Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at $-20^{\circ} \mathrm{C}$.
- Resuspended DNA is stable for 1 year at $-20^{\circ} \mathrm{C}$.
- Store Blasticidin at $4^{\circ} \mathrm{C}$ or $-20^{\circ} \mathrm{C}$. The expiry date is specified on the product label.


## Quality control

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


## PLASMID FEATURES

## New York Variant SARS-CoV-2 Spike cassette

- EF-1a/HTLV hybrid promoter is a composite promoter comprised of the Elongation Factor-1a (EF-1a) core promoter ${ }^{1}$ and the $5^{\prime}$ untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1a utilizes a type 2 promoter that encodes a "house-keeping"gene. It is expressed at high levels in all cell cycles and lower levels during the GO phase. Additionally, since the promoter is not tissue-specific it is highly expressed in all cell types. The R segment and part of the U5 sequence ( $\mathrm{R}-\mathrm{U} 5^{\prime}$ ) of the HTLV Type 1 Long Terminal Repeat ${ }^{2}$ has been coupled to the EF-1a promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency.
- Codon-optimized Spike ORF
pUNO1-SpikeV6 contains the Spike coding sequence from the New York (N.Y.) SARS-CoV-2 variant (B.1.526 lineage). This variant is characterized by a number of mutations within the the Spike coding sequence (see below) ${ }^{3}$. Additionally, to improve expression of the $S$ protein in cell lines, the gene is codon-optimized and the last 19 amino acids, which contain a endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed ${ }^{4,5}$.
pUNO1-SpikeV6 includes the following sequence features:
- S1 domain: L5F, T95I, D253G, D614G
- RBD: E484K
- S1/S2 boundary: Functional furin cleavage site
- S2 domain: A701V

Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and the proteolytic activity of host proteases such as furin and TMPRSS2 ${ }^{6}$.
For more information visit: https://www.invivogen.com/sars2-spike

- SV40 pAn is the Simian Virus 40 late polyadenylation (pAn) signal and it enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA ${ }^{\text {² }}$


## Antibiotic selection cassette

- hCMV (human cytomegalovirus) enhancer \& promoter drive the expression of the blasticidin resistance gene (bsr) in mammalian cells.
- EM7 is a bacterial promoter that enables the constitutive expression of the blasticidin resistance gene (bsr) in E. coli.
- bsr (blasticidin resistance gene) encodes a deaminase from Bacillus cereus that confers resistance to the antibiotic blasticidin. The expression of the bsr gene is driven by the CMV promoter/enhancer and the bacterial EM7 promoter. Therefore, Blasticidin can be used to select stable clones in mammalian cells 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 possible recombination events with the SV40 pAn signal ${ }^{8}$.


## General features of pUNO1-SpikeV6

- pMB1 ori is a minimal E. coli origin of replication.


## APPLICATIONS

Stable gene expression in mammalian cells.
pUNO1 plasmids are designed for both transient and stable transfection in mammalian cell lines by selection with Blasticidin. Furthermore, they faciliate high levels of expression of the gene of interest.

## Cell fusion assays

pUNO1-SpikeV6 has been specifically designed for mammalian cell expression of the SARS-CoV-2 S protein. This plasmid features a functional furin cleavage site, and therefore when expressed by a host cell (e.g. 293T cells) it facilitates cell-cell fusion assays with ACE2-expressing cells (e.g. HEK-Blue ${ }^{\text {TM }}$ hACE2 Cells)?

## METHODS

- Plasmid resuspension
- Quickly spin the tube containing the lyophilized plasmid to pellet the DNA.
- To obtain a plasmid solution at $1 \mu \mathrm{~g} / \mu \mathrm{l}$, resuspend the DNA in $20 \mu \mathrm{l}$ of sterile water.
- Store the resuspended plasmid at $-20^{\circ} \mathrm{C}$.
- 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 DH5a.

## - Blasticidin usage

Blasticidin should be used at 25-100 $\mu \mathrm{g} / \mathrm{ml}$ in bacteria and $1-30 \mu \mathrm{~g} / \mathrm{ml}$ in mammalian cells. Blasticidin is supplied as a $10 \mathrm{mg} / \mathrm{ml}$ colorless solution in HEPES buffer.

## REFERENCES

1. Kim D. et al., 1990. Use of the human elongation factor 1 a promoter as a versatile and efficient expression system. Gene 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. Annavajhala, M.K. et al. 2021. A Novel and Expanding SARS-CoV-2 Variant, B.1.526, Identified in New York. medRxiv doi: 10.1101/2021.02.23.21252259. 4. Johnson, M.C. et al. 2020. Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94. 5. Ou, X. et al. 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11, 1620. 6. Hoffmann M. et al., 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. 7. 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):424858. 8. Yu J. \& Russell J., 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. 9. Papa, G. et al. 2021. Furin cleavage of SARS-CoV-2 Spike promotes but is not essential for infection and cell-cell fusion. PLoS Pathog 17, e1009246.

## RELATED PRODUCTS

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| pUNO1-hACE2 | Expression vector | puno1-hace2 |
| pUNO1-hTMPRSS2a | Expression vector | puno1-htp2a |
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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
(1) Invivocen

InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com


21D03v09

201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGA

301 GAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC

401 GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTC
501 GCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGC

601 GTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC

701 CTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCAA

## L5F (813)

801 CATGTTTGTGTTCTTIGTGTTGCTTCCACTGGTCAGTTCCCAATGCGTTAATCTCACCACCCGAACTCAACTCCCACCCGCATATACAAATTCCTTCACC

1. M F V F F V L L P L V S S Q C V N L T T R T Q L P P A Y T 901 AGAGGAGTGTACTATCCTGACAAAGTGTTTCGGTCAAGTGTCCTCCACTCTACTCAGGACCTCTTTCTGCCTTTCTTTTCTAACGTTACATGGTTTCATG
 T95I (1083)
1001 CAATCCATGTGTCTGGGACAAACGGCACCAAACGCTTCGACAACCCTGTATTGCCATTCAATGATGGGGTGTACTTTGCCTCCATIGAGAAATCCAACAT 67. A I H V S G T N G T K R F D N P V L P F N D G V Y F A S I E K 1101 CATTCGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAACAACGCCACAAACGTTGTCATCAAAGTGTGCGAATTC 100. I R G W I F G T T L D S K T Q S L L I V N N A T N V V I K $V$ C 1201 CAGTTTTGCAATGATCCCTTCCTGGGAGTGTACTATCACAAGAATAACAAGTCCTGGATGGAGAGCGAATTTCGGGTCTACAGCAGCGCAAACAACTGCA
 1301 CCTTCGAGTACGTGAGTCAACCCTTTCTGATGGACCTGGAAGGGAAACAGGGAAACTTCAAGAACCTGAGAGAGTTTTGTCTTTAAGAACATCGACGGCTA
 1401 TTTTAAGATCTATAGTAAGCATACGCCTATCAACCTGGTAAGGGATCTTCCCCAGGGCTTTTCAGCCCTGGAACCTTTGGTTGACTTGCCTATTGGTATC
 D253G (1557)
1501 AATATCACCAGATTTCAGACCCTTCTGGCATTGCAICGGTCTTATCTTACTCCAGGTGGTTCCTCCTCCGGGTGGACTGCCGGCGCCGCTGCCTACTATG 234* $N$ I T R F Q T L L A L H R S Y L T $\begin{aligned} & \text { P }\end{aligned}$ 1601 TCGGCTATCTGCAACCAAGAACGTTCCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCCTCTGTCTGAGACTAA 267.V G Y L Q P R T F L L K Y N E 1701 ATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACCAGTAATTTCCGGGTCCAACCCACCGAAAGCATTGTGCGGTTCCCAAATATCACC 300. C T L K S F T V E K G I Y Q T

1801 AATCTGTGTCCCTTTGGCGAAGTGTTCAATGCTACAAGGTTTGCTTCTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGTGTCGCTGATTACTCCG


1901 TGCTGTACAATTCCGCCTCTTTCTCAACCTTCAAGTGTTATGGCGTTTCACCTACCAAACTTAACGACCTGTGCTTCACTAATGTGTATGCCGACTCTTT


2001 TGTGATACGAGGCGATGAAGTGAGACAGATTGCACCAGGGCAGACCGGCAAAATTGCCGACTACAACTACAAGCTTCCAGATGACTTTACCGGATGTGTT


2101 ATTGCATGGAACTCAAACAATCTGGATTCCAAGGTGGGTGGCAACTATAACTACCTGTATAGACTGTTCAGGAAATCCAACCTGAAACCATTCGAGCGAG


E484K (2250)
2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGAAAGGGTTCAACTGCTACTTCCCATTGCAGAGTTACGGATTCCAGCCTAC


2301 AAACGGGGTGGGTTACCAACCCTATCGTGTCGTAGTCCTGAGTTTTGAGCTCCTCCATGCCCCAGCCACAGTCTGTGGCCCCAAGAAAAGCACCAATCTG 500. $\mathrm{N} \quad \mathrm{G} \quad \mathrm{V} \quad \mathrm{G} \quad \mathrm{Y}$

2401 GTGAAGAACAAATGCGTGAACTTTAACTTTAACGGACTCACAGGAACCGGCGTATTGACGGAGAGTAACAAGAAGTTCCTGCCATTCCAGCAGTTCGGTC


2501 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCGGCGGAGTGAGCGTGATTACGCC
 D614G (2640)
2601 CGGAACCAATACCAGCAATCAGGTTGCCGTCCTGTATCAGGGTGTGAATTGCACCGAGGTACCTGTCGCCATCCACGCTGACCAACTTACACCCACATGG


2701 CGAGTATATTCCACCGGCTCCAACGTCTTTCAGACACGTGCTGGATGTCTGATCGGTGCAGAACACGTTAATAATAGCTACGAGTGTGATATCCCCATCG 634. R V Y S T G S N V F Q T R A G C L I G A E H V N N S Y E C D I P I Furin cleavage site (2853)
2801 GTGCTGGAATATGCGCCTCTTATCAAACTCAAACCAACTCTCCTAGGCGGGCACGTAGTGTAGCATCCCAAAGTATCATTGCCTACACAATGAGCCTCGG
 A701V (2901)
2901 TGIAGAGAATTCTGTCGCCTACAGCAACAACTCCATTGCTATCCCTACTAACTTCACAATCAGTGTGACAACTGAAATTCTGCCCGTATCTATGACCAAA 7001 V E N S V A Y S N N S I A I P T N F T I S V T T 3001 ACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCATTCTGCACTCAGCTGAATCGTGCCC 734. T S V D C T M Y I C G D S T 3101 TCACAGGTATTGCAGTTGAGCAGGACAAGAATACGCAGGAAGTGTTTGCCCAGGTGAAGCAAATCTACAAAACTCCACCCATAAAAGACTTTGGCGGATT
 3201 CAATTTCTCACAGATCCTGCCCGATCCCTCAAAACCCTCCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAACCCTCGCAGATGCCGGTTTC
 3301 ATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTCGGGACCTGATCTGTGCACAGAAGTTCAATGGACTGACCGTGCTGCCTCCCTTGCTGACCG 834. I K Q Y G D C L G D I A A $\quad$ R D D I 3401 ACGAGATGATAGCCCAATACACTAGCGCCCTGCTGGCCGGCACCATCACTTCTGGGTGGACATTCGGAGCTGGCGCTGCCCTTCAGATTCCTTTTTGCTAT 867. D E M I A Q Y T S A L L A G T I T S G W T F G A G A A L 3501 GCAGATGGCCTACCGCTTTAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACCAGAAACTCATCGCCAACCAGTTCAACAGTGCTATCGGTAAG
 3601 ATACAGGATAGCCTGTCATCCACTGCCAGCGCATTGGGAAAGTTGCAGGATGTAGTGAACCAGAATGCCCAGGCACTTAACACCCTGGTGAAACAGCTCT
 3701 CTTCAAATTTTGGTGCCATTTCTAGCGTGCTGAATGACATACTGAGCCGGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATAGGCTGATAACTGGGCG 967. S S N F G A I S S V L N D I L S R L D K V E A E V C ( 3801 CCTTCAGTCTCTTCAGACCTATGTGACCCAGCAGCTCATCCGCGCTGCTGAAATTCGCGCATCCGCTAACCTGGCAGCAACCAAAATGTCCGAGTGTGTG
 3901 CTGGGTCAGTCTAAGAGAGTGGACTTTTGCGGGAAGGGGTATCACCTGATGTCTTTTCCTCAGTCTGCACCCCATGGTGTGGTCTTTCTGCACGTGACTT
 4001 ATGTCCCAGCTCAGGAAAAGAACTTCACTACAGCCCCAGCCATCTGCCACGATGGGAAAGCCCACTTTCCCAGGGAAGGCGTATTCGTGTCCAATGGTAC
 4101 TCATTGGTTCGTCACTCAGAGAAATTTCTACGAGCCCCAGATTATAACCACTGACAATACATTTGTATCCGGCAATTGTGATGTGGTTATCGGGATTGTG
 4201 AATAATACTGTTTACGATCCTTTGCAGCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAATATTTTAAGAATCACACATCACCTGACGTCGACCTCG 1134. N N T V Y D P L Q P P E L D S F K 4301 GAGATATTTCAGGAATCAATGCTTCCGTGGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCT
 4401 GCAGGAGTTGGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTCATTGCTGGGCTGATAGCTATCGTCATGGTGACAATTATG
 Nhel (4567)
4501 TTGTGTTGCATGACATCCTGCTGTAGTTGTCTGAAGGGCTGCTGCTCATGCGGCAGCTGTTGCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGAT 1234* L C C M T S C C S C L K
4601 GAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATA
4701 AACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAT
4801 GGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTG
4901 TTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTAT
5001 GTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCA
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5201 GCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGG 141 • N R T Y K L P I L E E I T T
5301 AGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATG 111. A Y D S I L E R C M G C P 5401 GTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAG
 5501 AGATGATCTCCCCAGTCTTGGTCCTGATGGCCGCCCCGACATGGTGCTTGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTC 44 I I E G T K T R I A A G V H H K N D E Y L M T I K
5601 CAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAA 111 L D Q Q S I N F T K M «
5701 ACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAAT
5801 GGGGCGGAGTTGTTACGACATTTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTG
5901 AGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTC

6001 CCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCC
6101 AAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGC
BspLU11I (6271)
6201 GGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCC
6301 AGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC
6401 CGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCT
6501 CCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCC
6601 GTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA
6701 TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT
6801 GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACG
6901 CGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTA
7001 GTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTC -
7101 CATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA

