

pUNO1-SpikeV1

Expression vector containing the SARS-CoV-2 Wuhan-Hu-1 G614-variant Spike gene

Catalog code: p1-spike-v1

<https://www.invivogen.com/wuhan-spike-d614g-expression-vectors>

For research use only

Version 21C04-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pUNO1-SpikeV1 (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA is stable for 1 year at -20°C.
- Store Blasticidin at 4°C or -20°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

Wuhan-Hu-1 SARS-CoV-2 Spike (G614) cassette

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter¹ and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α 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 G0 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 (R-U5') of the HTLV Type 1 Long Terminal Repeat² 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.

- **Codon-optimized Spike ORF**

pUNO1-SpikeV1 contains the Spike coding sequence from the Wuhan-Hu-1 SARS-CoV-2 G614-variant. This variant is characterized by the D614G mutation³. This signature mutation has globally replaced the original sequence and is present in all subsequent variants of SARS-CoV-2. Additionally, to improve expression of the S protein in cell lines, the gene is codon-optimized and the last 19 amino acids, which contain an endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed^{4,5}.

pUNO1-SpikeV1 includes the following sequence features:

- **S1 domain:** D614G
- **S1/S2 boundary:** Functional furin cleavage site

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 TMPRSS2⁶.

Note: 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 mRNA⁷.

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 β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal⁸.

General features of pUNO1-SpikeV1

- **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 facilitate high levels of expression of the gene of interest.

Cell fusion assays

pUNO1-SpikeV1 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™ hACE2 Cells**)⁹.

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.

TECHNICAL SUPPORT

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REFERENCES

1. 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 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. Korber B. *et al.*, 2020. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell.* 182:1-16. 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):4248-58. 8. 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. 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

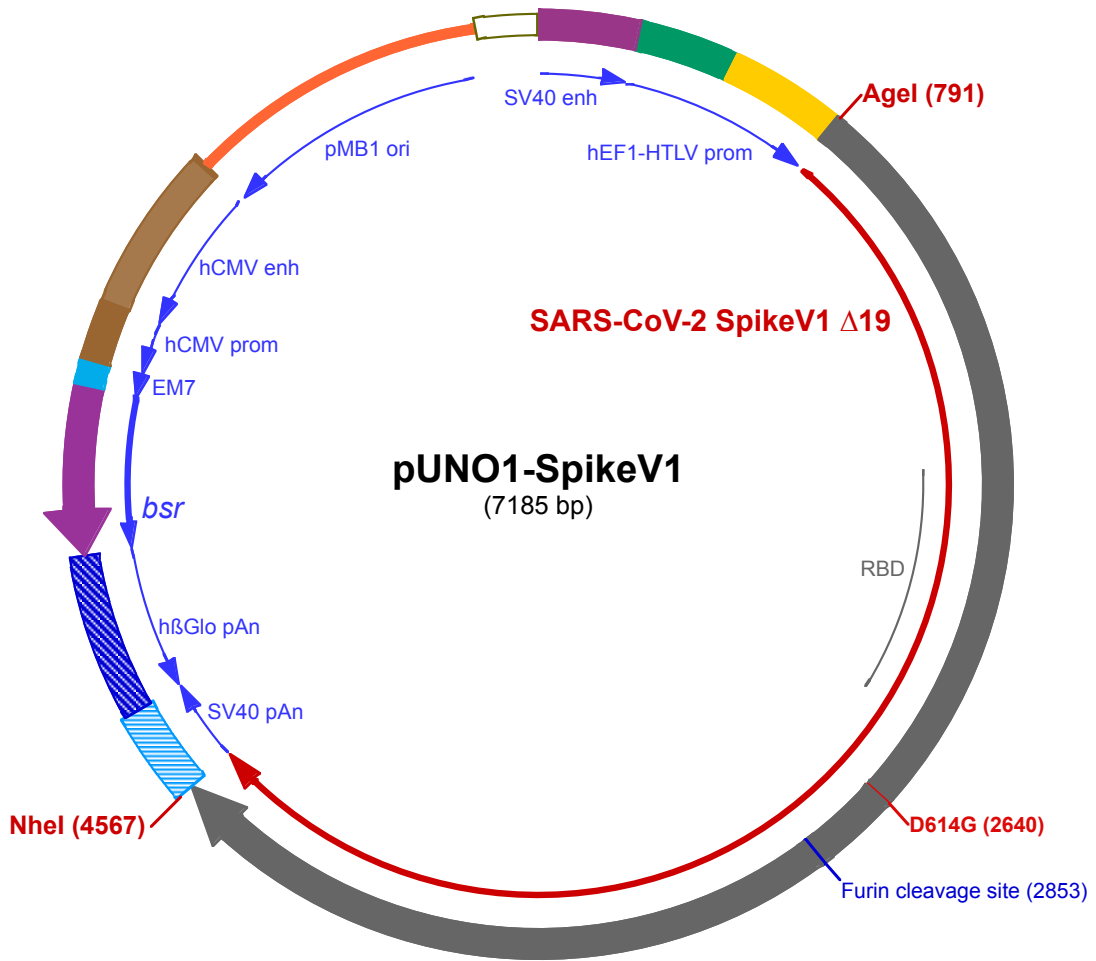
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
COVID-19 Product Range		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-c1-hlgG1	Recombinant Antibody	cov2rbdc1-mab1

For a complete list of InvivoGen's COVID-19 related products visit: <https://www.invivogen.com/covid-19>

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1 GGACCTGCAGGGCTGAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTCTGAGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTG
101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAG
201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCTCCGGTGGCCGTGAGTGGGAGAGCGCACATCGCCACAGTCCCCGA
301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCCC
401 GAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGGCTC
501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGC
601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC

Agel (791)

701 CTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCTGTTTCTGCGCAGTTACAGATCCAAGCTGTGACCGGGCCTACCTGAGATCACCGGTCAA
801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTAAATCTACCACCCGAACCTCAACTCCCACCCGCATATACAAATCCTTCACC
1 M F V F L V L L P L V S S Q C V N L T T R T Q L P P A Y T N S F T
901 AGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGCCTCCACTCTACTCAGGACCTCTTCTGCCTTTCTTTTCTAACGTTACATGGTTTCATG
34 R G V Y Y P D K V F R S S V L H S T Q D L F L P F F S N V T W F H
1001 CAATCCATGTGTCTGGGACAAACCGCACAAACGCTTCGACAACCCTGTATTGCCATTCAATGATGGGGTGTACTTTGCTCCACAGAGAAATCCAACAT
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 T E K S N I
1101 CATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGTGATCGTTAAACAACGCCACAAACGTTGTCATCAAAGTGTGCGAATTC
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 E F
1201 CAGTTTTGCAATGATCCCTTCTGGGAGTGTACTATACAAGAATAACAAGTCTGGATGGAGAGCGAATTTCCGGGTCTACAGCAGCGCAAACAACCTGCA
134 Q F C N D P F L G V Y Y H K N N K S W M E S E F R V Y S S A N N C
1301 CCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGGAAACTCAAGAACCTGAGAGAGTTTGTCTTTAAGAACATCGACGGCTA
167 T F E Y V S Q P F L M D L E G K Q G N F K N L R E F V F K N I D G Y
1401 TTTAAGATCTATAGTAAGCATAACGCTATCAACCTGGTAAGGGATCTTCCCAGGGCTTTTCCAGCCTGGAACCTTTGGTTGACTTGCCTATTGGTATC
200 F K I Y S K H T P I N L V R D L P Q G F S A L E P L V D L P I G I
1501 AATATCACCAGATTTCCAGACCCTTCTGGCATTGCATCGGTCTTATCTTACTCCAGGTATTCTCCTCCGGGTGGACTGCCGGCGCCGCTGCCTACTATG
234 N I T R F Q T L L A L H R S Y L T P G D S S S G W T A G A A A Y Y
1601 TCGGCTATCTGCAACCAAGAAGTTCCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCTCTGTCTGAGACTAA
267 V G Y L Q P R T F L L K Y N E N G T I T D A V D C A L D P L S E T K
1701 ATGCACCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACAGTAATTTCCGGGTCCAACCCACCGAAAGCATTGTGCGGTTCCCAAATATCACC
300 C T L K S F T V E K G I Y Q T S N F R V Q P T E S I V R F P N I T
1801 AATCTGTGTCCTTTGGCGAAGTGTTCATGCTACAAGTTTGTCTTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGTGTGCTGATTACTCCG
334 N L C P F G E V F N A T R F A S V Y A W N R K R I S N C V A D Y S
1901 TGCTGTACAATTCGCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTAACGACCTGTGCTTACTAATGTGTATGCCGACTCTTT
367 V L Y N S A S F S T F K C Y G V S P T K L N D L C F T N V Y A D S F
2001 TGTGATACGAGGGCATGAAGTGAAGCAGATTGCACCAGGGCAGACCGGCAAAATGCGGACTACAACATAAGCTTCCAGATGACTTTACCGGATGTGTT
400 V I R G D E V R Q I A P G Q T G K I A D Y N Y K L P D D F T G C V
2101 ATGATGGAACCTCAACAATCTGGATTCCAAGTGGGTGGCAACTATAACTACCTGTATAGACTGTTCCAGAAATCCAACCTGAAACCATTCCAGCGAG
434 I A W N S N N L D S K V G G N Y N Y L Y R L F R K S N L K P F E R
2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGAAGGGTTCAACTGCTACTTCCCATTGCAGAGTTACGGATTCCAGCCTAC
467 D I S T E I Y Q A G S T P C N G V E G F N C Y F P L Q S Y G F Q P T
2301 AAACGGGGTGGGTTACCAACCCTATCGTGTGCTAGTCTGAGTTTTGAGTCTCCTCATGCCCCAGCCACAGTCTGTGGCCCCAAGAAAAGCACCAATCTG
500 N G V G Y Q P Y R V V V L S F E L L H A P A T V C G P K K S T N L
2401 GTGAAGAACAATGCGTGAACCTTAACTTAAACGGACTCACAGGAACCGCGTATTGACGGAGAGTAACAAGAAGTCTGCTGCCATTCCAGCAGTTCGGTC
534 V K N K C V N F N F N G L T G T G V L T E S N K K F L P F Q Q F G
2501 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCCGGCGGAGTGAGCGTGATTACGCC
567 R D I A D T T D A V R D P Q T L E I L D I T P C S F G G V S V I T P
D614G (2640)
2601 CGGAACCAATACCAGCAATCAGGTTGCCGCTGTATCAGGCGTGAATTGCACCGAGGTACCTGTGCCATCCAGCTGACCAACTTACACCACATGG
600 G T N T S N Q V A V L Y Q G V N C T E V P V A I H A D Q L T P T W
2701 CGAGTATATTCCACCGGCTCCAACGCTTTTCCAGACAGTGTGGATGTCTGATCGGTGCAAGACAGTAAATAATAGCTACGAGTGTGATATCCCATCG
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 GTGCTGGAATATGCGCCTTTATCAAACCTCAAACCAACTCTCCTAGCGGGACCGTAGTGTAGCATCCCAAAGTATCATTGCCTACACAATGAGCCTCGG
667 G A G I C A S Y Q T Q T N S P R R A R S V A S Q S I I A Y T M S L G
2901 TGCTGAGAATTCGTGCGCTACGACCAACTCCATTGCTATCCCTACTAATCACAATCAGTGTGACAACCTGAAATTCGCCGATCTATGACCAA
700 A E N 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
3001 ACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCATTCTGCACTCAGCTGAATCGTGCCC
734 T S V 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
3101 TCACAGGTATTGAGTGTGAGCAGGACAAGAATACGAGGAAGTGTTCGCCAGGTGAAGCAAATCTACAAAACCTCCACCCATAAAAGACTTTGGCGGATT
767 L T G I 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
3201 CAATTTCTCACAGATCCTGCCGATCCCTCAAACCCCTCAAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAACCCCTCGCAGATGCCGGTTTC
800 N F S 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
3301 ATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTCGGGACCTGATCTGTGCACAGAAGTTCATGGACTGACCTGTGCTCCCTGCTGACCG
834 I K Q Y G D C L G D I A A R D L I C A A Q K F N G L T V L P P L L T
3401 ACGAGATGATAGCCAATACACTAGCGCCCTGTGGCCGGCACCATCACTTCTGGGTGGACATTGGGAGCTGGCGCTGCCCTCAGATTCCTTTTGTAT
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 Q I P F A M
3501 GCAGATGGCCTACCGTTTAAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACAGAACTCATCGCAACAGTTCACAGTGTATCGGTAAG
900 Q M A 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
3601 ATACAGGATAGCCTGTATCCACTGCCAGCGCATTGGGAAAGTTCAGGATGTAGTGAACCAGAATGCCAGGCACCTAACACCCTGGTGAACAGCTCT
934 I Q D 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
3701 CTTCAAATTTTGGTGCATTTTACGCTGCTGAATGACATACTGAGCCGTTGACAAAGTGGAGGCTGAAGTGCAGATTGATAGGCTGATAACTGGGCG
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 Q I D R L I T G R
3801 CCTTCAGTCTCTCAGACCTATGTGACCAGCAGCTCATCCGCGTGTGAAATTCGCGCATCCGCTAACCTGGCAGCAACAAAATGTCGAGTGTGTG
1000 L Q S 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
3901 CTGGGTCAGTCTAAGAGAGTGGACTTTTGGCGGAAGGGGTATCACCTGATGTCTTTTCTCAGTCTGCACCCCATGGTGTGGTCTTTCTGCAGTGACTT
1034 L G Q S K R V D F C G K G Y H L M S F P Q S A P H G V V F L H V T
4001 ATGTCACAGCTCAGGAAAAGAACTTACTACAGCCCGACCTGTCACAGTGGGAAAGCCACTTTCCAGGGAAGGCGTATTGCTGCAATGGTAC
1067 Y V P A 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
4101 TCATTGGTTCGCTACTCAGAGAAAATTTACGAGCCCGAGATTATAACCACTGACAATACATTTGTATCCGGCAATTGTGATGTGTTATCGGGATTGTG
1100 H W F 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
4201 AATAACTGTTTACGATCCTTTGCAGCCAGAGCTGGACTCCTCAAGGAGGAGCTTGACAAATATTTAAGAATCACACATCACCTGACGTGCACCTCG
1134 N N T 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
4301 GAGATATTTAGGAATCAATGCTTCCGTGGTCAATATTCAGAAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCT
1167 G D I S 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
4401 GCAGGAGTTGGCAAGTACGAACAGTATATCAATGGCCATGGTACATTTGGCTTGGGTTTATTGCTGGGCTGATAGCTATCGTATGGTGAACATATG
1200 Q E L 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

NheI (4567)

4501 TTGTGTTGCATGACATCCTGCTGTAGTTGTCTGAAGGGCTGCTGCTCATGCGGAGCTGTTGCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGAT
1234 L C C M T S C C S C L K G C C S C G S C C •
4601 GAGTTTGGACAAACCACAACCTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATA
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141 N R 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
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111 A Y D S I L E 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
5401 GTGTCAAAGTCTTCTGCCGTTGCTCAGCAGACCAATGGCAATGGCTTCAAGCAGACAGTACCCCTGCAATGTAGGCTCAATGTGGACAGCAG
77 T D F D K Q G 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
5501 AGATGATCTCCAGCTTGGTCTGATGGCCGCCGACATGGTGTCTGTTGCTCCTATAGAGCATGGTGTCTTCTCAGTGGCGACCTCCACCAGCTC
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 E T A V E V L E
5601 CAGATCCTGCTGAGAGATGTTGAAGGCTTCATGATGGCCCTCTATAGTGTGCTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAA
11 L D Q Q S I N F T K M
5701 ACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCATTTCGCTCAAT
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6201 GGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCC
6301 AGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGCAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC
6401 CGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCT
6501 CCCTTCGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC
6601 GTTCAGCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA
6701 TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT
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7001 GTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTC
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