

# pUNO1-SpikeV6-dfur

Expression vector encoding the SARS-CoV-2 N.Y. variant (B.1.569 lineage) Spike (delta furin) gene

Catalog code: p1-spike-v6-df

<https://www.invivogen.com/ny-b1526-spike-expression-vectors>

For research use only

Version 21E11-ED

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pUNO1-SpikeV6-dfur (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 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

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

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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 Repeat<sup>2</sup> 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-SpikeV6-dfur contains the Spike (S) coding sequence from the New York (N.Y.) variant (B.1.526 lineage). This variant is characterized by a number of mutations within the the Spike coding sequence (see below)<sup>3</sup>. The furin cleavage site in pUNO1-SpikeV4-dfur has been inactivated (dfur) by the inclusion of two mutations (R683/5A). Furthermore, to improve expression of the S protein in cell lines, the gene is codon-optimized and the last 19 amino acids, which contain an ER-retention motif (KxHxx), have been removed<sup>4,5</sup>.

pUNO1-SpikeV6-dfur includes the following sequence features:

- **S1 domain:** L5F, T95I, D253G, D614G
- **RBD:** E484K
- **S1/S2 boundary:** R683A, R685A
- **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<sup>6</sup>.

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<sup>7</sup>

### 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 signal<sup>8</sup>.

### General features of pUNO1-SpikeV6-dfur

- **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.

### Antibody screening by flow cytometry

pUNO1-SpikeV6-dfur has been specifically designed for mammalian cell expression of the SARS-CoV-2 S protein. Notably, due to the inactivated furin cleavage site, when this plasmid is expressed by a host cell (e.g. 293T cells) there is high surface expression of the full-length S protein<sup>4,9</sup>. Ideal for SARS-CoV-2 S-specific antibody screening by flow cytometry (*in-house data*).

## 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 the 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 $\alpha$  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):4248-58. 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. Walls, A.C. *et al.* 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell.*

## RELATED PRODUCTS

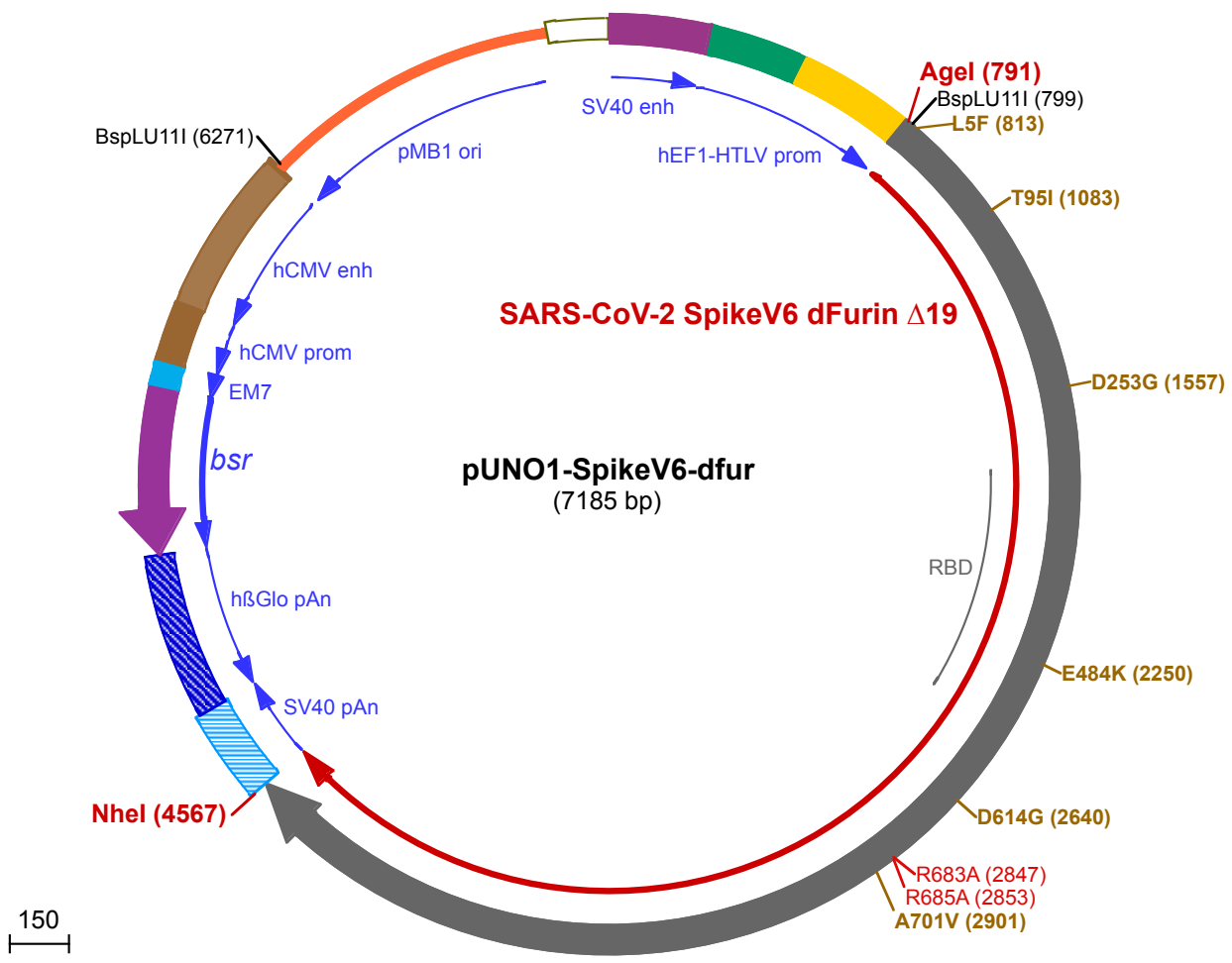
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
<b>COVID-19 Product Range</b>		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a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

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1 GGACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTAGTTAGGGTGTG  
 101 GAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG  
 201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTCTCGGTGCCCGTCACTGGGCGAGAGCGCACATCGCCACAGTCCCCGA  
 301 GAAGTTGGGGGGAGGGTTCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACAGTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCCC  
 401 GAGGGTGGGGGAGAACCGTATATAAGTCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACAGCTGAAGCTTCGAGGGGCTC  
 501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGC  
 601 GTCGCGCTTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC

Agel (791)

701 CTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCTGTTTCTGTCGCGAGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTCAA

L5F (813)

801 CATGTTTGTGTTCTTIGTGTGTTCCACTGGTCAGTTCCTCAATGCGTTAATCTACCACCCGAACTCAACTCCCACCCGCATATACAAATTCCTTACC  
 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 N S F T  
 901 AGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGTCTCCACTCTACTCAGGACCTTTTCTGCCTTTCTTTCTAACGTTACATGGTTTCATG  
 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

T95I (1083)

1001 CAATCCATGTGTCTGGGACAAACGGCACCAACCGTTCGACAAACCCTGTATTGCCATTCAATGATGGGGTGTACTTTGCCTCCATTGAGAAATCCAACAT  
 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 S N I  
 1101 CATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAAACAACGCCACAAACGTTGTCATCAAAGTGTGCGAATTC  
 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 CAGTTTTGCAATGATCCCTTCTGGGAGTGTACTATACAAGAATAACAAGTCTGGATGGAGAGCGAATTTCCGGGTCTACAGCAGCGCAAACAACACTGCA  
 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 CCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGGAAACTTCAAGAACCTGAGAGAGTTTGTCTTTAAGAACATCGACGGCTA  
 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 TTTAAGATCTATAGTAAGCATACGCCATCAACCTGTAAGGGATCTTCCCAAGGCTTTTCAGCCCTGGAACCTTTGGTTGACTTGCCTATTGGTATC  
 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

D253G (1557)

1501 AATATCACCAGATTTTCAGACCCTTCTGGCATTGCAICGGTCTTATCTTACTCCAGGTGGTTCCCTCCTCGGGTGGACTGCCGGCGCCGCTGCCTACTATG  
 234 N I T R F Q T L L A L H R S Y L T P G G S S S G W T A G A A A Y Y  
 1601 TCGGCTATCTGCAACCAAGAAGCTTCTGCTCAAGTACAAGCAAAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCCTGTCTGAGACTAA  
 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 C L S E T K  
 1701 ATGCACCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAAACAGTAATTTCCGGGTCCAACCCACGAAAGCATTGTGCGGTTCCCAAATATCACC  
 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 AATCTGTGTCCCTTTGGCGAAGTGTCAATGCTACAAGTTTCTTCTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGTGTGCTGATTACTCCG  
 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 TGTGATACGAGCGATGAAGTGAAGCAGATTGCACAGGGCAGACCGGCAAAATGCGGACTACAACCTACAAGCTTCCAGATGACTTTACCGGATGTGTT  
 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 ATTGCATGGAACCTCAAACAATCTGGATTCCAAGGTGGGTGGCAACTATAACTACCTGTATAGACTGTTTCAGGAAATCCAACCTGAAACCATTCCAGCGAG  
 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

E484K (2250)

2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGAAAGGGTTCAACTGCTACTTCCCATTGCAGAGTTACGGATTCCAGCCTAC  
 467 D I S T E I Y Q A G S T P C N G V K G F N C Y F P L Q S Y G F Q P T

2301 AAACGGGGTGGTTACCAACCCTATCGTGTGCTAGTCTGAGTGTGAGCTCCTCCATGCCAGCCACAGCTGTGGCCCAAGAAAAGCACCAATCTG  
 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 GTGAAGAACAATGCGTGAACCTTAACTTTAACGGACTCACAGGAACCGGCGTATTGACGGAGAGTAACAAGAAGTTCCTGCCATTCCAGCAGTTCGGTC  
 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 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCTGTAGTTTCCGGCGGAGTGAGCGTGATTACGCC  
 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 CGGAACCAATACCAGCAATCAGGTTGCCGCTGTATCAGGTTGTAATTGCACCGAGGTACCTGTCGCCATCCACGCTGACCAACTTACCCACATGG  
 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 CGAGTATATTCCACCGGCTCCAACGCTTTTCAGACACGTGCTGGATGTCTGATCGGTGCGAACACGTTAATAATAGCTACGAGTGTGATATCCCCATCG  
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  
R685A (2853)  
2801 GTGCTGGAATATGCGCCTCTTATCAAACCTCAAACCAACTCTCCTAGGCGGGCACTAGTGTAGCATCCCAAAGTATCATTGCCTACACAATGAGCCTCGG  
667▶ G A G I C A S Y Q T Q T N S P R A A A S V A S Q S I I A Y T M S L G  
A701V (2901)  
2901 GTAGAGAATTCTGTCGCTACAGCAACAACCTCCATTGCTATCCCTACTAACTTCACAATCAGTGTGACAACCTGAAATTTCTGCCGTATCTATGACCAAA  
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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 TCACAGGTATTGAGTGTGAGCAGGACAAGAATACGACAGGAAGTGTTCGCCAGGTGAAGCAAACTACAAAACCTCCACCCATAAAAGACTTTGGCGGATT  
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 CAATTTCTCACAGATCCTGCCGATCCCTCAAACCTCCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAACCTCGCAGATGCCGGTTTC  
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 ATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTGGGACCTGATCTGTGCACAGAAGTTCATGGACTGACCGTGTGCCTCCCTTGTGACCC  
834▶ I K Q Y G A 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  
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3701 CTTCAAATTTGGTGCCATTTCTAGCGTGTGAATGACATACTGAGCCGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATAGGCTGATAACTGGGG  
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3801 CCTTCAGTCTCTTCAGACCTATGTGACCCAGCAGCTCATCCGCGCTGCTGAAATTCGCGCATCCGCTAACCTGGCAGCAACCAAAATGTCGAGTGTGTG  
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 CTGGGTGAGTCTAAGAGAGTGGACTTTTGGGGAAGGGGTATCACCTGATGTCTTTTCTCAGTCTGCACCCATGGTGTGGTCTTTCTGCACGTGACTT  
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4001 ATGTCAGCTCAGGAAAAGAACTTCACTACAGCCAGCCATCTGCCACGATGGGAAAGCCACTTTCCAGGGAAGGCGTATTCTGTCCAATGGTAC  
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4101 TCATTGGTTCGCTACTCAGAGAAATTTCTACGAGCCCAAGATTATAACCACCTGACAATACATTTGTATCCGCAATTGTGATGTGGTTATCGGGATTGTG  
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 AATAATACTGTTTACGATCCTTTGACGCGAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAAATTTTAAAGAAATCACACATCACCTGACGTGACCTCG  
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 GAGATATTTAGGAATCAATGCTTCCGTGGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCT  
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 GCAGGAGTTGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTTATTGCTGGGCTGATAGCTATCGTATGGTGAACATATG  
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 TTGTGTTGCATGACATCCTGCTGTAGTTGTCTGAAGGCTGCTGCTCATGCGGAGCTGTTGCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGAT  
1234▶ L C C M T S C C S C L K G C C S C G S C C •  
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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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5801 GGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAACAACTCCCATGACGTCAATGGGGTGGAGACTTGAAATCCCGTG

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6001 CCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCC  
6101 AAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGC  
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6501 CCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC  
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