

# pLV-SpikeV8

Vector for lentiviral pseudotyping with SARS-CoV-2 Delta variant (B.1.617.2 lineage) Spike

Catalog code: plv-spike-v8

<https://www.invivogen.com/delta-b1617-spike-pseudotyping-vector>

For research use only

Version 21G07-ED

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pLV-SpikeV8 (plasmid DNA)

### 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 for at least 1 year.

### 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

- **hCMV (human cytomegalovirus) enhancer & promoter** drives high expression of the SARS-CoV-2 spike gene in mammalian cells.
- **Rabbit (rbt) β-Globin intron** enhances the expression of the SARS-CoV-2 spike gene in mammalian cells.

- **Codon-optimized Spike ORF**

pLV-SpikeV8 contains the Spike coding sequence from the Delta SARS-CoV-2 variant (B.1.617.2 lineage), first identified in India. This variant is characterized by a number of mutations and deletions within the the Spike coding sequence (*see below*)<sup>1</sup>. Additionally, to improve expression of the S protein in pseudovirions, the gene is codon-optimized and the last 19 amino acids, which contain a endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed<sup>2</sup>.

pLV-SpikeV8 includes the following sequence features:

- **S1 domain:** T19R, T95I, G142D, E156G, ΔF157-R158, D614G, P681R
- **RBD:** L452R, T478K
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** D950N

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>3</sup>.

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

- **Rabbit β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of the SARS-CoV-2 spike gene. It allows efficient transcription termination and polyadenylation of the mRNA.
- **bla (Ampicillin resistance gene)** encodes the β-lactamase enzyme, which confers resistance to the antibiotic ampicillin. Therefore, ampicillin can be used to select *E. coli* transformants.
- **pMB1 ori** is a minimal *E. coli* origin of replication.

## APPLICATION

pLV-SpikeV8 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (Delta variant). The basic strategy involves transfecting 293T cells with a lentiviral backbone plasmid encoding a fluorescent or luminescent reporter protein (e.g. GFP), a plasmid expressing the minimal set of lentiviral proteins necessary to assemble viral particles, and InvivoGen's pLV-SpikeV7. The transfected cells produce SARS-CoV-2 Spike-pseudotyped lentiviral particles, which can then be used to infect permissive cells.

## GENERAL PROTOCOL

For a detailed protocol for producing SARS-CoV-2 Spike (S)-pseudotyped lentiviral particles, please refer to the literature<sup>4</sup>. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:

- **InvivoGen's pLV-SpikeV8** plasmid
- Lentiviral backbone plasmid encoding a reporter protein (e.g. GFP or Luciferase)
- Plasmid/s encoding the necessary virion packaging proteins

2. After ~48 hours, collect the S-pseudotyped lentiviral particles by harvesting and filtering the cell culture supernatant.

3. Determine the titre of the S-pseudotyped lentiviral particles using a permissive cell line that express the SARS-CoV-2 host receptor (e.g. InvivoGen's **HEK-Blue™ hACE2 cells**) in a relevant assay.

## PLASMID PREPARATION

### • 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.

## REFERENCES

1. Davis, C. *et al.* 2021. Reduced neutralisation of the Delta (B.1.617.2) SARS-CoV-2 variant of concern following vaccination. medRxiv doi:10.1101/2021.06.23.21259327.
2. Johnson, M.C. *et al.* 2020. Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94.
3. 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.
4. Crawford, K.H.D. *et al.* 2020. Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. Viruses 12. doi: 10.3390/v12050513.

## TECHNICAL SUPPORT

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## RELATED PRODUCTS

Product	Description	Cat. Code
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-hIgG1	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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### TECHNICAL SUPPORT

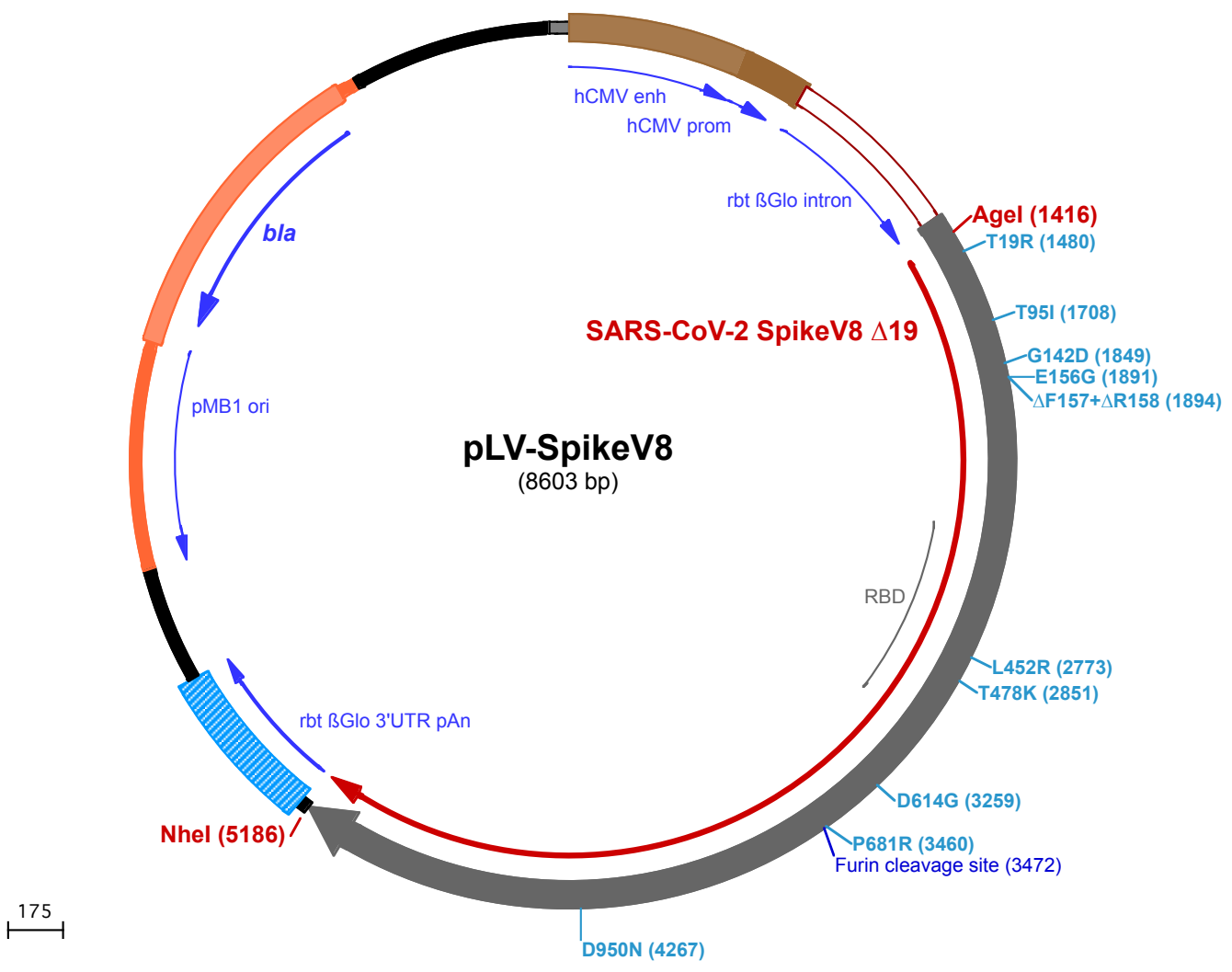
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1 GAGCTTGGCCCATTCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACGCCATGTTGACATTGATTATTGACTA  
101 GTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTACGGTAAATGGCCCGCTGGCTGACCGCC  
201 CAACGACCCCGCCCATTCAGGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA  
301 ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACA  
401 TGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA  
501 GCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAAC  
601 AACTCCGCCCATTCAGCCAAATGGGCGGTAGGCGGTACGGTGGGAGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC  
701 ATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCTCCGGTGCACCGATCCTGAGAACTCAGGgtgagtttggggacccttgattg  
801 ttctttcttttctgctattgtaaaattcatgttatatggagggggcaaagttttcagggtgttgttagaatgggaagatgtcccttgatcacctatgga  
901 ccctcatgataattttgtttctttcactttctactctgttgacaaccattgtctcctcttattttcttttcattttctgtaacttttctgtaaaccttta  
1001 gcttgcatttgtaacgaattttaattcacttttgtttatgttcagattgtaagtactttctctaatcactttttttcaaggcaatcagggtatatt  
1101 atattgtacttcagcacagtttagagaacaattgttataattaaatgataaggtagaatattttctgcatataaattctggctggcgtggaatatctt  
1201 attggtagaacaactacaccctggtcatcatcctgcctttctctttatggttacaatgatatacactgtttgagatgaggataaaaactctgagtcca  
1301 aaccgggcccctctgtaaccatgttcatgcctttctctttctacagCTCCTGGCAACGTGCTGTTGTTGTGCTGTCTCATATTTGGCAAAGA

**Agel (1416)**

**T19R (1480)**

1401 ATTCTCGACGGATCCACCGGTCAACATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTTAATCTCAGAACCCGAACCTCAACTCCC  
1501 ACCCGCATATACAAATTCCTTACCAGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGTCTCCACTCTACTCAGGACCTCTTTCTGCCTTTC  
1601 TTTTCTAACGTTACATGGTTTCATGCAATCCATGTGTCTGGGACAAACGGCACAAACGCTTCGACAAACCTGATTGCCATTCAATGATGGGGTGTACT  
1701 TTGCTCCATTGAGAAATCCAACATCATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAAACAACGCCACAAA

**T95I (1708)**

1701 TTGCTCCATTGAGAAATCCAACATCATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAAACAACGCCACAAA  
1801 CGTGTGCATCAAAGTGTGCGAATTCAGTTTTGAATGATCCCTTCCCTGGATGTACTATCACAGAATAACAAGTCTGGATGGAGAGCGGAGTCTAC

**G142D (1849)**

**ΔF157+ΔR158  
E156G (1891)**

1801 V V I K V C E F Q F C N D P F L D V Y Y H K N N K S W M E S G V Y  
1901 AGCAGCGCAAACAACTGCACCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGGAAACAGGGAACTTCAAGAACCCTGAGAGAGTTGTCT  
2001 TTAAGAACATCGACGGCTATTTAAGATCTATAGTAAGCATACGCCTATCAACCTGGTAAGGGATCTTCCCCAGGGCTTTTACGCCCTGGAACCTTTGGT  
2101 TGACTTGCCTATTGGTATCAATATCACCAGATTTGAGACCTTCTGGCATTGCAICGGTCTTACTTACTCCAGGTGATTCCTCCTCCGGGTGGACTGCC  
2201 GGC GCGCTGCTACTATGTCGCTATCTGCAACCAAGAAGCTTCTGCTCAAGTACAACGAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGG  
2301 ACCCTCTGTCTGAGACTAAATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACAGTAATTTCCGGGTCCAACCCACCGAAAGCATTGT  
2401 GCGTTCCCAAATATACCAATCTGTGTCCCTTTGGCGAAGTGTTCATGCTACAAGTTTGTCTTGTGTACGCATGGAATAGGAAACGCATCTCCAAT

2501 TGTGTCGCTGATTACTCCGTGCTGTACAATCCGCCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTTAAACGACCTGTGCTTCACTA  
2601 ATGTGTATGCCGACTCTTTTGTGATACGAGGCGATGAAGTGAACAGATTGCACCAGGGCAGACCGGCAAAATGCGGACTACAACCTACAAGCTTCCAGA  
2701 TGACTTTACCGGATGTGTTATTGCATGGAACCTCAAACAATCTGGATTCCAAGGTGGGTGGCAACTATAACTACCGCTATAGACTGTTCAAGAAATCCAAC

**L452R (2773)**

2701 D F T G C V I A W N S N N L D S K V G G N Y N Y R Y R L F R K S N

T478K (2851)

2801 CTGAAACCATTCGAGCGAGATATAAGCACAGAAATCTACCAGGCTGGAAGTAAAACCTGCAACGGCGTGAAGGGTTCAACTGCTACTTCCCATTGCAGA  
459▶ L K P F E R D I S T E I Y Q A G S K P C N G V E G F N C Y F P L Q

2901 GTTACGGATTCCAGCCTACAAACGGGGTGGGTTACCAACCCTATCGTGTCTAGTCTGAGTTTTGAGCTCCTCCATGCCACAGCAGTCTGTGGCCC  
492▶ S Y G F Q P T 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

3001 CAAGAAAAGCACCAATCTGGTGAAGAACAATGCGTGAACCTTAACTTAAACGGACTCACAGGAACCGCGTATTGACGGAGAGTAACAAGAAGTTCCTG  
525▶ K K S T N L 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

3101 CCATTCCAGCAGTTCGGTTCGCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCGGCG  
559▶ P F Q Q F G 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

D614G (3259)

3201 GAGTGAGCGTGATTACGCCCGGAACCAATACCAGCAATCAGGTTGCCGTCCTGTATCAGGGCGTGAATTGCACCGAGGTACCTGTCCGCATCCACGCTGA  
592▶ G V S V I T P 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

3301 CCAACTTACCCACATGGCGAGTATATTCCACCGGCTCCAACGCTTTTACAGACAGTGTGGATGTCTGATCGGTGCAGAACACGTTAATAATAGTAC  
625▶ Q L T P T W 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

P681R (3460) Furin cleavage site (3472)

3401 GAGTGTGATATCCCCATCGGTGCTGGAATATGCGCCTTTATCAAACCTCAAACCAACTCTCGTAGGGCGCACGTAGTATCCCAAAGTATCATTG  
659▶ E C D I P I G A G I C A S Y Q T Q T N S R R R A R S V A S Q S I I

3501 CCTACACAATGAGCCTCGGTGCTGAGAATTCTGTGCGCTACAGCAACAACCTCATTGCTATCCTACTAATTCACAATCAGTGTGACAACTGAAATCT  
692▶ A Y T M S L G 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

3601 GCCCGTATCTATGACCAAAACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTACCCGAATGTAGCAATCTCCTGCAATACGGATCATTCTGC  
725▶ P V S M T K 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

3701 ACTCAGCTGAATCGTGCCTCACAGGATTGAGTGGAGCAGGACAAGAATACGCAGGAAGTGTGGCCAGGTGAAGCAAATCTACAAAACCTCACCCA  
759▶ T Q L N R A 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

3801 TAAAAGACTTTGGCGGATTCAATTTCTACAGATCCTGCCGATCCCTCAAACCTCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAAC  
792▶ I K D F G G F 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

3901 CCTCGCAGATGCCGGTTTCATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTCGGGACCTGATCTGTGCACAGAAGTTCAATGGACTGACCGTG  
825▶ L A D A G F I K Q Y G D C L G D I A A R D L I C A Q K F N G L T V

4001 CTGCTCCCTTGTGACCGAGATGATAGCCCAATACACTAGCCCTGTGGCCGACCATCACTTCTGGGTGGACATTCCGAGCTGGCGCTGCC  
859▶ L P P L L T D E M I A Q Y T S A L L A G T I T S G W T I T S G A G A A

4101 TTCAGATTCCTTTTGTATGAGATGGCCTACCGCTTAAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACAGAACTCATCGCAACAGTT  
892▶ L Q I P F A M 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

D950N (4267)

4201 CAACAGTGTATCGGTAAGATACAGGATAGCCTGTCCACTGCCAGCGCATTGGGAAAGTTGCAGAAATGTAGTGAACCAGAAATGCCAGGCACTTAAC  
925▶ N S A I G K I Q D S L S S T A S A L G K L Q N V V N Q N A Q A L N

4301 ACCCTGGTAAACAGCTCTTCAAATTTGGTGCCATTTCTAGCGTCTGAATGACATACTGAGCCGGTTGGACAAGGTGGAGGCTGAAGTGCAGATTG  
959▶ T L V K Q L 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

4401 ATAGGCTGATAACTGGGCGCCTTCACTCTTTCAGACCTATGTGACCCAGCAGCTCATCCGCGCTGTGAAATTCGCGCATCCGCTAACCTGGCAGCAAC  
992▶ D R L I T G R 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

4501 CAAAATGTCCGAGTGTGTGCTGGGTGAGTCTAAGAGAGTGGACTTTTTCGGGGAAGGGGTATACCTGATGTCTTTTCTCAGTCTGCACCCCATGGTGTG  
1025▶ K M S E C V 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

4601 GTCTTTCTGCAGTGTACTTATGTCCAGCTCAGGAAAAGAATTCACAGCCCCAGCCATCTGCCACGATGGGAAAGCCCACTTTCCAGGGAAGGCG  
1059▶ V F L H V T 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

4701 TATTCGTGTTCAATGGTACTCATTGGTTCGTCACTCAGAGAAAATTTCTACGACCCCAAGATTATAACCACTGACAATACATTTGATCCGGCAATTGTGA  
1092▶ V F V S N G T 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

4801 TGTGGTTATCGGGATTGTGAATAACTGTTTACGATCCTTTGCAGCCAGAGCTGGACTCCTCAAGGAGGAGCTTGACAAATATTTAAGAATCACACA  
1125▶ V V I G I V 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

4901 TCACCTGACGTCGACCTCGGAGATATTTAGGAATCAATGCTTCCGTTGGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCCTCA  
1159▶ S P D V D L 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

5001 ACGAGTCTGTATCGATGTCAGGAGTTGGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTTCATTGCTGGGCTGATAGCTAT  
1192▶ N E S L I D L 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

NheI (5186)

5101 CGTCATGGTGACAATTATGTTGTGTTGCATGACATCCTGCTGTAGTTGTCTGAAGGGCTGCTGCTCATGCGGCAGCTGTTGCTAAAGCTAGCCTCGAGGG  
1225▶ V M V T I M L C C M T S C C S C L K G C C S C G S C C •

5201 ATCCGTCGAGGAATCACTCCTCAGGTGAGGCTGCCTATCAGAAGGTGGTGGCTGGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTT  
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5401 TTTTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCT  
5501 GGCTGCCATGAACAAAGTTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGTGTCCATTCTTATTCCATAGAAAAGCCTTGACTTGAGGT  
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6001 TCCTCGCTCACTGACTCGCTGCGCTCGGTCTGGCTGCGGCAGCGGTATCAGTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATA  
6101 ACGCAGGAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA  
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6501 ACACGACTTATCGCCACTGGCAGCAGCCACTGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTCTTGAAGTGGTGGCCTAACTAC  
6601 GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG  
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6901 TAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCT  
7001 GACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTT  
287 • W H K I L S A G I E A I Q R N R E D M T A Q  
264 S G T T Y I V V I R S P K G D P G L A A I I G R S G R E G A G S K  
7101 ATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGA  
231 D A I F W G A P L A S R L L P G A V K D A E M W D I L Q Q R S A L  
7201 GTAAGTAGTTCGCGAGTAAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGCTGTTGGTATGGCTTCATTAGCTCCG  
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164 E W R D L R T V H D G M N H L F A T L E K P G G I T T L L L N A A  
7401 AGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCA  
131 T N D S M T I A A S C L E R V T M G D T L H K E T V P S Y E V L D  
7501 TTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTAAAGTGCTCATCTTG  
97 N Q S Y H I R R G L Q E Q G A D I R S L V A G C L L V K F T S M M P  
7601 GAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTT  
64 F R E E P R F S E L I K G S N L D L E I Y G V R A G L Q D E A D K  
7701 TACTTTACCAGCGTTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTT  
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8601 AGA