pLV-SpikeV6

Vector for lentiviral pseudotyping with SARS-CoV-2 New York variant (B.1.526 lineage) Spike

Catalog code: plv-spike-v6

https://www.invivogen.com/ny-b1526-spike-pseudotyping-vector

Version 21E11-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pLV-SpikeV6** (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-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*)[1]. 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[2].

pLV-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[3].

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-SpikeV6 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (New York 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-SpikeV6. 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[4]. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
   - **InvivoGen's pLV-SpikeV6** plasmid
   - Lentiviral backbone plasmid encoding a reporter protein (e.g. GFP or Luciferase)
   - Plasmid/s encoding the neccessary 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 relevent 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.

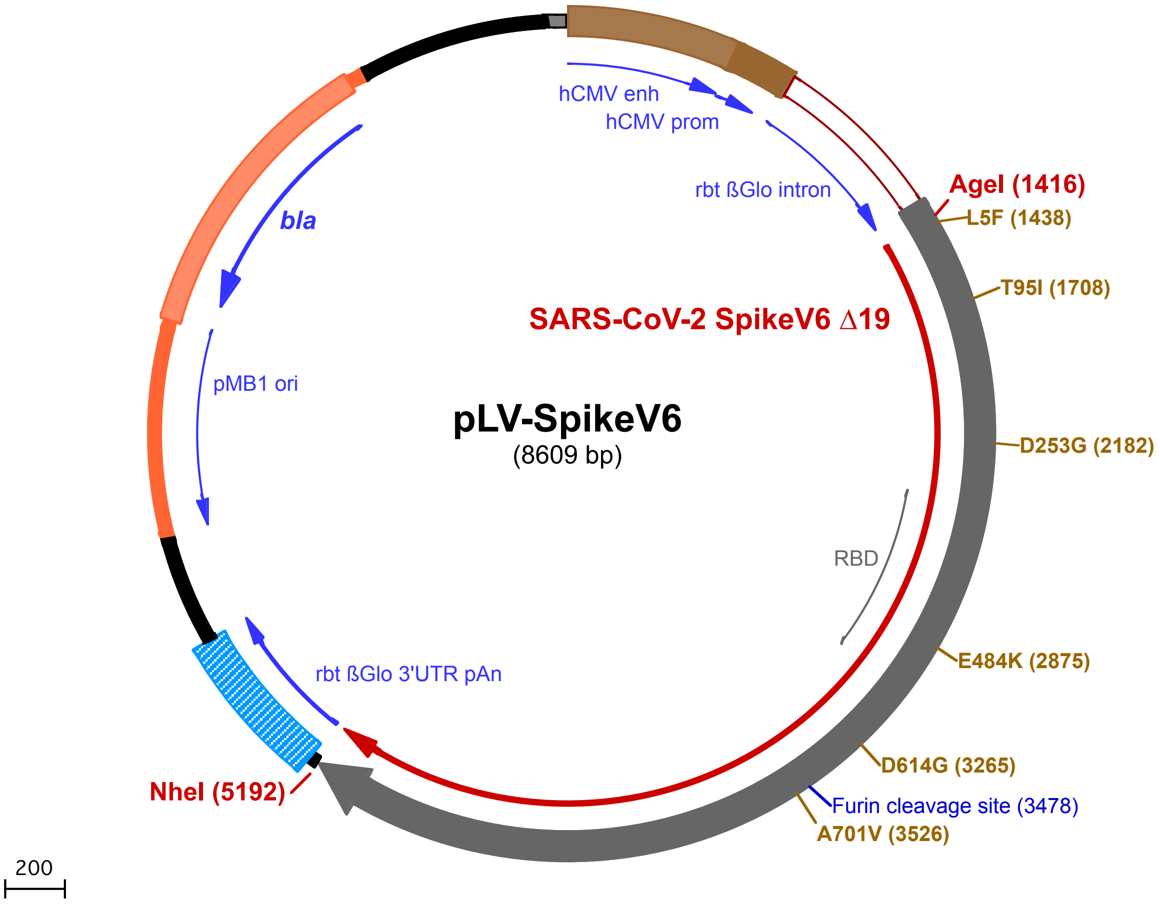
REFERENCES

1. 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. **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.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent *E. coli*	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-hIgG1	Recombinant Antibody	cov2rbdc1-mab1

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



InvivoGen

21D03v06

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   1 GAGCTTGGCCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTA

 101 GTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC

 201 CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA

 301 ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACA

 401 TGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA

 501 GCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAAC

 601 AACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC

 701 ATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGTCGACCGATCCTGAGAACTTCAGGgtgagtttggggacccttgattg

 801 ttctttctttttcgctattgtaaaattcatgttatatggagggggcaaagttttcagggtgttgtttagaatgggaagatgtcccttgtatcaccatgga

 901 ccctcatgataatttgtttctttcactttctactctgttgacaaccattgtctcctcttattttcttttcattttctgtaactttttcgttaaacttta

1001 gcttgcatttgtaacgaattttaaattcacttttgtttatttgtcagattgtaagtactttctctaatcactttttttttcaaggcaatcagggtatatt

1101 atattgtacttcagcacagttttagagaacaattgttataattaaatgataaggtagaatatttctgcatataaattctggctggcgtggaaatattctt

1201 attggtagaaacaactacaccctggtcatcatcctgcctttctctttatggttacaatgatatacactgtttgagatgaggataaaatactctgagtcca

1301 aaccgggcccctctgctaaccatgttcatgccttcttctctttcctacagCTCCTGGGCAACGTGCTGGTTGTTGTGCTGTCTCATCATTTTGGCAAAGA

                     AgeI (1416)           L5F (1438)
1401 ATTCCTCGACGGATCCACCGGTCAACATGTTTGTGTTCTTTGTGTTGCTTCCACTGGTCAGTTCCCAATGCGTTAATCTCACCACCCGAACTCAACTCCC
                             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
1501 ACCCGCATATACAATTCCTTCACCAGAGGAGTGTACTATCCTGACAAAGTGTTTCGGTCAAGTGTCCTCCACTCTACTCAGGACCTCTTTCTGCCTTTC
 25▸   P  A  Y  T  N  S  F  T  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
1601 TTTTCTAACGTTACATGGTTTCATGCAATCCATGTGTCTGGGACAAACGGCACCAAACGCTTCGACAACCCTGTATTGCCATTCAATGATGGGGTGTACT
 59▸  F  S  N  V  T  W  F  H  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
             T95I (1708)
1701 TTGCCTCCATTGAGAAATCCAACATCATTCGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAACAACGCCACAAA
 92▸ F  A  S  I  E  K  S  N  I  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
1801 CGTTGTCATCAAAGTGTGCGAATTCCAGTTTTGCAATGATCCCTTCCTGGGAGTGTACTATCACAAGAATAACAAGTCCTGGATGGAGAGCGAATTTCGG
125▸   V  V  I  K  V  C  E  F  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
1901 GTCTACAGCAGCGCAAACAACTGCACCTTCGAGTACGTGAGTCAACCCTTTCTGATGGACCTGGAAGGGAAACAGGGAAACTTCAAGAACCTGAGAGAGT
159▸  V  Y  S  S  A  N  N  C  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
2001 TTGTCTTTAAGAACATCGACGGCTATTTTAAGATCTATAGTAAGCATACGCCTATCAACCTGGTAAGGGATCTTCCCCAGGGCTTTTCAGCCCTGGAACC
192▸ F  V  F  K  N  I  D  G  Y  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
                                                                                       D253G (2182)
2101 TTTGGTTGACTTGCCTATTGGTATCAATATCACCAGATTTCAGACCCTTCTGGCATTGCATCGGTCTTATCTTACTCCAGGTGGTTCCTCCTCCGGGTGG
225▸   L  V  D  L  P  I  G  I  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
2201 ACTGCCGGCGCCGCTGCCTACTATGTCGGCTATCTGCAACCAAGAACGTTCCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTG
259▸  T  A  G  A  A  A  Y  Y  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
2301 CCCTGGACCCTCTGTCTGAGACTAAATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACCAGTAATTTCCGGGTCCAACCCACCGAAAG
292▸ A  L  D  P  L  S  E  T  K  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

2401 CATTGTGCGGTTCCCAAATATCACCAATCTGTGTCCCTTTGGCGAAGTGTTCAATGCTACAAGGTTTGCTTCTGTGTACGCATGGAATAGGAAACGCATC
325▸   I  V  R  F  P  N  I  T  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

2501 TCCAATTGTGTCGCTGATTACTCCGTGCTGTACAATTCCGCCTCTTTCTCAACCTTCAAGTGTTATGGCGTTTCACCTACCAAACTTAACGACCTGTGCT
359▸  S  N  C  V  A  D  Y  S  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

2601 TCACTAATGTGTATGCCGACTCTTTTGTGATACGAGGCGATGAAGTGAGACAGATTGCACCAGGGCAGACCGGCAAAATTGCCGACTACAACTACAAGCT
392▸ F  T  N  V  Y  A  D  S  F  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

2701 TCCAGATGACTTTACCGGATGTGTTATTGCATGGAACTCAAACAATCTGGATTCCAAGGTGGGTGGCAACTATAACTACCTGTATAGACTGTTCAGGAAA
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                                                                                  E484K (2875)
2801 TCCAACCTGAAACCATTCGAGCGAGATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGAAAGGGTTCAACTGCTACTTCCCAT
459▸  S  N  L  K  P  F  E  R  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
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 492▸ L  Q  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

3001  TGGCCCCAAGAAAAGCACCAATCTGGTGAAGAACAAATGCGTGAACTTTAACTTTAACGGACTCACAGGAACCGGCGTATTGACGGAGAGTAACAAGAAG
 525▸   G  P  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

3101  TTCCTGCCATTCCAGCAGTTCGGTCGCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTT
 559▸  F  L  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
                                                                                          D614G (3265)
3201  TCGGCGGAGTGAGCGTGATTACGCCCGGAACCAATACCAGCAATCAGGTTGCCGTCCTGTATCAGGGTGTGAATTGCACCGAGGTACCTGTCGCCATCCA
 592▸ F  G  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
3301  CGCTGACCAACTTACACCCACATGGCGAGTATATTCCACCGGCTCCAACGTCTTTCAGACACGTGCTGGATGTCTGATCGGTGCAGAACACGTTAATAAT
 625▸   A  D  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
                                                                                     Furin cleavage site (3478)
3401  AGCTACGAGTGTGATATCCCCATCGGTGCTGGAATATGCGCCTCTTATCAAACTCAAACCAACTCTCCTAGGCGGGCACGTAGTGTAGCATCCCAAAGTA
 659▸  S  Y  E  C  D  I  P  I  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
                               A701V (3526)
3501  TCATTGCCTACACAATGAGCCTCGGTGTAGAGAATTCTGTCGCCTACAGCAACAACTCCATTGCTATCCCTACTAACTTCACAATCAGTGTGACAACTGA
 692▸ I  I  A  Y  T  M  S  L  G  V  E  N  S  V  A  Y  S  N  N  S  I  A  I  P  T  N  F  T  I  S  V  T  T  E
3601  AATTCTGCCCGTATCTATGACCAAAACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCA
 725▸   I  L  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
3701  TTCTGCACTCAGCTGAATCGTGCCCTCACAGGTATTGCAGTTGAGCAGGACAAGAATACGCAGGAAGTGTTTGCCCAGGTGAAGCAAATCTACAAAACTC
 759▸  F  C  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
3801  CACCCATAAAAGACTTTGGCGGATTCAATTTCTCACAGATCCTGCCCGATCCCTCAAAACCCTCCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAA
 792▸ P  P  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
3901  GGTAACCCTCGCAGATGCCGGTTTCATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTCGGGACCTGATCTGTGCACAGAAGTTCAATGGACTG
 825▸   V  T  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
4001  ACCGTGCTGCCTCCCTTGCTGACCGACGAGATGATAGCCCAATACACTAGCGCCCTGCTGGCCGGCACCATCACTTCTGGGTGGACATTCGGAGCTGGCG
 859▸  T  V  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  F  G  A  G
4101  CTGCCCTTCAGATTCCTTTTGCTATGCAGATGGCCTACCGCTTTAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACCAGAAACTCATCGCCAA
 892▸ A  A  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
4201  CCAGTTCAACAGTGCTATCGGTAAGATACAGGATAGCCTGTCATCCACTGCCAGCGCATTGGGAAAGTTGCAGGATGTAGTGAACCAGAATGCCCAGGCA
 925▸   Q  F  N  S  A  I  G  K  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
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 959▸  L  N  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
4401  AGATTGATAGGCTGATAACTGGGCGCCTTCAGTCTCTTCAGACCTATGTGACCCAGCAGCTCATCCGCGCTGCTGAAATTCGCGCATCCGCTAACCTGGC
 992▸ Q  I  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
4501  AGCAACCAAAATGTCCGAGTGTGTGCTGGGTCAGTCTAAGAGAGTGGACTTTTGCGGGAAGGGGTATCACCTGATGTCTTTTCCTCAGTCTGCACCCCAT
1025▸   A  T  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
4601  GGTGTGGTCTTTCTGCACGTGACTTATGTCCCAGCTCAGGAAAAGAACTTCACTACAGCCCCAGCCATCTGCCACGATGGGAAAGCCCACTTTCCCAGGG
1059▸  G  V  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
4701  AAGGCGTATTCGTGTCCAATGGTACTCATTGGTTCGTCACTCAGAGAAATTTCTACGAGCCCCAGATTATAACCACTGACAATACATTTGTATCCGGCAA
1092▸ E  G  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
4801  TTGTGATGTGGTTATCGGGATTGTGAATAATACTGTTTACGATCCTTTGCAGCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAATATTTTAAGAAT
1125▸   C  D  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
4901  CACACATCACCTGACGTCGACCTCGGAGATATTTCAGGAATCAATGCTTCCGTGGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGA
1159▸  H  T  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
5001  ACCTCAACGAGTCTCTGATCGATCTGCAGGAGTTGGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTCATTGCTGGGCTGAT
1192▸ N  L  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
                                                                                                   NheI (5192)
5101  AGCTATCGTCATGGTGACAATTATGTTGTGTTGCATGACATCCTGCTGTAGTTGTCTGAAGGGCTGCTGCTCATGCGGCAGCTGTTGCTAAAGCTAGCCT
1225▸   A  I  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  CGAGGGATCCGTCGAGGAATTCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGAT

5301  CTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTT

5401  GGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCA

5501  TATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACT

5601  TGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCT

5701  CCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATCCCTCGACGGATCGGCCGCAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAA

5801  ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTT
5901  GCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCT
6001  TCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAG
6101  GGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCC
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6201  TGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGC

6301  TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTT

6401  CGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCC

6501  GGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT

6601  AACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA

6701  CCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA

6801  CGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAA

6901  TCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAG
                                        287◀ •  W  H  K  I  L  S  A  G  I  E  A  I  Q  R  N  R  E  D  M  T
7001  TTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCC
  266◀  A  Q  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
7101  AGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAA
  233◀ S  K  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
7201  GCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCA
  199◀A  L  T  L  L  E  G  T  L  L  K  R  L  T  T  A  M  A  V  P  M  T  T  D  R  E  D  N  P  I  A  E  N  L
7301  GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTT
  166◀  E  P  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
7401  GGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC
  133◀ A  A  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
7501  AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCA
   99◀L  D  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
7601  TCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGC
   66◀  M  P  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
7701  ATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATA
   33◀ D  K  V  K  V  L  T  E  P  H  A  F  V  P  L  C  F  A  A  F  F  P  I  L  A  V  R  F  H  Q  I  S  M
7801  CTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC
7901  CGCGCACATTTCCCCGAAAAGTGCCACCTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAA
8001  TAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACG
8101  TGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAA
8201  AGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCG
8301  GGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCA
8401  GGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAC
8501  GCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTAATACGACTCACTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGGC
8601  CGCTCTAGA
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