pLV-SpikeV7

Vector for lentiviral pseudotyping with SARS-CoV-2 Kappa variant (B.1.617.1 lineage) Spike

Catalog code: plv-spike-v7

https://www.invivogen.com/ind-b1617-spike-pseudotyping-vector

For research use only

Version 21F25-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pLV-SpikeV7** (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-SpikeV7 contains the Spike coding sequence from the Kappa SARS-CoV-2 variant (B.1.617.1 lineage), first identified in India. 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-SpikeV7 includes the following sequence features:

- **S1 domain:** *G142D, E154K, D614G, P681R*
- **RBD:** *L452R, E484Q*
- **S1/S2 boundary:** *Functional furin cleavage site*
- **S2 domain:** *Q1071H*

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-SpikeV7 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (Kappa 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[4]. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
   - **InvivoGen's pLV-SpikeV7** 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

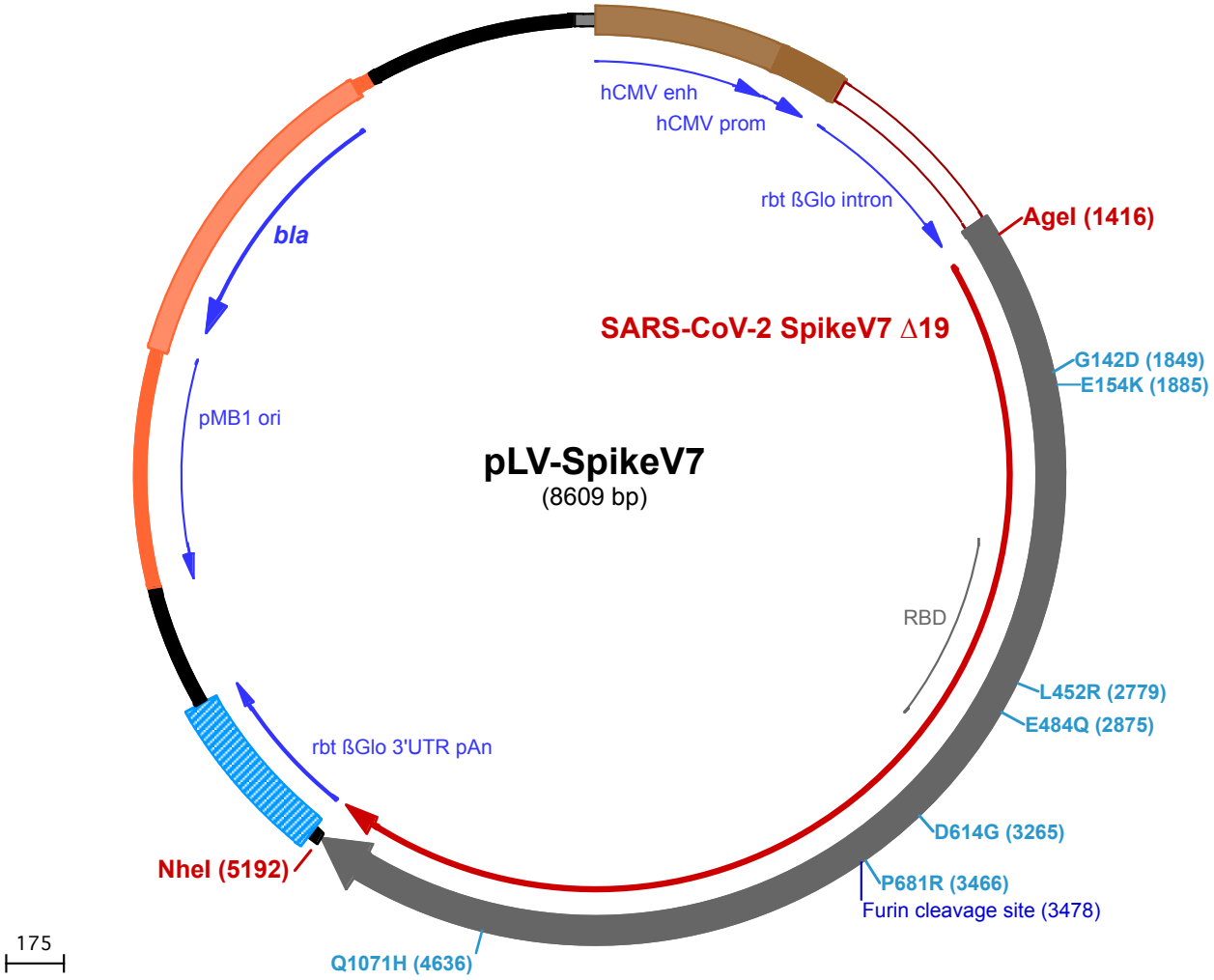
1. Liu, C. *et al.* 2021. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell, doi:10.1016/j.cell.2021.06.020. **2. Johnson, M.C. *et al.* 2020.** Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94. **3. Hoffmann M. *et al.*, 2020.** SARS-CoV-2 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

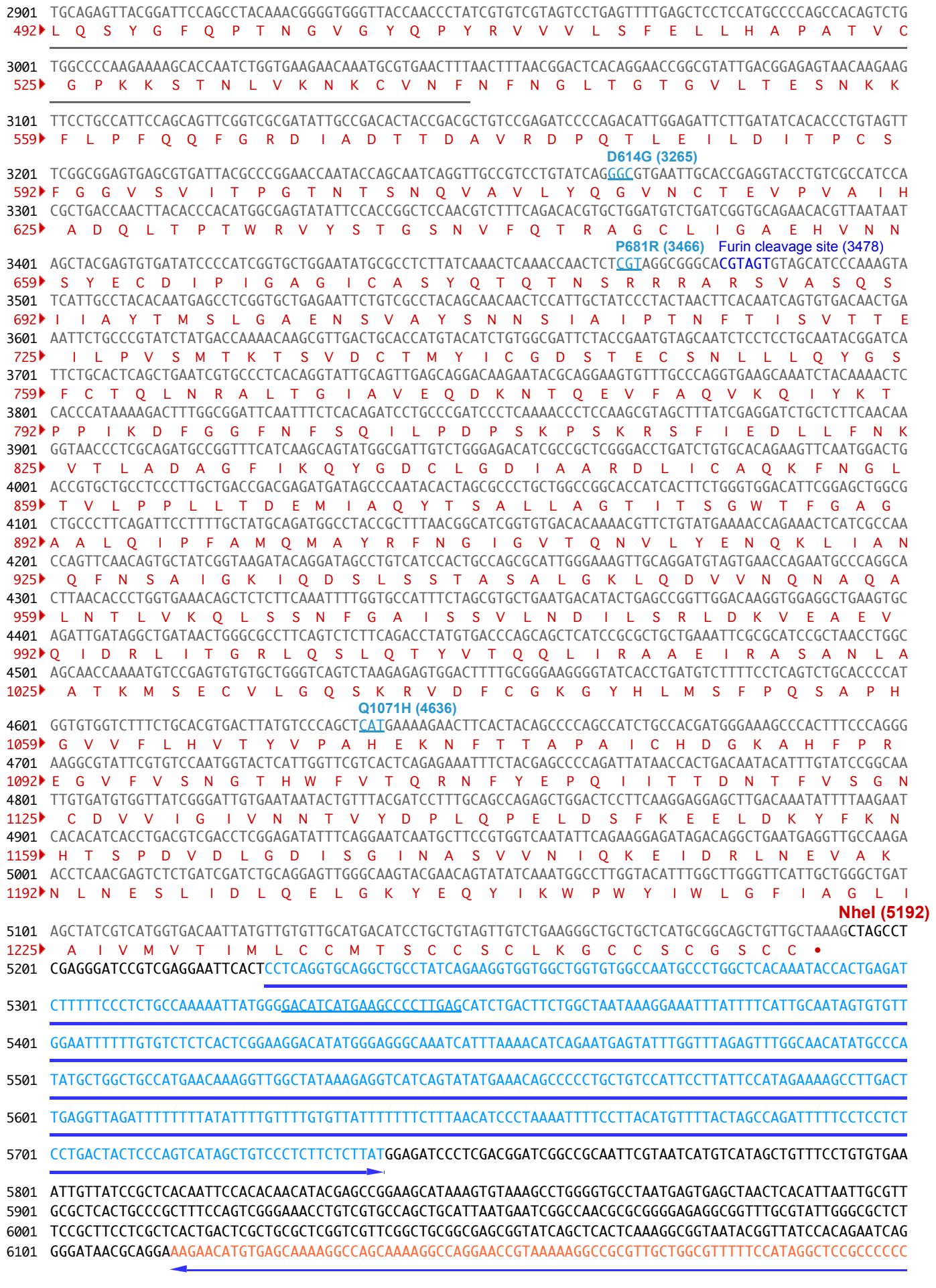
RELATED PRODUCTS

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


hCMV enh
hCMV prom
rbt ßGlo intron
AgeI (1416)
bla
SARS-CoV-2 SpikeV7 Δ19
G142D (1849)
E154K (1885)
pMB1 ori
pLV-SpikeV7
(8609 bp)
RBD
L452R (2779)
E484Q (2875)
rbt ßGlo 3'UTR pAn
D614G (3265)
P681R (3466)
Furin cleavage site (3478)
NheI (5192)
Q1071H (4636)
175


AgeI (1416)
G142D (1849)
E154K (1885)
L452R (2779)
E484Q (2875)


D614G (3265)
P681R (3466)
Furin cleavage site (3478)
Q1071H (4636)
NheI (5192)

```
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
```